Vavilovskii Zhurnal Genetiki i Selektsii
Vavilov Journal of Genetics and Breeding. 2026;30(3):391-402

doi 10.18699/vjgb-26-43

Factors of wheat frost hardiness – ice recrystallization inhibitor proteins

N.E. Korotaeva[1, 2], A.V. Fedyaeva[2], K.K. Musinov[2, 3], A.S. Surnachev[2, 3], G.B. Borovskii[1]

[1] Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia
[2] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[3] Siberian Research Institute of Plant Production and Breeding – Branch of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Krasnoobsk, Novosibirsk region, Russia
knev73@yandex.ru

Abstract. For winter wheat, winter hardiness is one of the complex traits that determine the successful cultivation of this crop, and the responsible genes are recognized as highly significant for breeding work. The accumulation of proteins that prevent ice recrystallization (ice recrystallization inhibition proteins, IRIP) correlates with the survival of winter wheat, which indicates the importance of taking this trait into account when obtaining more frost-resistant varieties. The importance of IRIPs is determined by their ability to integrate into growing ice crystals, which limits the formation of large ice conglomerates in the tissues of winter plants. Wheat IRIPs, which accumulate mainly in the apoplast of leaves and in the crowns during cold acclimation, are characterized by a typical duality of structural organization that determines both the manifestation of IRI activity and anti-pathogenic properties. The wheat IRIP molecule contains at the C-terminus a conserved NxVx(x)G fragment that repeats several times, forming a β-helix responsible for binding to the ice surface; at the N-terminus, there is an LRR sequence typical of pathogen-activated kinases, as well as a guiding signal peptide. The wheat genome contains up to eleven *IRI* genes. The *TaIRI* gene promoter contains typical basic *cis*-activating elements and some elements that respond to abiotic stress and hormones. Isoforms of proteins responsible for protecting against pathogens (pathogenesis related proteins, PRP), which accumulate in winter wheat during cold acclimation, also have IRI activity. The expression of the IRIP and PRP genes positively correlates with the cold resistance of winter wheat plants. According to modern data, the regulation of the IRIP genes and cold-activated PRP genes is ABA-independent, but depends on the presence of jasmonic acid and on some proteomic transcription factors. The review provides examples of the practical use of isolated winter wheat IRIPs. The issue of the factors regulating the activity of the IRIP genes and cold-activated PRPs is the least developed to date. The association of these proteins with the winter hardiness of wheat indicates the prospects for their further study.

Key words: ice recrystallization inhibition proteins; *Triticum aestivum* L.; winter hardiness; regulation of gene activity

For citation: Korotaeva N.E., Fedyaeva A.V., Musinov K.K., Surnachev A.S., Borovskii G.B. Factors of wheat frost hardiness – ice recrystallization inhibitor proteins. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov J Genet Breed*. 2026;30(3): 391-402. doi 10.18699/vjgb-26-43

Funding. This research was funded by a grant of Russian Science Foundation No. 25-24-00117 (https://rscf.ru/project/25-24-00117/).

Факторы морозоустойчивости пшеницы – белки-ингибиторы рекристаллизации льда

Н.Е. Коротаева[1, 2], А.В. Федяева[2], К.К. Мусинов[2, 3], А.С. Сурначёв[2, 3], Г.Б. Боровский[1]

[1] Сибирский институт физиологии и биохимии растений Сибирского отделения Российской академии наук, Иркутск, Россия
[2] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[3] Сибирский научно-исследовательский институт растениеводства и селекции – филиал Федерального исследовательского центра Институт цитологии и генетики Сибирского отделения Российской академии наук, р. п. Краснообск, Новосибирская область, Россия
knev73@yandex.ru

Аннотация. Для озимой пшеницы зимостойкость – один из комплексных признаков, определяющих успешное возделывание этой культуры, а отвечающие за нее гены признаны высоко значимыми для селекционных работ. Накопление белков, препятствующих рекристаллизации льда (ice recrystallization inhibition proteins, IRIP), коррелирует с выживаемостью озимой пшеницы, что указывает на важность учета этого признака при получении более морозоустойчивых сортов. Значение IRIP для выживаемости определяется их способностью встраиваться в растущие кристаллы льда, что ограничивает формирование крупных ледяных конгломератов в тканях озимых растений. IRIP пшеницы, накапливающиеся преимущественно в апопласте листьев и узлов кущения в период холодовой акклимации, характеризуются типичной двойственной структурной организацией, определяющей как проявление IRI-активности, так и антипатогенные свойства. Молекула IRIP пшеницы содержит на С-конце консервативный несколько раз повторяющийся фрагмент NxVx(x)G, формирующий ответственную за связывание с поверхностью льда β-спираль; на N-конце расположены типичная для активируемых патогенами киназ LRR-последовательность, а также направляющий сигнальный пептид. Геном пшеницы содержит до 11 *IRI*-генов. Промотор генов *TaIRI* содержит типичные основные *цис*-активирующие элементы и некоторые элементы, реагирующие на абиотический стресс и гормоны. Изоформы ответственных за защиту от патогенов белков (pathogenesis related proteins, PRP), накапливающихся у озимой пшеницы в период холодовой акклимации, также обладают IRI-активностью. Экспрессия генов IRIP и PRP положительно коррелирует с холодоустойчивостью растений озимой пшеницы. Регуляция генов IRIP и генов PRP, активируемых холодом, согласно современным данным, АБК-независимая, но зависит от присутствия жасмоновой кислоты и от некоторых протеомных факторов транскрипции. В обзоре приведены примеры практического использования изолированных IRIP озимой пшеницы. Вопрос о факторах регуляции активности генов IRIP и активируемых холодом PRP является наименее разработанным на сегодняшний день. Связь этих белков с зимостойкостью пшеницы указывает на перспективность их дальнейшего изучения.

Ключевые слова: белки-ингибиторы рекристаллизации льда; *Triticum aestivum* L.; зимостойкость; регуляция активности генов

Introduction

Winter wheat is an economically valuable cereal crop that is of special interest for plant breeders. This crop is cultivated worldwide in regions with significantly different combinations of climatic conditions, which requires a highly diverse selection of high-yielding, regionally adapted varieties. The availability of a large set of studied traits and their associated genes significantly expands the potential for regional adaptation of wheat and for increasing its yield under specific regional conditions.

Active vegetative growth ensuring high yield of winter wheat depends on the overwintering success rate of winter cereal crops. Traits ensuring high survival during the winter-spring period are of special importance. Winter hardiness is a complex trait responsible for stability against several environmental stresses, including soil freezing (Ambroise et al., 2020).

Soil freezing, which leads to ice formation in the crowns of winter wheat, is one of the most significant damaging factors contributing to survival and yield reduction. The accumulation of antifreeze proteins (AFPs) protects the crowns and leaf parts of the plants from freezing and acts as an indicator of increased frost hardiness. The results of studies on AFPs in cereals were presented earlier in the review by J.G. Duman and M.G. Wisniewski (2014). The reviews available to us mostly focus either on cold tolerance or AFPs in plants in general. The information obtained so far on these proteins in wheat remains fragmented.

The goal of this paper is to provide a comprehensive overview of the currently available literature data on AFPs in soft wheat *Triticum aestivum* L. Here, a general characterization of these proteins will be presented, their molecular organization at the proteomic and genomic levels will be examined, and current knowledge on the regulation of their gene activity and practical applications of AFPs in *T. aestivum* will also be presented.

General characterization of antifreeze proteins

AFPs were first described in Antarctic fish more than fifty years ago (DeVries, Wohlschlag, 1969). Since then, the body of knowledge about these proteins has significantly expanded, and methods for their practical application have been proposed and tested (Voets, 2017; Liu et al., 2021). Several terms are used in research papers to refer to these proteins, including ice-structuring proteins (ISP) (Kontogiorgos et al., 2007; Wang X. et al., 2024), ice-binding proteins (IBP) (Janech et al., 2006), thermal hysteresis proteins (THP) (Ewart et al., 1999), or ice recrystallization inhibitor proteins (IRIP) (Knight et al., 1995). Some plant AFPs are glycosylated proteins, commonly referred to as antifreeze glycoprotein proteins (AFGP) (Griffith et al., 1992). The term “IRI proteins” (IRIP) is currently accepted for wheat AFPs with annotated genes, as it largely reflects their functional activity (Wisniewski et al., 2020).

AFPs in plants were first described in bittersweet nightshade (Urrutia et al., 1992) and winter rye (Griffith et al., 1992). It was later found that plant AFPs had a specific distinctive feature compared to those of other living organisms. Plant AFPs are characterized by a weak ability to lower the freezing point of water below its melting point, a property known as thermal hysteresis. Only a few plant species exhibit thermal hysteresis up to +2 °C (Jarząbek et al., 2009; Bayer-Giraldi et al., 2011). In contrast, this property is strongly expressed in animal AFPs (Kristiansen et al., 1999, 2011). In wheat leaves, thermal hysteresis measured in February is about 0.2 °C (Duman, Olsen 1993).

Ice recrystallization occurs during one of the water freezing stages, when latent heat is slowly released as a result of ice formation (Puchkov, 2017). The ability of plant IRI proteins (IRIPs) to inhibit ice recrystallization lies in the binding of a protein molecule to the surface of a growing ice crystal and its incorporation into the crystal, which shapes the crystal as a hexagonal disk (at low IRIP concentrations) or a bipyramidal prism (at high IRIP concentrations) (Griffith et al., 2005). Importantly, this prevents the formation of large ice conglomerates (Yang et al., 2024), which are likely to damage the living tissues.

The level of IRI activity depends on the concentration of IRIPs in the freezing solution (Regand, Goff, 2006). The ability of plant IRIPs to inhibit ice recrystallization was first described in rye (Griffith et al., 1992) and later in other cold-tolerant species (Antikainen, Griffith, 1997). This property turned out to only be characteristic of frost-hardy plants (Hon et al., 1995; Antikainen, Griffith, 1997).

As an overwintering cold-tolerant species, wheat contains *IRI* genes. The accumulation of IRIPs in wheat is especially relevant in late autumn and early winter, preceded by cold acclimation at low positive temperatures. At temperatures ranging from –6 to –8 °C, cold-acclimated wheat plants exhibit intercellular ice formation in the vascular transition zone of crowns, where large quantities of water migrate from the meristem zone. This water redistribution contributes to the better protection of the meristem zone from ice damage and ensures winter survival (Willick et al., 2019). IRIPs accumulate primarily in the vascular transition zone of crowns compared to the stem meristem zone (Willick et al., 2018).

It is worth noting that the minimum temperature at which meristematic cells survive in the overwintering wheat is close to the LT50 (Lethal Temperature 50 – the temperature at which 50 % of the test plant population dies), whereas IRIP accumulation correlates with the overall winter survival (Chun et al., 1998). In spring, *IRI* genes affect growth, heading, and the onset of flowering in wheat by stimulating the activity of genes responsible for vernalization (Cao et al., 2021).

Snow cover creates favorable conditions for the survival of pathogenic fungi (Gaudet, Laroche, 1997). As a result of tissue damage caused by growing ice conglomerates, there is a high risk of pathogenic infection in winter wheat plants after the loss of snow cover at the end of winter. In response to pathogen attack, plants synthesize PR proteins (PRPs), which are released into the apoplast upon infection to inhibit fungal enzymes or enzymatically degrade their cell walls (Jain, Khurana, 2018).

The LRR amino acid sequence, which is common among PRPs, is also contained in wheat IRIP molecules (Tremblay et al., 2005), where it is responsible for the PR activity (Juurakko et al., 2022). Some wheat PRPs, such as β-1,3-glucanases, chitinases, thaumatin-like proteins, and polygalacturonase-inhibition proteins, exhibit antifreeze activity (Hon et al., 1995; Kontogiorgos et al., 2007). In response to cold, wheat accumulates specific PRP isoforms acting as IRIPs, modifying ice growth during freezing, and ensuring pathogen resistance even before infection (Griffith, Yaish, 2004).

Wheat IRIPs have been found in plant organs such as roots, stems, leaves, and crowns (Duman, Olsen, 1993; Willick et al., 2018, 2019; Jin et al., 2022; Vaitkevičiūte et al., 2024). The initial activation of *TaIRI* genes after cold exposure occurs in the leaves and then propagates to roots and stems (Jin et al., 2022), which is likely due to leaves being above-ground organs, and as such, the first to be affected by freezing. For example, the *TaIRI1* transcript was detected in leaves, crowns, and roots, while the *TaIRI2* transcript was only found in leaves (Tremblay et al., 2005).

A significant accumulation of IRIPs in the extracellular space is observed in winter cereals during cold acclimation (Antikainen, Griffith, 1997). PRPs, the genes of which are regulated by cold acclimation/deacclimation, are likely to act as IRIPs and may be localized in the extracellular space, plasma membrane, nucleus, or cytoplasm (Vaitkevičiūte et al., 2024).

Amino acid sequence of *T. aestivum* IRIPs

It was previously shown that the mature *T. aestivum* IRIPs TaIRI-1 and TaIRI-2 with molecular masses of 26.8 and 40.7 kDa respectively, share 40.4 % sequence identity (Tremblay et al., 2005). A conserved fragment located at the C-terminus and consisting of NxVxxG or NxVxG repeat motifs, where “x” represents a non-conserved residue, is recognized as the main conserved fragment of plant IRIPs, including those of wheat (Tremblay et al., 2005; Middleton et al., 2012; Jin et al., 2018). This conserved amino acid sequence, repeated several times at the C-terminal region, forms a β-helix structure. The two sides of this helix make it possible for IRIPs to bind to the surfaces of two forming ice crystals (Middleton et al., 2009).

Presumably, the number of repeats of this conserved sequence evolved through duplication events during the acquisition of cold tolerance in plants, and an increased number of repeats is likely associated with enhanced resistance to ice formation (Sandve et al., 2008). The number of repeats of the conserved C-terminal motif may vary. For example, TaIRI-1 and TaIRI-2, the two IRIPs of *T. aestivum*, exhibit five and six repeats, respectively (Tremblay et al., 2005). In a later study, the six identified *T. aestivum* IRIPs were found to contain 9 to 13 repeats (Jin et al., 2018).

The N-terminal region of IRIPs contains a leucine-rich segment (Jin et al., 2018). The LRR amino acid sequence, which is common among both eukaryotic and prokaryotic organisms, is known to determine the properties of protein-protein and ligand-binding interactions. In plants, it is incorporated into protein molecules synthesized in response to stress caused by pathogenic organisms (McHale et al., 2006). The LRR domain of *T. aestivum* proteins TaIRI-1 and TaIRI-2 turned out to be homologous to receptor-like kinases (Tremblay et al., 2005) involved in signaling pathways triggered by the emergence of pathogen-associated elicitor molecules (He, Wu, 2016).

Another region of TaIRI molecules turned out to be homologous to the receptor kinase of the phytosulfokine peptide phytohormone (Tremblay et al., 2005) involved in differentiation of the plant immune response depending on the pathogen type (Sauter, 2015). *T. aestivum* proteins coded for by genes *Tr001_B19* and *Tr001_M19* showed high similarity to cold-regulated IRIP sequences and were annotated as protein kinases with LRR sequences (Monroy et al., 2007). Although IRIPs with kinase domains do not exhibit protein kinase activity (Tremblay et al., 2005), these findings indicate a similarity between IRIPs and PRPs. The N-terminal region of *T. aestivum* IRIPs also contains a signal peptide directing proteins to the extracellular space or to the membrane (Tremblay et al., 2005).

Eight wheat IRIPs were grouped based on the similarity of their amino acid sequences. According to phylogenetic analysis, the amino acids located at the C- or N-terminal regions determine the classification of some wheat IRIPs into group 2 (TaIRI-6, TaIRI-7, and TaIRI-8). Group 1 includes the proteins TaIRI-1, TaIRI-3, TaIRI-4, and TaIRI-5. TaIRI-2 protein having a distinct LRR domain was attributed to the third group (Jin et al., 2018).

T. aestivum IRIP gene organization

The IRI gene family in cold-tolerant grasses of the Pooideae subfamily began to form and acquire specific features approximately 75 Mya, following the divergence of this subfamily into a separate taxon (Sandve et al., 2008). According to the prevailing evolutionary hypothesis with regard to *LRR-IRI*-like genes, the most recent common ancestor of *IRI* genes in Pooideae was the *OsLRR-PSR*-like gene identified in rice. This gene contained at its 5′ end an LRR sequence characteristic of the phytosulfokine receptor kinase. Notably, this sequence is also present in *TaIRI* genes (Houde et al., 2006). It is assumed that the IRI-coding fragment appeared in *TaIRI* genes as a result of an insertional event (Tremblay et al., 2005). The major expansion of the IRI gene group in Pooideae occurred around 36 Mya due to duplications of a specific IRI region within the genes (Sandve et al., 2008). In various studies, *T. aestivum* was found to possess two (Tremblay et al., 2005), six (Jin et al., 2018), or eleven *IRI* genes (Sandve et al., 2008) containing both LRR and IRI coding fragments. The sequence identity between *TaIRI1* and *TaIRI2* is 74.7 and 54.1 % at the 5′ and 3′ ends, respectively (Tremblay, 2005).

With few exceptions, the grouping of *IRI* genes in monocotyledons based on genomic sequence similarity generally corresponds to genus affiliation and phylogenetic relationships between the taxons. It is noteworthy that the organization of *IRI* genes in *Triticum* is closely related to that of *Aegilops* genus plants, which are the progenitors of allohexaploid wheat. Differences in characteristic regions of *TaIRI* (*T. aestivum*) gene sequences were found when compared against *TdiIRI1* (*T. dicoccoides*), *TmIRI1* (*T. monococcum*) and *TuIRI1* (*T. urartu*), but not against *TdIRI1* (*T. durum*) (Jin et al., 2018).

Eight *TaIRI* gene sequences are divided into four groups, with one gene each in groups III and IV (*TaIRI6* and *TaIRI2*, respectively), four genes in group I (*TaIRI1*, *TaIRI3*, *TaIRI4*, and *TaIRI5*), and two genes in group II (*TaIRI7* and *TaIRI8*) (Sandve et al., 2008; Jin et al., 2018).

The promoters of *TaIRI* genes contain typical core *cis*-acting elements as well as elements responsive to abiotic stress and hormones. These responsive elements include three elements in charge of responses to low temperature and drought stresses, one element specifically in charge of stress response at low temperatures, two elements involved in methyl jasmonate signaling response, four light-responsive elements, one abscisic acid-responsive element (ABRE), and several additional regulatory elements (ARE, CAT-box, O2-site and TC-rich repeats) (Jin et al., 2022).

Mapping of *T. aestivum* IRIP and cold-activated PRP genes

To date, a substantial amount of information has been obtained regarding the chromosomal localization of traits associated with wheat frost hardiness. Relevant traits for frost hardiness are located in QTL (Quantitative Trait Loci) regions on chromosomes 1D, 2A, 2B, 6D, and 7B (Båga et al., 2007); 5B and 5D (Chun et al., 1998); 5A, 2D, 2A, and 4B (Case et al., 2014); 5A and 4B (Kruse et al., 2017); as well as in chromosomes from group 5, 2B, and 4B (Sutka, 1994, 2001). The *Fr1* and *Fr2* genes controlling frost hardiness have been mapped on chromosomes 5A and 5D, respectively (Sutka et al., 1997). Substitutions of chromosomes from the Cheyenne variety for chromosomes 4A, 6A, 3B, 5D, and 3D of the frost-sensitive Chinese Spring variety resulted in increased protein accumulation in the apoplast, while the substitutions of chromosomes 1A, 5A, 4B, 5B, 6B, 1D, and 5D resulted in increased antifreeze activity.

The key regulatory genes responsible for increasing both the antifreeze activity and protein accumulation in the leaf apoplast were observed on chromosomes 5B and 5D (Chun et al., 1998). Two *TaIRI* genes were mapped on the same chromosomes obtained from the leaves of the cold-acclimated Zhoumai variety of *T. aestivum* (Zheng et al., 2020). The *Tr015_M14* and *Tr017_M15* genes coding for IRIPs in the Chinese Spring variety, were located in the 4A locus (Monroy et al., 2007). Notably, the analysis was conducted using plant parts that included both the crown and leaves, despite the fact that the highest IRIP content in wheat plants exposed to freezing is found in the vascular transition zone of crowns, while the meristematic zone and leaves exhibit their own specific responses to freezing (Willick et al., 2018).

Since IRIPs contain a fragment related to PRPs, it is reasonable to search for loci that simultaneously control responses to cold and pathogen attack. QTL regions in *T. aestivum* associated with resistance to both stress factors have been identified on chromosome 5A and are closely linked to the *Fr2* locus. Separate QTLs associated with freezing hardiness were found on chromosomes 5A and 4B, while a QTL associated with snow mold resistance was identified on chromosome 6B (Kruse et al., 2017). The accumulation of β-1,3-glucanases, chitinases, and thaumatin-like proteins under freezing conditions was reduced in plants with chromosome substitutions 2A, 3A, 6B, and 7A. Substitution of chromosome 7A led to a significant prolongation of the freezing period followed by PRP gene activation (Chun et al., 1998).

It can be seen that the composition of QTL regions responsible for frost hardiness and antifreeze activity in wheat varies among varieties. In addition, the specific plant part used for mapping plays a significant role. Therefore, when genome organization of wheat plants is analyzed in studies of proteins with IRI activity, it is essential to take these factors into account.

Effect of low temperatures on the activity of *T. aestivum* IRIP genes

To date, substantial evidence has been accumulated showing that the expression of *T. aestivum* AFPs is induced by cold exposure (see the Table) and is associated with cold and frost hardiness of the variety.

The expression of three *TaIRI* genes (*TaIRI16*, *TaIRI17*, and *TaIRI18*) in stem, leaf, and root tissues of young *T. aestivum* plants was induced by cold (+4 °C, from 2 to 72 hours), and *TaIRI16* showed the most significant induction among them (Jin et al., 2018, 2022). The *TaIRI1* transcript in soft wheat begins to accumulate after low temperature exposure (+4 °C) and reaches its maximum level after 36 days of acclimation. Further exposure to low temperature does not lead to a higher transcript level. The *TaIRI2* transcript also accumulates immediately after the onset of cold hardening, but it reaches its peak earlier in the acclimation period. After deacclimation, transcript levels return to those of non-acclimated plants (Tremblay et al., 2005).

Cold hardening (1–6 days) of seedlings from the Jagger and Alabaskaya varieties showed increased transcript levels of the *Tr001_B19* (GB ID CK197231) and *Tr001_M19* (GB ID CK201227) genes exhibiting high sequence similarity with cold-regulated IRIPs (Monroy et al., 2007). The activity of *TaIRI* genes related to genes coding for *Lolium perenne* IRIPs more than doubled after moving cold-hardened *T. aestivum* plants to subzero temperatures (Herman et al., 2006). The activity of the AFP I precursor gene (represent sequence ID CK197682) was more than eight times as high within one day of freezing young wheat plants at –5 °C and reached even higher levels by the third day of freezing (Kang et al., 2013).

Levels of *TaIRI-1* and *TaIRI-2* transcripts increase significantly in response to both gradual and abrupt temperature drops. Their induction occurs later than that of PRP-encoding genes. The highest accumulation levels are observed in leaves, especially when it comes to the *TaIRI-2* transcript (Winfield et al., 2010).

Much of the evidence for the role of IRIPs in cold and frost hardiness has been obtained using wheats varying in their cold tolerance. Enhanced expression of *TaIRI-1* and *TaIRI-2* in response to low temperatures was observed in winter wheat cultivars compared to spring ones (Winfield et al., 2010). The more cold-tolerant cultivar Mironovskaya 808 has demonstrated higher *TaIRI* gene activity under all tested cooling regimes (Jin et al., 2022). After the first six hours of cold acclimation, transcript levels of *Tr015_M14* and *Tr017_M15* coding for AFPs were lower in the winter cultivar CDC Clair (LT50 –17 °C) than in the spring cultivar Quantum (LT50 –8 °C). However, the gene activity in the winter cultivar exceeded that in the spring cultivar after the first day of hardening, with almost three times the difference in *Tr017_M15*. Expression of the *Tr015_J17* gene, annotated as IRIP-encoding, was two times as high in winter wheat seedlings after one day of acclimation at +4 °C compared to the spring cultivar, and this difference increased with longer acclimation up to day 14. Transcript levels of *Tr001_B19* in the winter cultivar CDC Clair exceeded those in the spring cultivar Quantum at all stages of acclimation (from 6 hours to 14 days) (Monroy et al., 2007).

Additional evidence for the role of IRIPs in *T. aestivum* cold tolerance was obtained through gene transfer experiments. Expression of the *TaIRI4* and *TaIRI6* genes from the frost-resistant cultivar Mironovskaya 808 in transgenic tobacco under freezing conditions led to reduced membrane permeability (with the transformation of *TaIRI4*) and decreased lipid peroxidation activity (with transformations in both *TaIRI4* and *TaIRI6*) (Jin et al., 2018).

These studies provide convincing evidence that the expression of *T. aestivum* IRIP genes is activated by both subzero and low positive temperatures and contributes to the development of frost hardiness in this species.

IRIP- and PR-encoding genes, the activity of which increases in response to cold exposure

Gene name	Treatment conditions	Stage of development or plant organ	Reference
IRIP			
TaIRI4, *TaIRI16*, *TaIRI17*, *TaIRI1*	+4 °C, 2–72 h	leaves, stems, roots	Jin et al., 2018, 2022
TaIRI-1	+4 °C, 36 d	leaves, roots crowns	Tremblay et al., 2005
TaIRI-2	+4 °C, 6 d	leaves	
Tr001_B19 (GB ID K197231); *Tr001_M19* (GB ID K201227); *Tr015_M14* (GB ID Y742123); *Tr017_M15* (GB ID Y742648)	+4 °C, 1–14 d	seedlings	Monroy et al., 2007
Tr015_J17	+4 °C, 1–6 d	seedlings	
Contig3670_at; *Contig3668_at*; *Contig7221_s_at*	+3 °C, 3 weeks → –3 °C, 6 h–3 d	–	Herman et al., 2006
Ta.12663.1.S1_at (CK197682)	–5 °C, 1–3 d	leaves	Kang et al., 2013
TaIRI-1; *TaIRI-2*	"shock" (temperature decline from +16 to +4 °C during 2 d); "acclimation" (temperature decline from +16 to +4°C during 5–9 weeks)	leaves	Winfield et al., 2010
TaIRI-1	+3 °C, 3 weeks	leaves	Livingston et al., 2021
PRP			
Pathogenesis-related protein 1-21 (TraesCS7B03G0275300)	+2 °C, 7 weeks → +10 °C, 24 h–1 weeks → + 2 °C, 24 h–2 weeks	crowns	Vaitkevičiūte et al., 2024
PRP – chitinases			
*CL386Contig5** *CL386Contig1** *CL1911Contig1** *CL40Contig11** *CL40Contig9** *CL754Contig1**	+4 °C, 14 d	–	Houde et al., 2006
JC5845	+3 °C, 3 weeks → –3 °C, 6 h–3 d	–	Herman et al., 2006
Chitinase (ID AB029936.1)	–5 °C, 1–3 d	leaves	Kang et al., 2013
Chitinase (class II)	+2 °C, 1–28 d	crowns	Gaudet et al., 2000
TaCHT-1	+3 °C, 3 weeks	leaves	Livingston et al., 2021
Chitinase EC 3.2.1.14 (TraesCS1B03G0732400) (TraesCS3A03G0663800) (TraesCS4D03G0756500)	+2 °C, 7 weeks → +10 °C, 24 h–1 week → + 2 °C, 24 h–2 weeks	crowns leaves	Vaitkevičiūte et al., 2024
PRP – thaumatin-like proteins			
TraesCS5B03G1182300 TraesCS2A03G0227600 TraesCS4A03G0142000 TraesCS4A03G0750400 TraesCS4A03G1252200 TraesCS4D03G0549700 TraesCS5A03G0810000 TraesCS5A03G0043700 TraesCS7B03G1303000 TraesCS7B03G1122500 TraesCS5B03G0034800 TraesCS7A03G1360800 TraesCS6B03G1288000	+2 °C, 7 weeks → +10 °C, 24 h–1 week → + 2 °C, 24 h–2 weeks	crowns leaves	Vaitkevičiūte et al., 2024
PRP – glucanases			
TaGLB2b	+16 → +4 °C during 5–9 weeks	leaves	Winfield et al., 2010
β-1,3-glucanase	+2 °C, 1–28 d	crowns	Gaudet et al., 2000

* The Arabidopsis TAIR database was used to find homologues (Hannah et al., 2005).
GB ID – GenBank Identification Description.

Regulation of *T. aestivum* PRP gene activity by low temperatures

A number of PRP-encoding wheat genes are activated by low temperatures (see the Table). It is assumed that such genes code for PRP isoforms with IRI activity. M. Houde et al. (2006) obtained high-quality ESTs (Expressed Sequence Tags) from wheat cDNA libraries associated with cold stress. A set of 75,488 unique sequences (31,580 contigs and 43,908 singletons/singlets) enriched with stress-regulated genes was obtained. Among them were ESTs containing contigs coding for chitinases, β-1,3-glucanase, and thaumatin-like proteins. Fifteen of these sequences were annotated as precursors or actual chitinases, six of which being cold-inducible. The transcript level of the *TaGLB2b* gene coding for glucanase significantly increases only in response to gradual temperature decrease, while no increase is observed under sudden cold shock. Its induction occurs earlier than that of *TaIRI* genes, i. e. between the third and the fifth weeks of cooling (Winfield et al., 2010).

The activity of the *JC5845* gene coding for chitinase more than doubles after moving cold-hardened *T. aestivum* plants to subzero temperatures (Herman et al., 2006). The gene coding for chitinase 3 (represent sequence ID AB029936.1) was among those, the activity of which was more than eight times as high in response to freezing young wheat plants at –5 °C. Its expression level rose significantly depending on the duration of exposure, with nearly a 20 times difference between the measurements taken on days 1 and 3 of freezing (Kang et al., 2013).

Differences in PRP gene expression levels in response to cooling were observed between wheats varying in PR and frost hardiness. When comparing the genomes of spring and winter wheat cultivars under cold acclimation (+4 °C, from 6 hours to 14 days), no significant differences were found in the activity of genes coding for thaumatin or chitinase. However, the increase in transcript levels of the *Tr017_C17* gene coding for chitinase II precursor in response to hardening was higher in the winter cultivar (Monroy et al., 2007).

A number of chitinase and β-1,3-glucanase genes are regulated by autumn cold acclimation: their expression begins in late autumn, peaks in mid-winter, then decreases, and reaches maximum levels again in spring. Under cold acclimation, β-1,3-glucanase gene transcripts are weakly expressed during short-term exposure, but the expression increases significantly with prolonged exposure and remains elevated after returning to control conditions. The chitinase-encoding gene begins active expression immediately after the onset of cooling, and its expression decreases after returning to control conditions. Differences in expression levels of these genes between cultivars resistant and less resistant to snow mold began to manifest themselves during autumn cold acclimation, with higher expression observed in the more resistant cultivar (Gaudet et al., 2000).

D.P. Livingston et al. (2021) found that cold acclimation of young wheat plants enhances the expression of genes coding for chitinase-1 (*TaCHT-1*) and IRIPs. However, the relationship between the freezing temperature of leaves from pre-acclimated plants and the expression levels of these genes turned out to be negative, i. e. the expression was lower in leaves that froze at lower temperatures.

Since plant AFPs lower the freezing point by only a fraction of a degree (Tremblay et al., 2005), the absence of a direct positive correlation between *TaIRI-1* and *TaCHT-1* expression and leaf freezing temperature is understandable. Expression levels of *TaIRI-1* and *TaCHT-1* show positive correlation with the abundance and complexity of bacterial and fungal communities, likely due to the structural and functional similarities between AFPs and PRPs.

These findings indicate the existence of differential expression of PRP genes regulating their activity depending on the wheat cultivar's cold tolerance, the duration and direction of exposure, and organ-specific features.

G. Vaitkevičiūte et al. (2024) studied the effects of cold deacclimation and reacclimation on genome activity in frost-hardy and frost-sensitive *T. aestivum* cultivars. It was shown that a significant number of genes associated with pathogen response reacted to both deacclimation and repeated cold exposure, with gene activity affected by the duration of exposure. For example, both short- and long-term deacclimation of crowns led to reduced activity of the thaumatin-like protein gene, while longer exposure additionally reduced the activity of chitinase-encoding genes.

During reacclimation, differential expression of genes coding for PRPs with similar action was observed. Among the genes coding for chitinase in crowns, some exhibited decreased activity during reacclimation, while others showed increased activity. Reacclimation in crowns also resulted in differential activity in thaumatin-like protein genes depending not only on the direction of change but also on the duration of exposure.

A significantly more diverse set of genes coding for thaumatin-like protein and chitinase in *T. aestivum* leaves compared to crowns responded with differential expression to deacclimation and reacclimation depending on the duration of exposure. The activity of PR genes in the crowns of the resistant cultivar Lakaja DS was less affected by temperature fluctuations compared to the more sensitive cultivar KWS Ferrum. In contrast, a greater number of genes were regulated by temperature fluctuations in the leaves of the more resistant cultivar compared to the more sensitive one, many of them being thaumatin-like protein genes (Vaitkevičiūte et al., 2024). These findings reveal a link between wheat cold tolerance and the transcription of PRP genes presumably also acting as IRIPs.

Given the above, we can conclude that wheat genes coding for PRPs are activated by cold and reacclimation and contribute to enhanced cold and frost hardiness. At the same time, genes belonging to the same PRP subgroup (chitinases, thaumatin-like proteins) are regulated differentially, and not all of them are activated by cooling.

Regulatory pathways of *T. aestivum* IRIP and cold-induced PRP gene expression

It has been shown that wheat *IRI* genes begin to express in response to decreasing temperatures. The activity of plant genes responsible for cold tolerance is regulated through both ABA-dependent and ABA-independent pathways. Among the latter, the ICE–CBF–COR signaling pathway is considered the primary one. This includes cold-regulated genes (COR – COld Related), which activated by C-repeat Binding Factor (CBF) with the activity of CBF controlled by a dedicated inducer (ICE – Inducer of CBF Expression) (Chinnusamy et al., 2003). The role of this pathway in activating cold adaptation genes in *T. aestivum* is widely recognized. However, the regulation of *IRI* genes by the ICE–CBF–COR transcription factor group has not been definitively proven, despite the fact that ICE proteins in *T. aestivum* bind to MYC elements (Badawi et al., 2008) present in *TaIRI* promoters (Jin et al., 2022). The literature search revealed one research suggesting a possible link between the ICE–CBF–COR pathway and *IRI* regulation in *T. aestivum* (Tchagang et al., 2017).

Another known regulatory pathway for cold adaptation is ABA-dependent (Xue-Xuan et al., 2010). Although ABA plays an important role in the development of cold and frost hardiness in *T. aestivum* (Zheng et al., 2020), some factors responsible for these traits are activated independently of ABA (Wang W. et al., 2017). The ABA involvement in the activation of *TaIRI* and PRP genes has not been confirmed, although *TaIRI* gene promoters contain elements associated with ABA-responsiveness (Jin et al., 2022). No activation of the *TaIRI1* and *TaIRI2* genes was observed after spraying 6-day-old *T. aestivum* seedlings with ABA (Tremblay et al., 2005).

In *T. monococcum*, ABA levels in leaves and crowns increase during the initial phase of non-lethal cold exposure, but the adaptation begins on the third day of exposure. Notably, the decrease in ABA levels coincides with an increase in salicylic acid content. Thus, it is possible that ABA's influence on *TaIRI* and cold-induced PRP gene expression during cold exposure is limited based on exposure duration, and ABA may not be involved in the adaptive response during later phases requiring IRIP accumulation, when the regulatory role in adaptive changes may shift to other factors (Vanková et al., 2014).

Wheat IRIPs are structurally similar to PRPs, and its PRPs (β-1,3-glucanases, thaumatin-like proteins, and chitinases) also exhibit AFP properties. It is reasonable to assume that PR-associated factors, particularly the WRKY transcription factor family, may act as gene transcription factors for plant AFPs (Pandey, Somssich, 2009). Several findings indirectly support this possibility. For example, WRKY gene expression is associated with increased wheat resistance during the early stages of cold acclimation (Talanova et al., 2008; Winfield et al., 2010); some WRKY genes are activated by cold only in winter wheat (Winfield et al., 2010); and WRKY gene activity increases at subzero temperatures and depends on exposure duration (Herman et al., 2006). In addition, some wheat genes coding for glucanases, chitinases, and thaumatin-like proteins are co-regulated with genes coding for WRKY transcription factors (Winfield et al., 2010). The *TaWRKY1* gene is activated by both pathogen attack and cold (Wang W. et al., 2017), and some WRKY proteins exhibit IRIP-like properties on their own (Huang, Duman, 2002).

It was shown that phytohormones involved in pathogen response, such as ethylene, jasmonic acid, and salicylic acid, also participate in the AFP gene regulation. These phytohormones are also involved in regulating IRI gene activity in wheat (Tremblay et al., 2005), although their role in cold and frost hardiness is not always definitively confirmed (Kurepin et al., 2013). In addition, it has been shown that ethylene is not associated with wheat cold tolerance (Macháčcková et al., 1989).

Expression of the *TaIRI17*, *TaIRI18*, and especially *TaIRI16* genes in stem, root, and leaf tissues of young *T. aestivum* plants increased after exogenous treatment of wheat shoots, roots, and leaves with methyl jasmonate combined with cold exposure (+4 °C, 24 h) (Jin et al., 2022). Exogenous methyl jasmonate treatment also stimulated the activity of *TaIRI* gene promoters (Jin et al., 2022). However, jasmonic acid had different effects on *TaIRI1* and *TaIRI2* expression in 6-day-old seedlings of a frost-hardy *T. aestivum* cultivar, it induced *TaIRI1* expression but not *TaIRI2* (Tremblay et al., 2005). Analysis of motif activity of *TaIRI* gene promoters in tobacco and their expression in *A. thaliana* after the respective transformations showed that MYB, Mybb, MYC, CGTCA, and TGACG motifs from the *TaIRIp1* promoter were highly active and inducible by cold and methyl jasmonate (Jin et al., 2022).

The role of salicylic acid in AFP gene regulation still remains to be confirmed. No activation of *TaIRI1* and *TaIRI2* was observed after spraying 6-day-old seedlings of the frost-hardy *T. aestivum* cultivar with salicylic acid (Tremblay et al., 2005). Treatment of young wheat plants of cultivar Yangmai 16 with salicylic acid solution followed by 6 days of field freezing (at the average temperatures of +5.3 °C and minimum of –7 °C) did not increase *IRI2* (AY968589) expression compared to control temperature conditions (average +12 °C) (Wang W. et al., 2021). At the same time, freezing without salicylic acid solution treatment led to a twofold increase in gene expression compared to control. According to the authors, previous experiments showed that pretreatment with salicylic acid could increase *TaIRI2* expression during 48-hour cold exposure (Wang W. et al., 2020). Thus, it may be suggested that salicylic acid regulation of *TaIRI2* expression may depend on exposure duration, though this requires further study.

Ethylene is involved in regulating antifreeze activity in winter rye in response to cold and drought, with β-1,3-glucanases, chitinases, and thaumatin-like proteins accumulating in the leaf apoplast (Yu et al., 2001). In *T. aestivum*, spraying 6-day-old seedlings of a frost-hardy cultivar with ethylene solution led to differential regulation of the *TaIRI1* and *TaIRI2* genes: specifically, it induced *TaIRI1* expression but not that of *TaIRI2* (Tremblay et al., 2005). However, ethylene turned out to have a negative association with wheat cold tolerance (Macháčková et al., 1989).

Overall, data on transcription factor involvement in AFP gene expression regulation remain limited, and information on phytohormonal regulation is often contradictory. It appears that the duration, intensity, and phase of cold exposure play a significant role in phytohormonal regulation. It also remains unclear why the studied phytohormones do not enhance wheat cold tolerance despite their ability to activate *TaIRI* genes. This aspect of antifreeze activity regulation requires further investigation.

Practical application of *T. aestivum* IRIPs and cold-induced PRPs

The use of plant AFPs is limited by their reduced thermal hysteresis capacity compared to animal AFPs and is primarily justified by their ice recrystallization inhibition (IRI) activity. One of the most promising directions for the practical application of AFPs is the development of more frost-hardy plants and cultivars. Although gene transfer studies involving wheat AFPs have yielded encouraging results (Jin et al., 2022), practical use of the available knowledge on wheat IRIPs remains limited.

In present, the main area of practical application for plant AFPs is cryopreservation in medicine and food industry. Naturally derived AFPs are less toxic than dimethyl sulfoxide (DMSO), which is commonly used today, making them promising cryoprotectants (Chow-Shi-Yée et al., 2020). A crude extract from wheat seedlings turned out to protect rat hepatocytes during cryopreservation; the effect could be successfully enhanced by further purification of the extract or by using recombinant TaIRI-2 protein, which enabled longer storage of hepatocytes and recovery of a greater number of viable cells restoring their functions upon thawing (Grondin et al., 2009).

When recombinant TaIRI-2 was used for cryopreserving insulin-secreting INS832/13 cells, it was found that TaIRI-2 could penetrate these cells as well as hepatocytes. This likely contributed to improved cell survival after thawing and reduced cell death (Chow-Shi-Yée et al., 2016). However, it is important to consider that plant-based cryoprotectants may have immunogenic properties, potentially triggering unwanted immune or allergic reactions, which is a significant limitation for medical cryopreservation uses (Kostyaev et al., 2016).

In the food industry, natural AFPs are widely used in ice cream production, with several patents granted, including one for the use of AFPs from winter rye (Boonsupthip, Lee, 2003). AFPs extracted from cold-acclimated wheat significantly improved ice cream texture, and this effect was not diminished by shock freezing (Regand, Goff, 2006). Adding AFPs extracted from flour of frost-hardy winter wheats to bread dough reduces water flow and migration during freeze-thaw cycles, helping to preserve the dough's internal structure and gluten network, increase viscosity and elasticity, and improve textural properties (Wang X. et al., 2024).

Direct use of raw plant extracts in the food industry is preferable to using purified extracts due to their low yield and the complexity of the purification process. However, using concentrated extracts can be problematic due to the presence of enzymes that may negatively affect the taste or texture of the final product. Undesired enzymatic activity in the extract can be eliminated by heat treatment, so thermal stability becomes necessary for natural AFPs used in food production. A promising thermostable thaumatin-like AFP was identified in the leaf apoplast extract of cold-acclimated *T. aestivum* (Kontogiorgos et al., 2007).

Conclusion

The relationship between the expression of AFP genes (with the subsequent accumulation of their respective products) and increased frost hardiness in winter wheat has been convincingly demonstrated in a number of model experiments. It has been shown that the accumulation of apoplastic proteins and AFPs correlates with the survival of winter wheat plants under field conditions (Chun et al., 1998). However, it remains unclear how significant the contribution of antifreeze activity is to plant survival compared to other known factors (e. g., accumulation of free proline and oligosaccharides, depth of occurrence of the crown). Therefore, including AFP accumulation as a target trait in breeding programs still requires more solid justification. Wheat AFPs exhibit dual antifreeze and antipathogenic activity. On the one hand, this duality complicates the assessment of the specific contribution of AFPs to wheat survival; on the other hand, it makes the presence of this trait particularly valuable, as it reflects the presence of two types of resistance and yield-enhancing factors.

In the vast majority of model experiments, the effect of low positive temperatures on the expression of IRIP and cold-induced PRP genes was assessed, while only a few studies examined the impact of subzero temperatures (Herman et al., 2006; Kang et al., 2013; Willick et al., 2018). As a result, researchers can currently draw conclusions only about the association of these genes with cold tolerance. However, the actual need for these proteins arises under freezing conditions. Simulating field-like subzero temperatures in experimental setups could not only deepen our understanding of IRIP and PRP gene expression but also bring us closer to understanding how their functions are realized in real-world conditions.

Understanding the regulatory mechanisms controlling AFP gene activity remains a relevant problem, as the overall picture of their regulation is still unclear, especially regarding the protein factors involved in gene expression. The hormonal regulation patterns of wheat AFPs are also not fully understood. It is highly likely that the dual nature of plant AFPs has led to the development of a regulatory system in which signaling pathways for both biotic and abiotic stress responses are involved. This hypothesis requires particularly thorough investigation.

The promising results of practical applications of wheat AFPs, along with evidence linking cold tolerance, gene expression, and protein accumulation in winter wheat, support the need for continued research into this group of low-temperature adaptation proteins.

References

Ambroise V., Legay S., Guerriero G., Hausman J.F., Cuypers A., Sergeant K. The roots of plant frost hardiness and tolerance. *Plant Cell Physiol*. 2020;61(1):3-20. doi 10.1093/pcp/pcz196

Antikainen M., Griffith M. Antifreeze protein accumulation in freezing-tolerant cereals. *Physiol Plant.* 1997;99(3):423-432. doi 10.1111/j.1399-3054.1997.tb00556.x

Badawi M., Reddy Y.V., Agharbaoui Z., Tominaga Y., Danyluk J., Sarhan F., Houde M. Structure and functional analysis of wheat ICE (inducer of CBF expression) genes. *Plant Cell Physiol.* 2008;49(8):1237-1249. doi 10.1093/pcp/pcn100

Båga M., Chodaparambil S.V., Limin A.E., Pecar M., Fowler D.B., Chibbar R.N. Identification of quantitative trait loci and associated candidate genes for low-temperature tolerance in cold-hardy winter wheat. *Funct Integr Genomics.* 2007;7(1):53-68. doi 10.1007/s10142-006-0030-7

Bayer-Giraldi M., Weikusat I., Besir H., Dieckmann G. Characterization of an antifreeze protein from the polar diatom *Fragilariopsis cylindrus* and its relevance in sea ice. *Cryobiology*. 2011;63(3):210-219. doi 10.1016/j.cryobiol.2011.08.006

Boonsupthip W., Lee T.-C. Application of antifreeze protein for food preservation: effect of type III antifreeze protein for preservation of gel-forming of frozen and chilled actomyosin. *J Food Sci*. 2003;68(5):1804-1809. doi 10.1111/j.1365-2621.2003.tb12333.x

Cao Y., Hu G., Zhuang M., Yin J., Wang X. Molecular cloning and functional characterization of *TaIRI9* gene in wheat (*Triticum aestivum* L.). *Gene*. 2021;791:145694. doi 10.1016/j.gene.2021.145694

Case A.J., Skinner D.Z., Garland-Campbell K.A., Carter A.H. Freezing tolerance-associated quantitative trait loci in the brundage × coda wheat recombinant inbred line population. *Crop Sci.* 2014;54(3):982-992. doi 10.2135/cropsci2013.08.0526

Chinnusamy V., Ohta M., Kanrar S., Lee B.H., Hong X., Agarwal M., Zhu J.K. ICE1: a regulator of cold-induced transcriptome and freezing tolerance in *Arabidopsis*. *Genes Dev*. 2003;17(8):1043-1054. doi 10.1101/gad.1077503

Chow-Shi-Yée M., Briard J.G., Grondin M., Averill-Bates D.A., Ben R.N., Ouellet F. Inhibition of ice recrystallization and cryoprotective activity of wheat proteins in liver and pancreatic cells. *Protein Sci.* 2016;25(5):974-986. doi 10.1002/pro.2903

Chow-Shi-Yée M., Grondin M., Ouellet F., Averill-Bates D.A. Control of stress-induced apoptosis by freezing tolerance-associated wheat proteins during cryopreservation of rat hepatocytes. *Cell Stress Chaperones*. 2020;25(6):869-886. doi 10.1007/s12192-020-01115-y

Chun J., Yu X., Griffith M. Genetic studies of antifreeze proteins and their correlation with winter survival in wheat. *Euphytica.* 1998;102:219-226. doi 10.1023/A:1018333730936

DeVries A.L., Wohlschlag D.E. Freezing resistance in some Antarctic fishes. *Science.* 1969;163(3871):1073-1075. doi 10.1126/science.163.3871.1073

Duman J.G., Olsen T.M. Thermal hysteresis protein activity in bacteria, fungi, and phylogenetically diverse plants. *Cryobiology*. 1993;30(3):322-328. doi 10.1006/cryo.1993.1031

Duman J.G., Wisniewski M.J. The use of antifreeze proteins for frost protection in sensitive crop plants. *Env Exp Bot*. 2014;106:60-69. doi 10.1016/j.envexpbot.2014.01.001

Ewart K.V., Lin Q., Hew C.L. Structure, function and evolution of antifreeze proteins. *Cell Mol Life Sci*. 1999;55(2):271-283. doi 10.1007/s000180050289

Gaudet D.A., Laroche A. Winter survival of cereals parasitized by Snow Mold. In: Li P.H., Chen T.H.H. (Eds) Plant Cold Hardiness. Boston: Springer, 1997;331-342. doi 10.1007/978-1-4899-0277-1_31

Gaudet D.A., Laroche A., Frick M., Davoren J., Puchalski B., Ergon Å. Expression of plant defence-related (PR-protein) transcripts during hardening and dehardening of winter wheat. *Physiol Mol Plant Pathol.* 2000;57(1):15-24. doi 10.1006/pmpp.2000.0275

Griffith M., Yaish M.W. Antifreeze proteins in overwintering plants: a tale of two activities. *Trends Plant Sci.* 2004;9(8):399-405. doi 10.1016/j.tplants.2004.06.007

Griffith M., Ala P., Yang D.S., Hon W.C., Moffatt B.A. Antifreeze protein produced endogenously in winter rye leaves. *Plant Physiol*. 1992;100(2):593-596. doi 10.1104/pp.100.2.593

Griffith M., Lumb C., Wiseman S.B., Wisniewski M., Johnson R.W., Marangoni A.G. Antifreeze proteins modify the freezing process in planta. *Plant Physiol*. 2005;138(1):330-340. doi 10.1104/pp.104.058628

Grondin M., Hamel F., Averill-Bates D.A., Sarhan F. Wheat proteins improve cryopreservation of rat hepatocytes. *Biotechnol Bioeng*. 2009;103(3):582-591. doi 10.1002/bit.22270

Hannah M.A., Heyer A.G., Hincha D.K. A global survey of gene regulation during cold acclimation in *Arabidopsis thaliana*. *PLoS Genet*. 2005;1(2):e26. doi 10.1371/journal.pgen.0010026

He K., Wu Y. Receptor-like kinases and regulation of plant innate immunity. *Enzymes*. 2016;40:105-142. doi 10.1016/bs.enz.2016.09.003

Herman E.M., Rotter K., Premakumar R., Elwinger G., Bae H., Ehler-King L., Chen S., Livingston D.P. 3rd. Additional freeze hardiness in wheat acquired by exposure to –3 degrees C is associated with extensive physiological, morphological, and molecular changes. *J Exp Bot.* 2006;57(14):3601-3618. doi 10.1093/jxb/erl111

Hon W.C., Griffith M., Mlynarz A., Kwok Y.C., Yang DSC. Antifreeze proteins in winter rye are similar to pathogenesis-related proteins. *Plant Physiol.* 1995;109(3):879-889. doi 10.1104/pp.109.3.879

Houde M., Belcaid M., Ouellet F., Danyluk J., Monroy A.F., Dryanova A., Gulick P., Bergeron A., Laroche A., Links M.G., MacCarthy L., Crosby W.L., Sarhan F. Wheat EST resources for functional genomics of abiotic stress. *BMC Genomics*. 2006;7:149. doi 10.1186/1471-2164-7-149

Huang T., Duman J.G. Cloning and characterization of a thermal hysteresis (antifreeze) protein with DNA-binding activity from winter bittersweet nightshade, *Solanum dulcamara*. *Plant Mol Biol*. 2002; 48(4):339-350. doi 10.1023/A:1014062714786

Jain D., Khurana J.P. Role of pathogenesis-related (PR) proteins in plant defense mechanism. In: Singh A., Singh I. (Eds) Molecular Aspects of Plant-Pathogen Interaction. Singapore: Springer, 2018;265-281. doi 10.1007/978-981-10-7371-7_12

Janech M.G., Krell A., Mock T., Raymond J.A. Ice-binding proteins from sea ice diatoms (Bacillariophyceae). *J Phycol*. 2006;42(2):410-416. doi 10.1111/j.1529-8817.2006.00208.x

Jarząbek M., Pukacki P.M., Nuc K. Cold-regulated proteins with potent antifreeze and cryoprotective activities in spruces (*Picea* sp.). *Cryobiology*. 2009;58(3):268-274. doi 10.1016/j.cryobiol.2009.01.007

Jin Y.N., Bai L.P., Guan S.X., Zhong M., Ma H., Wang S., Guo Z.F. Identifcation of an ice recrystallisation inhibition gene family in winter-hardy wheat and its evolutionary relationship to other members of the *Triticeae*. *J Agron Crop Sci.* 2018;204(4):400-413. doi 10.1111/jac.12270

Jin Y., Ding X., Li J., Guo Z. Isolation and characterization of wheat ice recrystallisation inhibition gene promoter involved in low temperature and methyl jasmonate responses. *Physiol Mol Biol Plants.* 2022;28(11-12):1969-1979. doi 10.1007/s12298-022-01257-6

Juurakko C.L., diCenzo G.C., Walker V.K. *Brachypodium* antifreeze protein gene products inhibit ice recrystallisation, attenuate ice nucleation, and reduce immune response. *Plants.* 2022;11(11):1475. doi 10.3390/plants11111475

Kang G., Li G., Yang W., Han Q., Ma H., Wang Y., Ren J., Zhu Y., Guo T. Transcriptional profile of the spring freeze response in the leaves of bread wheat (*Triticum aestivum* L.). *Acta Physiol Plant.* 2013;35(02):575-587. doi 10.1007/s11738-012-1099-3

Knight C.A., Wen D., Laursen R.A. Nonequilibrium antifreeze peptides and the recrystallization of ice. *Cryobiology*. 1995;32(1):23-34. doi 10.1006/cryo.1995.1002

Kontogiorgos V., Regand A., Yada R., Goff H.D. Isolation and characterization of ice structuring proteins from cold-acclimated winter wheat grass extract for recrystallization inhibition in frozen foods. *J Food Biochem*. 2007;31(2):139-160. doi 10.1111/j.1745-4514.2007.00112.x

Kostyaev A.A., Martusevich A.K., Andreev A.A. Toxicity of cryoprotectants and cryoconservants on their basis for blood components and bone marrow (review article). *Nauchnoe Obozrenie. Meditsinskie Nauki = Scientific Review. Medical Sciences.* 2016;6:54-74 (in Russian)

Kristiansen E., Pedersen S.A., Ramløv H., Zachariassen K.E. Antifreeze activity in the cerambycid beetle Rhagium inquisitor. *J Comp Physiol B.* 1999;169:55-60. doi 10.1007/s003600050193

Kristiansen E., Ramløv H., Højrup P., Pedersen S.A., Hagen L., Zachariassen K.E. Structural characteristics of a novel antifreeze protein from the longhorn beetle *Rhagium inquisitor*. *Insect Biochem Mol Biol.* 2011;41(2):109-117. doi 10.1016/j.ibmb.2010.11.002

Kruse E.B., Carle S.W., Wen N., Skinner D.Z., Murray T.D., Garland-Campbell K.A., Carter A.H. Genomic regions associated with tolerance to freezing stress and snow mold in winter wheat. *G3* (*Bethesda*). 2017;7(3):775-780. doi 10.1534/g3.116.037622

Kurepin L.V., Dahal K.P., Savitch L.V., Singh J., Bode R., Ivanov A.G., Hurry V., Hüner N.P. Role of CBFs as integrators of chloroplast redox, phytochrome and plant hormone signaling during cold acclimation. *Int J Mol Sci*. 2013;14(6):12729-12763. doi 10.3390/ijms140612729

Liu X., Pan Y., Liu F., He Y., Zhu Q., Liu Z., Zhan X., Tan S. A review of the material characteristics, antifreeze mechanisms, and applications of cryoprotectants (CPAs). *J Nanomater*. 2021;2021(1):9990709. doi 10.1155/2021/9990709

Livingston D.P. 3rd, Bertrand A., Wisniewski M., Tisdale R., Tuong T., Gusta L.V., Artlip T. Factors contributing to ice nucleation and sequential freezing of leaves in wheat. *Planta*. 2021;253(6):124. doi 10.1007/s00425-021-03637-w

Macháčcková I., Hanisova A., Krekule J. Levels of ethylene, ACC, MACC, ABA and proline as indicators of cold hardening and frost resistance in winter wheat. *Physiol Plant*. 1989;76(4):603-607. doi 10.1111/j.1399-3054.1989.tb05486.x

McHale L., Tan X., Koehl P., Michelmore R.W. Plant NBS-LRR proteins: adaptable guards. *Genome Biol.* 2006;7(4):212. doi 10.1186/gb-2006-7-4-212

Middleton A.J., Brown A.M., Davies P.L., Walker V.K. Identification of the ice-binding face of a plant antifreeze protein. *FEBS Lett*. 2009; 583(4):815-819. doi 10.1016/j.febslet.2009.01.035

Middleton A.J., Marshal C.B., Faucher F., Bar-Dolev M., Braslavsky I., Campbell R.L., Walker V.K., Davies P.L. Antifreeze protein from freeze-tolerant grass has a beta-roll fold with an irregularly structured ice-binding site. *J Mol Biol*. 2012;416(5):713-724. doi 10.1016/j.jmb.2012.01.032

Monroy A.F., Dryanova A., Malette B., Oren D.H., Ridha Farajalla M., Liu W., Danyluk J., Ubayasena L.W., Kane K., Scoles G.J., Sarhan F., Gulick P.J. Regulatory gene candidates and gene expression analysis of cold acclimation in winter and spring wheat. *Plant Mol Biol.* 2007;64(4):409-423. doi 10.1007/s11103-007-9161-z

Pandey S.P., Somssich I.E. The role of WRKY transcription factors in plant immunity. *Plant Physiol*. 2009;150(4):1648-1655. doi 10.1104/pp.109.138990

Puchkov E.O. Biogenic control of ice formation. *Priroda.* 2017;2:27-37 (in Russian)

Regand A., Goff H.D. Ice recrystallization inhibition in ice cream as affected by ice structuring proteins from winter wheat grass. *J Dairy Sci*. 2006;89(1):49-57. doi 10.3168/jds.S0022-0302(06)72068-9

Sandve S.R., Rudi H., Asp T., Rognli O.A. Tracking the evolution of a cold stress associated gene family in cold tolerant grasses. *BMC Evol Biol*. 2008;8:245. doi 10.1186/1471-2148-8-245

Sauter M. Phytosulfokine peptide signaling. *J Exp Bot*. 2015;66(17): 5161-5169. doi 10.1093/jxb/erv071

Sutka J. Genetic control of frost tolerance in wheat (*Triticum aestivum* L.). *Euphytica*. 1994;77:277-282. doi 10.1007/BF02262642

Sutka J. Genes for frost resistance in wheat. *Euphytica.* 2001;119:169-177. doi 10.1023/A:1017520720183

Sutka J., Galiba G., Snape J.W. Inheritance of frost resistance in wheat (*Triticum aestivum* L.). *Acta Agron Hung.* 1997;45:257-263

Talanova V.V., Titov A.F., Topchieva L.V., Malysheva I.E., Venzhik Y.V., Frolova S.A. Expression of genes encoding the WRKY transcription factor and heat shock proteins in wheat plants during cold hardening. *Dokl Biol Sci*. 2008;423:440-442. doi 10.1134/s0012496608060215

Tchagang A.B., Fauteux F., Tulpan D., Pan Y. Bioinformatics identification of new targets for improving low temperature stress tolerance in spring and winter wheat. *BMC Bioinformatics*. 2017;18(1):174. doi 10.1186/s12859-017-1596-x

Tremblay K., Ouellet F., Fournier J., Danyluk J., Sarhan F. Molecular characterization and origin of novel bipartite cold-regulated ice recrystallization inhibition proteins from cereals. *Plant Cell Physiol.* 2005;46(6):884-891. doi 10.1093/pcp/pci093

Urrutia M.E., Duman J.G., Knight C.A. Plant thermal hysteresis proteins. *Biochem Biophys Acta.* 1992;1121(1-2):199-206. doi 10.1016/0167-4838(92)90355-h

Vaitkevičiūte G., Aleliūnas A., Brazauskas G., Armonienè R. Deacclimation and reacclimation processes in winter wheat: novel perspectives from time-series transcriptome analysis. *Front Plant Sci.* 2024; 15:1395830. doi 10.3389/fpls.2024.1395830

Vanková R., Kosová K., Dobrev P., Vítámvás P., Trávníčková A., Cvikrová M., Pešek B., Gaudinová A., Přerostová S., Musilová J., Galiba G., Prasil I.T. Dynamics of cold acclimation and complex phytohormone responses in *Triticum monococcum* lines G3116 and DV92 differing in vernalization and frost tolerance level. *Env Exp Bot*. 2014;101:12-25. doi 10.1016/j.envexpbot.2014.01.002

Voets I.K. From ice-binding proteins to bio-inspired antifreeze materials. *Soft Matter*. 2017;13(28):4808-4823. doi 10.1039/c6sm02867e

Wang W., Hao Q., Wang W., Li Q., Wang W. The genetic characteristics in cytology and plant physiology of two wheat (*Triticum aestivum*) near isogenic lines with different freezing tolerances. *Plant Cell Rep*. 2017;36(11):1801-1814. doi 10.1007/s00299-017-2195-z

Wang W., Wang X., Zhang J., Huang M., Cai J., Zhou Q., Dai T., Jiang D. Salicylic acid and cold priming induce late-spring freezing tolerance by maintaining cellular redox homeostasis and protecting photosynthetic apparatus in wheat. *Plant Growth Regul.* 2020;90:109-121. doi 10.1007/s10725-019-00553-8

Wang W., Wang X., Huang M., Cai J., Zhou Q., Dai T., Jiang D. Alleviation of field low-temperature stress in winter wheat by exogenous application of salicylic acid. *J Plant Growth Regul*. 2021;40:811-823. doi 10.1007/s00344-020-10144-x

Wang X., Geng H., Wu D., Wang L., Zhang N., Wang W., Yu D. Isolation of ice structuring proteins from winter wheat in frigid region (Dongnongdongmai1) and the effect on freeze-thaw stability of dough. *Food Res Int.* 2024;197(Pt. 2):115295. doi 10.1016/j.foodres.2024.115295

Willick I.R., Takahashi D., Fowler D.B., Uemura M., Tanino K.K. Tissue-specific changes in apoplastic proteins and cell wall structure during cold acclimation of winter wheat crowns. *J Exp Bot*. 2018;69(5):1221-1234. doi 10.1093/jxb/erx450

Willick I.R., Gusta L.V., Fowler D.B., Tanino K.K. Ice segregation in the crown of winter cereals: evidence for extraorgan and extratissue freezing. *Plant Cell Environ*. 2019;42(2):701-716. doi 10.1111/pce.13454

Winfield M.O., Lu C., Wilson I.D., Coghill J.A., Edwards K.J. Plant responses to cold: transcriptome analysis of wheat. *Plant Biotechnol J*. 2010;8(7):749-771. doi 10.1111/j.1467-7652.2010.00536.x

Wisniewski M., Willick I.R., Duman J.G., Livingston D.P. 3rd, Newton S.S. Plant antifreeze proteins. In: Ramløv H., Friis D. (Eds) Antifreeze Proteins. Vol. 1. Cham: Springer, 2020;189-226. doi 10.1007/978-3-030-41929-5_7

Xue-Xuan X., Hong-Bo S., Yuan-Yuan M., Gang X., Jun-Na S., Dong-Gang G., Cheng-Jiang R. Biotechnological implications from abscisic acid (ABA) roles in cold stress and leaf senescence as an important signal for improving plant sustainable survival under abiotic-stressed conditions. *Crit Rev Biotechnol*. 2010;30(3):222-230. doi 10.3109/07388551.2010.487186

Yang T., Zhang Y., Guo L., Li D., Liu A., Bilal M., Xie C., Yang R., Gu Z., Jiang D., Wang P. Antifreeze polysaccharides from wheat bran: the structural characterization and antifreeze mechanism. *Biomacromolecules*. 2024;25(7):3877-3892. doi 10.1021/acs.biomac.3c00958

Yu X.M., Griffith M., Wiseman S.B. Ethylene induces antifreeze activity in winter rye leaves. *Plant Physiol*. 2001;126(3):1232-1240. doi 10.1104/pp.126.3.1232

Zheng X., Shi M., Wang J., Yang N., Wang K., Xi J., Wu C., Xi T., Zheng J., Zhang J. Isoform sequencing provides insight into freezing response of common wheat (*Triticum aestivum* L.). *Front Genet*. 2020;11:462. doi 10.3389/fgene.2020.00462

Conflict of interest. The authors declare no conflict of interest.

Received May 13, 2025. Revised September 15, 2025. Accepted September 24, 2025.