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REVIEW

The relationship between vernalization- and photoperiodically-regulated genes and the development of frost tolerance in wheat and barley

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Abstract

The review summarizes the level of current knowledge of impacts of vernalization and photoperiod on the induction and maintenance of frost tolerance (FrT) in wheat and barley. The phenomenon of vernalization is briefly described and the major vernalization (*VRN*) loci are characterised. Vernalization requirement and the three major growth habits of *Triticeae* (facultative, winter and spring) are defined on the basis of the two-locus *VRN-2*/*VRN-1* epistatic model. Major photoperiodically regulated genes, which influence the transition to flowering, are characterised and their interactions with *VRN* genes are briefly discussed. The phenomenon of induction of FrT during the process of cold acclimation (CA) is described and the major cold-induced *Cor*/*Lea* genes are listed. Important regulatory mechanisms, *i.e.*, CBF pathway, controlling the expression of *Cor*/*Lea* genes under cold, are discussed. The major loci affecting the development of FrT in *Triticeae*, the *Fr* loci, are characterised. In conclusion, current progress in this research field is summarized and new questions arising in the area are formulated.

Additional key words: cold acclimation, *Hordeum*, *Triticum.*

Introduction

Low temperatures (LTs) not only induce a plant direct response *via* cold acclimation (CA), they also profoundly affect the plant developmental programme. Conversely, plant developmental stage has a crucial impact on a plant ability to cope with the unfavourable effects of LTs. The transition from vegetative phase into reproductive phase is generally associated with a decline in plant potential to resist the impacts of unfavourable environment. Plants originating from higher latitudes and/or altitudes had to adapt to relatively long periods of cold occurring regularly throughout the year. They thus had incorporated the requirement of a sufficiently long period of cold treatment into their individual developmental programme to prevent an early transition into the more cold-susceptible reproductive phase. This phenomenon, *i.e.*, the requirement of a sufficiently long cold period prior to the transition to flowering, is called vernalization. The process of CA is induced rapidly within a few days after the beginning of LT influence while the vernalization response develops for weeks and months of LT treatment. A comparison of the rates of plant responses to LT *via* CA and vernalization is given in Sung and Amasino (2004, 2005). In addition to cold, the developmental transition into the reproductive phase is regulated photoperiodically in some plants. In higher latitudes, the warm period of the year (summer) is associated with long

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Abbreviations: aa - amino acid; ABA - abscisic acid; *AP1* - *apetala 1* (gene); CA - cold acclimation; *CBF* - *C-repeat binding factor* (gene); *CO* - *constans* (gene); *Cor/Lea* - *cold-regulated/late embryogenesis abundant* (genes); *Dhn* - *dehydrin* (gene); *FLC* - *FLOWERING LOCUS C* (gene); *Fr* - *frost-resistance locus(i)*; FrT - frost tolerance; *FT* - *FLOWERING LOCUS T* (gene); *ICE-1* - *inducer of CBF expression 1* (gene); LD - long day; LT - low temperature; LT_{50} - lethal temperature when 50 % of the samples die; Mr - relative molecular mass; NIL - near isogenic line; pI - protein isoelectric point; *Ppd* - *photoperiod locus(i)*; *PRR* - *pseudo-response regulator* (gene); QTL - quantitative trait locus(i); ROS - reactive oxygen species; SD - short day; SDS-PAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis; TF - transcription factor; *VRN* - *vernalization* (gene); *Vrn* - a dominant allele of *VRN* gene; *vrn* - a recessive allele of *VRN* gene; *Wcor* - *wheat cold-regulated* (gene); *Wcs* - *wheat cold-specific* (gene); *Wrab* - *wheat response-to-ABA* (gene)

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days (LDs) while unfavourable cold period of the year (winter) is connected with relatively short days (SDs). It became obvious that the photoperiod influences the expression of many regulatory genes including the genes which control the developmental transition into flowering.

The genera belonging to the tribe *Triticeae*, *i.e.*, wheat (*Triticum*), barley (*Hordeum*) and rye (*Secale*) are important cultivated cereals and form a homogeneous genetic system exhibiting colinearity of their genomes (*i.e.*, A, B and D genomes in cultivated wheat *T. aestivum*, H genome in *H. vulgare* and R genome in *S. cereale*). This means that genes found in one *Triticeae* genome have their counterparts located at the same chromosomal position in the other ones (apart from some exceptions caused by deletions, duplications, insertions or translocations, *e.g.*, different positions of *VRN-2* candidate genes in *T. monococcum* A^m genome and barley H genome). As a consequence of this important fact, the results obtained on one *Triticeae* member can be extrapolated to the others.

The aim of this review is to summarize the current state of knowledge of the connections between vernalization and photoperiod and the ability to induce a sufficient level of frost tolerance (FrT) in wheat and barley. In addition, some basic characteristics of wheat and barley vernalization loci, *Ppd* loci and photoperiodically-regulated genes, as well as cold-induced *Cor*/*Lea* genes are given in the form of tables.

The phenomenon of vernalization

Vernalization is an ecophysiological adaptation of plants originating from higher latitudes and/or altitudes which enables them to survive relatively long cold periods occurring regularly during a year. Vernalization has been rigorously defined as 'the acquisition or acceleration of the ability to flower by a chilling treatment' (Chouard 1960). Plants which require vernalization need exposure to a sufficiently long period of cold prior to 'switching' their individual developmental programme from the vegetative phase to the reproductive phase. Frost-tolerant plants in the vegetative phase can induce a sufficiently high level of FrT, while the same plants in the reproductive phase cannot since the generative organs (flowers and fruits) are generally more susceptible to cold in comparison with the vegetative ones (Sakai and Larcher 1985). Vernalization thus functions as an important control mechanism preventing the early transition into the less cold-tolerant reproductive phase. During vernalization, significant changes occur on the molecular level in the plants. In *Arabidopsis thaliana*, it was shown by Bastow *et al.* (2004) that vernalization leads to epigenetic silencing of the major flowering repressor *FLC via* histone methylation.

Winter cultivars belonging to the tribe *Triticeae* do not have an absolute requirement for vernalization, *i.e.*, they eventually flower without any period of cold; however, the time of flowering is significantly delayed (Krekule 1987).

Vernalization genes and growth habits

In wheat and barley, the existence of three vernalization loci has been shown. The loci have been named *VRN-1*, *VRN-2* and *VRN-3*. All vernalization loci encode transcription factors (TFs) affecting the regulation of other genes.

VRN-1 locus encodes the major vernalization gene which controls the transition to flowering. If the gene is active, *i.e.*, its gene product is present in the nucleus, profound changes in the expression of many TFs which eventually result in the formation of flower meristem are induced. The *VRN-1* gene can be present in the cereal genome in two types of alleles – repressible and irrepressible. The irrepressible allele *Vrn-1* is dominant, *i.e.*, if present in the genome, it has the major effect on plant development irrespective to the other allele(s). The repressible allele *vrn-1* can be inactivated by the binding of some TFs to either the CArG box in the promoter region (Yan *et al.* 2004a), or to a specific 436 bp 'vernalization critical region' in the first intron (Fu *et al.* 2005). It has been proposed that the CArG box in the promoter can function as a potential binding site for a MADS-box TF (Yan *et al.* 2004a). In contrast, the 'vernalization critical region' in the first intron is predicted to form four Dof sites which can be potential targets for a TF with a zinc-finger DNA binding motif (Von Zitzewitz *et al.* 2005). The irrepressible dominant *Vrn-1* allele has deletions in these critical regions (Fu *et al.* 2005). Thus, repressors cannot bind to them. Apart from the variability described above, Cockram *et al.* (2007a) have recently found out that some spring barley genotypes contain an insertion of a transposable element in the intron I region, upstream of the vernalization critical region. Analogously, an insertion of a transposon in the promoter region has been reported for the *VRN-B3* gene by Yan *et al.* (2006) (see below for more details). Thus, insertions of transposons seem to present another type of allelic variation in *VRN* loci.

The *VRN-1* gene encodes a MADS-box TF of the AP1 family. The same gene has also been named *HvBM5A* in barley (*H. vulgare*) (Von Zitzewitz *et al.* 2005), *TmAP1* gene in diploid wheat (*T. monococcum*) (Yan *et al.* 2003) and *WAP1* (Murai *et al.* 2003) and *TaVRT-1* (Danyluk *et al.* 2003) genes in hexaploid wheat (*T. aestivum*). The *VRN-1* gene is located on the long arm of chromosome 5. In barley genome, there is only one *VRN-1* gene located on 5HL; this gene is described as *VRN-H1*. Analogously,

in diploid wheat (*T. monococcum*), the only *VRN-1* gene is located on 5A^{m}L and described as *VRN-A^{m}1*. In hexaploid wheat (*T. aestivum*), there are three *VRN-1* genes described as *VRN-A1* on 5AL, *VRN-B1* on 5BL and *VRN-D1* on 5DL, respectively. While the dominant *Vrn-A1* allele completely reduces vernalization in hexaploid wheat, cultivars carrying only dominant *Vrn-B1* or *Vrn-D1* alleles usually show some residual vernalization requirement. This hypothesis has already been explained by Loukoianov *et al.* (2005) as differences in transcription: transcripts of dominant *Vrn-A1* allele appear earlier and in larger quantities in leaf tissue than transcripts of dominant *Vrn-B1* and *Vrn-D1* alleles during the plant development. The authors have also suggested a feedback loop in which winter alleles of *VRN-B1* and *VRN-D1* become induced without vernalization if a spring *Vrn-A1* allele is present in the genome of common wheat. This mechanism is probably mediated via a *Vrn-A1*-dependent downregulation of the expression of *VRN-2*. Moreover, the *Vrn-1* alleles are not completely dominant over the recessive *vrn-1* alleles at the same locus because it has been shown by some researchers (*e.g.*, Kóti *et al.* 2006) that heterozygotes head significantly later than *Vrn-H1/Vrn-H1* homozygotes in a barley cross between Hardy (winter) and Jubilant (spring) cultivars. Košner and Pánková (1998) have also hypothesized that an allelic variation in recessive *vrn-1* alleles may exist which could affect the length of vernalization in various winter wheat cultivars.

The *VRN-1* genes in *Triticeae* are closely related to *Arabidopsis* MADS-box genes of the *AP1/SQUA* subfamily, especially to the flower meristem identity genes *AP1* (*APETALA1*), *CAL* (*CAULIFLOWER*) and *FUL* (*FRUITFULL*) (Yan *et al.* 2003, Laurie *et al.* 2004). However, the *VRN-1* genes which are often described as *Arabidopsis AP1* homologues in the *Triticeae* differ from the *Arabidopsis AP1* gene in some significant aspects: they are sequentionally more related to *A. thaliana FUL* gene than to the *A. thaliana AP1* gene, they are expressed not only in the flower meristem (as is the case for *Arabidopsis AP1*), but also in some types of the vegetative tissue such as leaves. Moreover, their potential function in the formation of flower meristem and determination of floral organs (which is precisely described for *Arabidopsis AP1* gene – a flower meristem identity gene and a gene of the A-type function in the classical ABC model of flower development) still remains to be elucidated (Schmitz *et al.* 2000). Recent advances in the elucidation of the roles of *AP1*/*FUL* genes in the morphogenesis of floral meristems in *Poaceae* can be found in Preston and Kellogg (2007).

The *VRN-2* locus is located on chromosome 4HL in barley. However, in the A genome, the part of 4AL with the *VRN-2* locus had been translocated to 5A^{m}L (Cattivelli *et al.* 2002, Yan *et al.* 2004b). Thus, the *VRN-2* locus is found at 5A^{m}L in *T. monococcum*. The *VRN-2* locus in barley consists of three tightly linked genes *ZCCT-Ha*, *ZCCT-Hb* and *ZCCT-Hc* (Dubcovsky *et al.* 2005). In *T. monococcum*, two tightly linked genes named *ZCCT-1* and *ZCCT-2* have been identified at *VRN-2* locus (Yan *et al.* 2004b). *ZCCT-Ha* in barley and *ZCCT-1* in *T. monococcum* have been reported to be the most likely candidates for the *VRN-2* gene (Yan *et al.* 2004b, Dubcovsky *et al.* 2005). All these genes encode *ZCCT* (zinc-finger, *CONSTANS*, *CONSTANS*-like, and *TOC*) TFs which are down-regulated by vernalization. The *ZCCT* TFs contain two important binding domains, the C_2H_2 (2 cysteine 2 histidine) zinc-finger domain, which can bind both DNA and protein and which is encoded by the first exon, and the CCT domain, which controls nuclear localization of the TF (Robson *et al.* 2001) and binds to the CCAAT box binding factors, which mediate the interactions between *CONSTANS*-like proteins and DNA (Ben-Naim *et al.* 2006). The CCT domain is encoded by the second exon. The *VRN-2* gene can also be present in dominant (functional) and recessive (loss-of-function point mutation – *vrn-2a*; complete deletion – *vrn-2b*) alleles. The loss-of-function mutations in *ZCCT-1* are associated with a substitution of a single conserved arginine to a tryptophan at position 35 of the CCT domain which correlates with the spring growth habit in *T. monococcum* (Yan *et al.* 2004b).

In *T. aestivum*, the *VRN-2* loci have not yet been mapped nor further characterised due to the absence of trait variation. A possible explanation of the failure in mapping of the *VRN-2* loci in common wheat may lie in the fact that the recessive phenotype in *VRN-2* in hexaploid wheat would require complete deletion or loss-of-function mutation in the *ZCCT* genes in all three genomes. However, it can be expected that the winter cultivars of common wheat contain functional *ZCCT* genes analogously to the situation in *T. monococcum* and in barley; otherwise, Yan *et al.* (2004b) did not prove the role of the *ZCCT1* gene as a repressor of the *VRN-1* gene by an RNAi experiment in a winter cultivar of common wheat.

The *VRN-3* locus encodes a TF orthologous to *Arabidopsis FT* (*FLOWERING LOCUS T*) (Yan *et al.* 2006) and is located on chromosome 7HS in barley and 7BS in wheat, where it was formerly described as *VRN-B4* locus. The candidate genes have been named *HvFT1* in barley and *TaFT1* in wheat. The dominant allele enhances flowering *via* up-regulation of the *VRN-1* gene expression. In barley, the dominant allele has a deletion in its first intron with respect to the recessive one, whereas in wheat, the dominant *Vrn-3* allele has an insertion of a retroelement in the promoter in contrast to the recessive one. The dominant alleles in both wheat and barley enhance flowering under LDs as it does *FT* TF in *A. thaliana* and it is proposed that they are under regulation of *Ppd-1* locus (Fig. 1, Table 1).

Since no allelic variation at the *VRN-3* locus has been observed in most accessions belonging to cultivated wheat and barley, a two-gene epistatic model of vernalization considering *VRN-1* and *VRN-2* genes only has been proposed (Yan *et al.* 2003, Szücs *et al.* 2007). *VRN-2* gene is a repressor of *VRN-1*, *i.e.*, it inhibits the expression of the *VRN-1* gene product. In this model, the

VRN-1 gene acts as the major developmental gene 'switching' the individual plant development from the vegetative phase to the reproductive phase. Cultivars carrying at least one dominant *Vrn-1* allele do not have any vernalization requirement. If the cultivars carry only recessive *vrn-1* alleles, these alleles can be repressed by the *VRN-2* gene product (Fu *et al.* 2005, Dubcovsky *et al.* 2006). The *VRN-2* gene can also be present in two alleles – dominant (functional repressor) and recessive (the repressor is absent or unfunctional). It has been shown by Yan *et al.* (2004b) that the *VRN-2* gene product is down-regulated by vernalization. The function of *VRN-2* as a repressor of *VRN-1* has been proposed by Yan *et al.* (2004b) on the basis of an RNAi experiment: the insertion of an RNAi segment complementary to the *ZCCT1* gene in the hexaploid winter wheat cultivar Jagger resulted in the up-regulation of *VRN-1* and reduction of the vernalization requirement. Thus, the winter growth habits which contain only recessive *vrn-1* alleles and at least one dominant *Vrn-2* allele do have a vernalization requirement. Later, it was shown by some researchers (Dubcovsky *et al.* 2006, Trevaskis *et al.* 2006) that this model is valid only under LD conditions, while SD conditions lead to the down-regulation of *VRN-2* expression regardless of the temperature which does not result in the up-regulation of *VRN-1* expression in *vrn-1vrn-1* homozygous genotypes. Therefore, the existence of at least one other repressor of *VRN-1*, which may repress its expression under SDs, has been postulated.

The *VRN-2* gene as a central repressor of the *VRN-1* gene down-regulated by vernalization has no clear orthologues in *Arabidopsis.* In *A. thaliana*, the central flowering repressor *FLC* is a MADS-box TF of a special subclass, which is not found in grasses, and analogously, *ZCCT* TFs, which are candidate genes for the *VRN-2* locus in *Triticeae*, have not been identified in the vernalization response pathway in *Arabidopsis* yet (Kane *et al.* 2005). Moreover, the CCT domains from the cereal *ZCCT* genes belong to a specific sub-group which does not occur in *Arabidopsis* (Yan *et al.* 2004b). These findings support the thesis that the vernalization regulatory pathways in *Arabidopsis* and cereals had developed independently.

Based on the *VRN-1* and *VRN-2* alleles and their interactions, three major growth habits have been defined in wheat and barley: the winter habit, which has vernalization requirement, the spring habit, which does not have vernalization, and the facultative habit, which also does not have vernalization, but is strongly photoperiodically sensitive. The spring habit has at least one dominant *Vrn-1* allele, *i.e.*, the transition to the reproductive phase cannot be repressed by vernalization. The facultative habit has the allelic constitution *vrn-2vrn-2*/*vrn-1vrn-1* which means that the *vrn-1* allele is not repressed by *vrn-2* (and thus the facultative growth habit does not have any vernalization requirement), but it can be repressed by other genes, *e.g.*, by photoperiodically activated TFs which cause that facultative habit is very photoperiodically sensitive. Therefore, the facultative growth habit remains in the vegetative phase unless stimulated to flower by LDs. The winter habit has the allelic constitution *Vrn-2_*/*vrn-1vrn-1* which means that it has a vernalization requirement to down-regulate the expression of the *Vrn-2* allele in order to de-repress the *vrn-1* allele (Von Zitzewitz *et al.* 2005, Szücs *et al.* 2007).

The relationship between *VRN-1* and *VRN-2* genes is epistatic, *i.e.*, if a dominant *Vrn-1* allele is present in the genome, its presence results in a spring growth habit regardless of the allelic constitution at the *VRN-2* locus (dominant epistasis of *VRN-1* over *VRN-2*). It has been suggested by some authors (Loukoianov *et al.* 2005, Trevaskis *et al.* 2006) that the dominant *Vrn-1* allele could act as a repressor of the dominant *Vrn-2* alleles, *i.e.*, if a dominant *Vrn-1* allele is present in the genome, it could downregulate the expression of *Vrn-2*.

Most of the wild *Triticeae* belong to the winter growth habit. It seems to be unlikely that a vernalization requirement would have developed independently at the same locus in all *Triticeae*. Thus, it could be proposed that a winter growth habit and the recessive *vrn-1* allele are ancestral, and the dominant spring allele *Vrn-1* has evolved independently as a loss-of-function allele (loss of vernalization requirement) via mutations in its regulatory sites – the promoter CArG box and the 'vernalization critical region' in the first intron (Yan *et al.* 2003).

Table 1. The list of vernalization loci and their candidate genes characterised in barley (*H. vulgare*), in einkorn wheat (*T. monococcum*) and common wheat (*T. aestivum*).

Species	Locus	Gene - transcription factor	Location	Reference
H. vulgare	*VRN-H1* (formerly *Sh 2*)	*HvBM5A* – MADS-box	5HL	Von Zitzewitz *et al.* (2005)
	VRN-H2 (formerly *Sh*)	*ZCCT-Ha*, *ZCCT-Hb*, *ZCCT-Hc* – zinc-finger TF with CCT domain	4HL	Dubcovsky *et al.* (2005)
	VRN-H3 (formerly *Sh 3*)	*HvFT* - orthologue of *FT* in *A. thaliana*	7HS	Yan *et al.* (2006)
T. monococcum	*VRN-A^{m}1*	*TmAP1* – MADS box	5A^{m}L	Yan *et al.* (2003)
	VRN-A^{m}2	*ZCCT-1, ZCCT-2* (zinc-finger and CCT TF)	5A^{m}L	Yan *et al.* (2004b)
T. aestivum	*VRN-A1* (formerly *VRN1*)	*WAP1*- MADS-box	5AL	Murai *et al.* (2003)
	VRN-B1 (formerly *VRN2*)	*TaVRT-1*- MADS-box	5BL	Danyluk *et al.* (2003)
	VRN-D1 (formerly *VRN3*)		5DL	
	VRN-B3 (formerly *VRN-B4*)	*TaFT* - orthologue of *FT* in *A. thaliana*	7BS	Yan *et al.* (2006)

Photoperiodically-responsive loci and genes involved in the transition to flowering

Certain developmental processes in plants are initiated when the photoperiod (day-length) is either longer or shorter than a certain day-length which is called 'critical'. The critical photoperiod is not the same in all cases – it depends on the plant species and the developmental process it regulates. According to the effects of the photoperiod on the induction of flowering, plants can be divided into three major groups: long-day (LD) plants, short-day (SD) plants and neutral plants. LD plants flower when the day-length is longer than a certain critical photoperiod while SD plants flower when the day-length is shorter than a critical photoperiod. Neutral plants are photoperiodically insensitive, *i.e.*, they flower independently on the day-length. For photoperiod sensing, phytochrome photo-receptors are crucial.

Cereals belonging to the tribe *Triticeae* are photoperiodically-sensitive; the transition to flowering is promoted by LDs. In barley, the major photoperiodically-sensitive genes controlling the transition to flowering are located at two *Ppd* (*Photoperiod*) loci, *Ppd-H1* and *Ppd-H2* (Laurie 1997). The *Ppd-H1* locus is found on 2HS and is a principal inducer of flowering under LDs (Laurie *et al.* 1994, Karsai *et al.* 1997). The *Ppd-H2* locus is found on 1HL and is a principal repressor of flowering under SDs (Laurie *et al.* 1995). Previously, *CO*-like (*CONSTANS*-like) genes, photoperiodically regulated genes which control the transition to flowering in *Arabidopsis* (Putterill *et al.* 1995), potato (González-Schain and Suárez-López 2008) and many other plants, have been proposed to be likely candidate genes for *Ppd* loci. Nine *CO*-like genes, *HvCO1* to *HvCO9*, have been identified in barley (Griffiths *et al.* 2003); however, they are not located at the *Ppd* loci in the barley genome.

Later, Turner *et al.* (2005) have identified a *ppd-H1* mutant of barley which exhibits a reduced photoperiodical responsiveness, reduced expression of *HvFT1*, and an altered circadian timing of *CO* expression. The *ppd-H1* mutation is associated with a substitution of a single conserved glycine residue to tryptophan in the CCT domain of a *PRR* (*PSEUDO-RESPONSE REGULATOR*) gene. *PRR* genes like *CO* genes are involved in the timing of the internal circadian clock or are involved in output pathways from the clock and both gene families share the CCT domain. Apart from the CCT domain, all *PRR* genes contain a pseudoreceiver domain with similarities to bacterial two-component signalling systems. It is well known from *Arabidopsis* that *PRR7* gene has a relatively strong influence on the expression of *FT* (Nakamichi *et al.* 2005) which is the major determinant of flowering in *Arabidopsis*. Thus the *PRR* gene presents a promising candidate for the *Ppd-H1* locus in barley. In common wheat, three homoeologous *Ppd* loci named *Ppd-A1*, *Ppd-B1* and *Ppd-D1* have been identified on the short arms of homoeologous group 2 chromosomes. Recently, Beales *et al.* (2007) have reported a photoperiodically-insensitive mutant of common wheat cv. Ciano 67 which carries a *Ppd-D1a* allele that has a 2 kb deletion upstream of the coding region of a *PRR* gene and is expressed under both LD and SD regimes. Therefore, it enhances flowering irrespective of the day-length, probably *via* a positive induction of *TaFT1* (*VRN-B3*), a major positive regulator of *VRN-1* expression, which is normally repressed under SD conditions.

Recently, Faure *et al.* (2007) have proposed that *HvFT3* gene, which belongs to the *HvFT* family (analogously as *HvFT1*, a candidate gene for *VRN3* locus) and which is involved in the regulation of flowering under SDs, can be a candidate gene for the *Ppd-H2* locus. It is also very probable that other HvFT TFs (the family of *HvFT* genes includes five members named *HvFT1* to *HvFT5* which encode proteins with a phosphatidyl-ethanolamine binding domain) are involved in photoperiodical regulation of the transition into flowering.

It has been demonstrated by many authors (Karsai *et al.* 2005, Dubcovsky *et al.* 2006) that the expression of *VRN* genes is affected by photoperiod. Dubcovsky *et al.* (2006) have found out that the expression of *VRN-2* gene is down-regulated by SDs. However, its down-regulation does not result in the up-regulation of *VRN-1* expression. Thus, the authors have postulated the existence of at least one other repressor of *VRN-1*. Kane *et al.* (2005) have described the *TaVRT-2* gene in wheat and its orthologue, *HvVRT-2* in barley, which is a MADS-box TF up-regulated by SDs and down-regulated by LDs in barley cv. Dicktoo. They have recently proven (Kane *et al.* 2007) that *TaVRT-2*, also a MADS-box TF, can bind to both the CArG box in the promoter of *VRN-1* and the *VRN-2* gene product. Thus, the model of *VRN-1* regulation becomes more complex and it seems evident that both vernalization and photoperiod affect the repression (de-repression) of *VRN-1*, and thus transition to flowering in many cultivars of wheat and barley. This model can also explain the long-known fact that facultative barley cultivars, which have a deletion in the *VRN2* locus, begin to flower significantly earlier under LDs than under SDs (Table 2, Fig. 1). A very informative

Table 2. List of the major *Ppd* loci (their candidate genes, respectively) and photoperiodically regulated genes in barley which participate in the regulation of transition into flowering. In reference, *means discovery, **means mapping.

Locus	Gene	Location	Reference
Ppd-H1	*PRR*	2HS	Laurie *et al.* (1994) Karsai *et al.* (1997) Turner *et al.* (2005)
Ppd-H2	*HvFT3*	1HL	Laurie *et al.* (1995) Faure *et al*. (2007)
	HvVRT-2	7HS	Kane *et al.* (2005)* Szücs *et al.* (2006)**
	HvCO1-9	1H,2H,5H,6H,7H	Griffiths *et al.* (2003)

scheme of the relationships between individual vernalization- and photoperiodically-regulated loci (their candidate genes, respectively) in wheat and barley, compared with the analogous relationships in rice and *Arabidopsis*, has been published by Cockram *et al.* (2007b).

The induction of frost tolerance: the phenomenon of cold acclimation

Plants growing in regions where regular long periods of cold and/or frost during the year occur had adapted their life cycle to these unfavourable growth conditions. Annuals usually survive these periods as seeds, biennials and perennials survive them in the vegetative phase when their vegetative organs exhibit a sufficiently high level of FrT.

FrT is defined as the ability of plants to survive the impacts of frost (temperatures below zero) and is usually determined as LT_{50} values (the lethal temperature when 50 % of samples die). FrT is often inducible in many plant species including *Triticeae*; even very frost-tolerant plants are susceptible to frost when they are suddenly transferred from their optimum growth temperature to an environment with temperatures below zero. The distinction between frost-sensitive and frost-tolerant plants lies in the fact that frost-tolerant plants can induce a high level of FrT when exposed to a sufficiently long period of low, but above-zero temperatures, whereas frost-sensitive plants cannot. Non-acclimated rye, for instance, is killed by freezing at about -5 °C, but after a period of exposure to low nonfreezing temperatures the same rye can survive freezing down to about -30 °C (Thomashow 1999). Moreover, it usually takes quite a long time to reach the maximum FrT in highly frost-tolerant cultivars, *e.g.*, the plants of the highly frost-tolerant winter wheat Mironovskaya 808 reach the maximum FrT after a 4-week LT treatment at 3 °C (Prášil *et al.* 2004). This type of treatment, *i.e.*, the impact of low, above-zero temperatures on a plant, is called a process of cold acclimation (CA).

Moreover, the plant´s ability to induce a sufficiently high level of FrT is dependent on its developmental stage. Species belonging to the tribe *Triticeae* are able to induce a high level of FrT only when they are in the vegetative phase of their individual development. The developmental transition into the reproductive phase, indicated in *Triticeae* by a double-ridge formation in the apical meristem, is usually accompanied by a significant decline in this ability (Fowler *et al.* 1996, 2001).

During CA, many physiological and biochemical changes in plant cells occur. They are aimed at the compensation of the loss of water in cell cytoplasm during LT treatment and at the avoidance of intracellular freezing. The osmotic potential of cell cytoplasm decreases due to the accumulation of osmotically active molecules of compatible solutes such as sugars (glucose, fructose, sucrose, mellibiose, raffinose, stachyose, verbascose), sugar alcohols (mannitol, sorbitol, pinitol), quaternary ammonium compounds (glycine betaine, alanine betaine), polyamines (spermine, spermidine, putrescine), and immino acid proline. In membranes, a fraction of unsaturated fatty acids increases in order to retain sufficient fluidity of the membranes and transmembranaceous protein complexes (Sakai and Larcher 1985, Guy 1990). Moreover, many cold-specific proteins are synthesized. These proteins can act either as enzymes – scavenging reactive oxygen species (ROS) which are produced in relatively high amounts under cold, synthesizing molecules of compatible solutes or unsaturated fatty acids, or they can have structural (protective) functions, *i.e.*, can act as chaperones to prevent other proteins and endomembranaceous structures from unfavourable structural changes. One important group of these structural proteins are COR/LEA (cold-regulated/late embryogenesis abundant) proteins which accumulate in both cell cytoplasm and nucleus under the conditions associated with cellular dehydration – environmental stress conditions (drought, enhanced salinity, enhanced evaporation, cold, frost) and the physiological conditions of embryo maturation and desiccation in the late stages of embryogenesis (hence the name LEA proteins).

As a consequence of the biochemical changes described above, the process of CA has increased demands on plant energetic metabolism. Therefore, the *Triticeae* are generally able to induce higher FrT levels under LD than under SD conditions when they are in the vegetative phase, since they can synthesize more assimilates under these conditions (Limin *et al.* 2007).

Cold-induced COR/LEA proteins, *CBF* genes and loci for frost-resistance (*Fr* loci)

COR/LEA proteins are a large group of important structural proteins that accumulate during cellular dehydration. They are usually highly hydrophilic and can protect other proteins and / or membranaceous structures against the loss of their hydration envelopes. The loss of water is associated with unfavourable structural and functional changes of the biomolecules. LEA proteins are usually divided into three major sub-groups based on their unique sequence characteristics (Ingram and Bartels 1996, Cattivelli *et al.* 2002). LEA I sub-group includes glycine-rich, highly hydrophilic proteins which contain one to four copies of a conserved 20 aa motif which consists of an N-terminal (GETWPGGTGGK) and a C-terminal (EGIDIDESKF) consensus sequences. LEA II sub-group which is also called LEA D11 and whose members are named dehydrins includes all proteins with

at least one copy of a lysine-rich sequence, the K-segment (consensus sequence EKKGIMDKIKEKLPG) which is the major antigen determinant of dehydrins (Close *et al.* 1993) and which can form a class A2 amphipathic α-helix under conditions of reduced hydration (Close 1996, 1997). LEA III sub-group is characterised by the presence of a tandem repeat of an 11 aa sequence (consensus ΦΦE/QxΦKE/QKΦxE/D/Q where Φ represents a hydrophobic aa), which can form an amphipathic α-helix (Dure 1993).

In wheat and barley, the cold-induced *Lea* genes include many *Lea II* genes – dehydrins; in barley, the induction by cold has been described for *Dhn5* (K_n type) and *Dhn8* (acidic SK_3 type) (Choi *et al.* 1999); in common wheat, the induction by cold has been described for *Wcs120* gene family including *Wcs200*, *Wcs180*, *Wcs66*, *Wcs120*, *Wcor825* and *Wcor726* genes (all K_n type; for review, see Sarhan *et al.* 1997), acidic SK_3-type *Wcor410* gene family including *Wcor410a*, *Wcor410b* and *Wcor410c* genes (Danyluk *et al.* 1994; 1998); small K_n-type dehydrin *Wdhn13* (Ohno *et al.* 2003), *etc.* From *Lea III* sub-group, induction by cold has been described for chloroplast-located *Wcs19*, *Wcor14a,b* and *Wcor15* in wheat and *Cor14b* in barley and for many other *Cor* and *Rab* genes. Some of the cold-induced *Cor*/*Lea* genes can be regarded as markers of FrT, *i.e.*, their amounts accumulated under cold correspond quantitatively with the level of FrT in different cultivars of wheat and barley (*e.g.*, the WCS120 proteins in wheat – Houde *et al.* 1992b, Vítámvás *et al.* 2007, DHN5 protein in barley – Kosová *et al.* 2008, *Cor14b* in barley and wheat – Crosatti *et al.* 1995, Vágújfalvi *et al.* 2000; for review on the roles of dehydrins upon cold, see Kosová *et al.* 2007). A positive effect of the expression of some wheat *Cor/Lea* genes on the enhancement of FrT has also been proven by studies using transgenic techniques (*e.g.*, NDong *et al.* 2002, Shimamura *et al.* 2006).

The expression of *Cor*/*Lea* genes is regulated by several regulatory pathways which can be basically divided into ABA-dependent and ABA-independent (Table 3). The *Cor*/*Lea* genes whose expression is predominantly regulated by ABA contain several ABRE regulatory elements in their promoter regions which serve as a binding site for bZIP TFs. The ABRE elements possess two fragments: TACGTCC (the G-box) and GGCCGCG (GC-motif) (Thomashow 1999, Allagulova *et al.* 2003, Yamaguchi-Shinozaki and Shinozaki 2005).

One of the most important ABA-independent regulatory pathways is the CBF pathway. In *Arabidopsis*, four *CBF* TFs (*CBF1* - *CBF4*) have been identified; three of them (*CBF1*/*DREB1B*, *CBF2*/*DREB1C* and *CBF3*/*DREB1A*) are cold-induced and tandemly arranged on chromosome 4 while *CBF4*/*DREB1D* is drought-induced. The CBF TFs bind to CRT/DRE/LTRE regulatory elements in promoter regions of their effector genes (*e.g.*, many *Cor*/*Lea* genes). The CRT/DRE/LTRE elements contain a characteristic sequence GCCGAC which serves as a binding site for the AP2 domain of the *CBF* TF (Yamaguchi-Shinozaki and Shinozaki 2005). The expression of *CBF* TFs in *Arabidopsis* is controlled partly by *ICE1* TF (inducer of *CBF* expression 1; TF with bHLH DNA-binding domain) which binds to MYC elements in the promoter regions of the *CBF* genes (Chinnusamy *et al.* 2003); by *ICE1*, the expression of *CBF3* gene is predominantly regulated while *CBF1* and *CBF2* genes are influenced only very slightly. In addition to *ICE1*, it has been shown by Vogel *et al.* (2005) that *ZAT12* regulates the expression of *CBF* genes in *A. thaliana*. Recently, one *AtICE1* homologue and two *AtZAT12* homologues have been identified in barley by Skinner *et al.* (2006) by *BLAST* search of EST sequences. Zarka *et al.* (2003) have found threshold induction temperature for *CBF* genes in *A. thaliana* around 14 °C. Jaglo-Ottosen *et al.* (1998) have clearly shown the relationship between the expression of *CBF* genes and *Cor* genes in *A. thaliana* – the overexpression of *CBF1* led to enhanced expression of four *Cor* genes. In common wheat, a positive relationship between the activity of *CBF* genes and the expression of *Cor*/*Lea* genes has been confirmed by Kobayashi *et al.* (2005), Kume *et al.* (2005), and others.

The majority of *CBF* genes in *Triticeae* has been mapped to the long arm of homoeologous group 5 chromosomes at the *Fr-2* locus (Choi *et al.* 2002, Francia *et al.* 2004), one of the two major QTLs for FrT which is also one of the two major QTLs regulating the expression of *Cor14b* gene (Vágújfalvi *et al.* 2000, 2003). It has also become evident that the *CBF* genes in *Triticeae* are more numerous [barley – 20 *CBFs* (Skinner *et al.* 2005), *T. monococcum* – 13 *CBFs* (Miller *et al.* 2006), *T. aestivum* – up to 25 *CBFs* proposed (Badawi *et al.* 2007)] and diverse than in *Arabidopsis*. In barley and in *T. monococcum*, the *CBFs* have been divided into three distinct phylogenetic sub-groups (Skinner *et al.* 2005, Miller *et al.* 2006). In *T. aestivum*, the *CBFs* have been divided into ten sub-groups (Badawi *et al.* 2007), six of them have been characterised as *Pooideae*-specific. It is also interesting that in both wheat and barley, the members of the sub-groups, which are most closely sequentionally related to the *Arabidopsis CBF1 – CBF3* genes, are located in chromosomal regions other than the *Fr-2* loci.

This large diversity of *CBFs* in *Triticeae* may be a consequence of their adaptation to temperate climate habitats. Expression studies carried out by Badawi *et al.* (2007) showed that five of the *Pooideae*-specific sub-groups display higher constitutive and CA-inducible expression levels in the winter wheat cultivar Norstar when compared to the spring wheat cultivar Manitou. The higher constitutive and inducible expression levels probably present an inherited trait of the winter cultivars, which may form the basis of the higher FrT capacity of the winter cultivars when compared to the spring ones. The quantitative differences in *CBF* expression between differently frost-tolerant cultivars have also been observed by other researchers in einkorn wheat (Vágújfalvi *et al.* 2005, Miller *et al.* 2006) and in barley (Stockinger *et al.* 2007). The last authors mentioned

above have also confirmed differences in constitutive expression of some *CBFs* at the *Fr-2* locus. They have also found out that the quantitative expression of some barley *CBFs* is dependent on photoperiod. However, other studies determining the expression levels of individual *CBFs* under cold and investigating the redundancy between them will be needed to characterise the contribution of individual CBFs to the expression of *Cor* genes and induction of FrT.

Other studies have dealt with the affinity of CBFs to various CRT motifs occurring in the promoters of *Cor* genes. Xue (2002) demonstrated the interaction of HvCBF1 with the (G/a)(C/t)CGAC sequence present in the promoters of *Cor* genes. The activation of the wheat *Cor* genes *Wdhn13* and *Wrab17* by the wheat WCBF2 was demonstrated by Takumi *et al.* (2008) in transgenic tobacco. Skinner *et al.* (2005) have confirmed a great variability in the binding affinity of different CBFs to individual CRT motifs which depends on the sequence motifs flanking the CRT core sequence CCGAC. In addition, Xue (2003) has shown that the binding affinity of the AP2 domains of the CBFs is also temperature-dependent; the author has found out that a temperature decline leads to the enhanced affinity of HvCBF2 to the GCCGAC core motif of the CRT/DRE/LTRE elements. Thus, it can be proposed that a temperature-dependent binding affinity of CBFs can present another level of regulation of *Cor* gene expression.

Apart from the *Fr-2* locus, the major frost-resistance loci named *Fr-1* have been mapped to the long arms of homoeologous group 5 chromosomes in the *Triticeae* genomes far from the *Fr-2* loci, but in a tight linkage with the *VRN-1* loci. Thus, in barley, the *Fr-H1* locus is tightly linked to the *VRN-H1* locus, and in hexaploid wheat, the *Fr-A1* locus is linked to the *VRN-A1* locus (interval of 2 cM – Galiba *et al.* 1995), the *Fr-B1* locus is linked to the *VRN-B1* locus (interval of 40 cM – Tóth *et al.* 2003), and the *Fr-D1* locus is linked to the *VRN-D1* locus (interval of 10 cM – Snape *et al.* 1997). The two *Fr* loci on the long arm of chromosome 5 have also been described by Vágújfalvi *et al.* (2000) as QTLs affecting the expression of *Cor14b* gene in wheat. The expression study carried out by Kobayashi *et al.* (2005) in *T. aestivum* suggested that the *Fr-1* loci can control *Wcbf2* gene expression and thus the up-regulation of the downstream *Cor*/*Lea* gene expression at least partly through the CBF pathway.

In wheat, it is suggested that the spring-type *Vrn-A1* allele is associated with a spring-type *Fr-A1* allele and conversely, the winter-type *vrn-A1* allele is tightly linked to winter-type *Fr-A1* allele. In the B and D genomes, the situation is proposed to be analogous. As *Vrn-A1* is the major *VRN-1* gene in common wheat, it is also proposed that the *Fr-A1* gene has the major effect on the expression of cold-induced genes when compared with the *Fr-B1* and *Fr-D1* genes. Kobayashi *et al.* (2005) used a set of near isogenic lines (NILs) of common wheat and found out that the *Vrn-B1* NIL showed a higher FrT than the *Vrn-A1* NIL because the *Vrn-B1* NIL probably possessed a dominant winter-type *Fr-A1* allele while the *Vrn-A1* NIL possessed a spring-type *Fr-A1*. Similarly, Sutka *et al.* (1999) showed that the lines possessing a functional *Fr-A1* locus showed a 13 % higher survival rate in frost tolerance tests compared to the lines lacking it. Some authors (Miller *et al.* 2006) have even proposed that in genome A, the *Fr-A1* locus is so tightly linked to the *Vrn-A1* locus that they may be identical and that the *Vrn-A1* gene product could regulate the expression of *CBF* genes. However, it has been shown by Ishibashi *et al.* (2007) that wheat cultivars possessing either a dominant *Vrn-D1* allele or a recessive *vrn-D1* allele can exhibit no significant differences in FrT. Therefore, the authors have suggested that in the D genome, the *Vrn-D1* and *Fr-D1* loci are not tightly linked together, and as a consequence, a similar level of acquired FrT can be found in the winter and spring wheat cultivars differing only in the allelic constitution of the *VRN-D1*. Recently, it has been found out that the *Fr* locus described originally as *Fr-B1* locus by Tóth *et al.* (2003) is orthologous to the *Fr-A2* locus in wheat; thus, its name has been corrected to *Fr-B2* (McIntosh *et al.* 2004). Analogously to wheat, the *Fr-H1* locus in barley is tightly linked to the *VRN-H1* locus and it has been shown by Francia *et al.* (2004) that the QTL for FrT on 5 HL cannot be clearly separated from the QTL for vernalization requirement in the Nure (winter) × Tremois (spring) barley mapping population. The candidate genes for the *Fr-1* locus in *Triticeae*, the major locus controlling the induction of FrT, have not been identified yet.

Studies dealing with the relationships between the activity of the VRN-1 gene products and *Cor*/*Lea* gene expression have clearly shown that the induction of the formation of flower meristem and the induction of *VRN-1* gene expression lead to the decline in *Cor*/*Lea* gene expression (Fowler *et al.* 2001, Danyluk *et al.* 2003, Kane *et al.* 2005, Kobayashi *et al.* 2005, Kume *et al.* 2005). Recently, Stockinger *et al.* (2007) have reported that the *VRN-H1/Fr-H1* locus affects the expression of multiple *CBF* genes at the *Fr-H2* locus in the Nure × Tremois barley mapping population. Prášil *et al.* (2005) have also shown that different *vrn-1* alleles affect the ability to maintain a sufficient level of FrT during the conditions of CA in winter wheat. However, the interactions between the activity of *VRN-1* and *Cor*/*Lea* gene expression and the development of FrT have not been precisely elucidated yet.

A scheme describing the possible regulatory relationships between the vernalization loci (the candidate genes), the photoperiod loci (the candidate genes) and the frost-resistance loci (the candidate genes) is given in Fig 1.

Table 3. The list of major cold-induced *Cor*/*Lea* genes in wheat and barley. Genes and corresponding proteins are characterised by their accession numbers from NCBI (state in March 2008), pI and M_r of the proteins were calculated by a calculation tool in *ExPASy* (Swiss protein database). The values of Mr in brackets were determined by SDS-PAGE.

Gene (accession number)	Protein (accession number)	Group LEA	Number of aa	pI	M_r [kDa]	Reference
Triticum aestivum						
Wcs200	WCS200 (AAB31285)	II		6.50	(200)	Quellet *et al.* (1993)
Wcs180	WCS180	II				Houde *et al.* (1995)
Wcs66 (L27516)	WCS66/CS66 (AAA21819)	II	469	6.74	46.8	Chauvin *et al.* (1994)
Wcs120 (M93342)	WCS120/CS120 (AAA34261)	II	390	7.02	39 (50)	Houde *et al.* (1992a)
Wcs40	WCS40	II		7.30	(40)	Houde *et al.* (1995)
Wcs726/*Wcor726* (U73213)	WCS726/WCOR726 (AAB18204)	II	124	7.04	12.7	Danyluk and Sarhan (1996 – NCBI)
Wcs80/*Wcor80* (U73212)	WCS80/WCOR80 (AAB18203)	II	93	8.05	9.6	Danyluk and Sarhan (1996 – NCBI)
Cor39 (AF058794)	COR39 (AAC14297)	II	391	6.92	39 (50)	Guo *et al.* (1992)
Wdhn13 (AB076807)	WDHN13 (BAC01112)	II	124	8.01	12.8	Ohno *et al.* (2003)
Wcor410a (L29152)	WCOR410a (AAA20189)	II	262	5.19	28.0	Danyluk *et al.* (1994)
Wcor410b (U73210)	WCOR410b (AAB18201)	II	268	5.25	28.8	Danyluk and Sarhan (1996 – NCBI)
Wcor410c (U73211)	WCOR410c (AAB18202)	II	259	5.20	27.9	Danyluk and Sarhan (1996 – NCBI)
Wcor825 (U73215)	WCOR825 (AAB18206)	II	73	8.08	8.1	Danyluk and Sarhan (1996 – NCBI)
Wcor14a (AF207545)	WCOR14a (AAF17098)	III	140	4.86	13.5	Tsvetanov *et al.* (2000)
Wcor14b (AF207546)	WCOR14b (AAF17099)	III	137	9.10	13.6	Tsvetanov *et al.* (2000)
Wcor15b(AB095006)	WCOR15 (BAC56935)	III	147	5.12	14.7	Takumi *et al.* (2003)
Wcor615 (U73217)	WCOR615 (AAB18208)	III	175	4.92	17.8	Danyluk and Sarhan (1996 – NCBI)
Wlt10 (AF271260)	WLT10 (AAF75555)	III	101	6.52	9.9	Tsvetanov *et al.* (2000)
Wcs19 (L13437)	WCS19 (AAA16282)	III	147	5.59	14.6	Chauvin *et al.* (1993)
Wrab15 (AB115913)	WRAB15 (BAC80265)	III	130	7.90	15.0	Kobayashi *et al.* (2004)
Wrab17 (AF255053)	WRAB17 (AAF68628)	III	166	4.85	17.2	Tsuda *et al.* (2000)
Wrab18 (AB115914)	WRAB18 (BAC80266)	III	169	5.95	17.5	Kobayashi *et al.* (2004)
Wrab19 (AF255052)	WRAB19 (AAF68627)	III	179	8.63	18.3	Tsuda *et al.* (2000)
Hordeum vulgare						
Dhn5 (AF181455)	DHN5 (AAF01695)	II	575	6.65	58.5 (86)	Close *et al.* (1995)
Dhn8 (AF181458)	DHN8 (AAF01696)	II	255	5.21	27.7	Choi *et al.* (1999)
Cor14b (AJ512944)	COR14b (CAD55692)	III	142	4.84	13.9	Dal Bosco *et al.* (2003)

Future perspectives

In recent years, significant progress in the research dealing with developmental and environmental regulation of FrT in *Triticeae* has been made. However, many crucial questions still remain unanswered. Now, we would like to discuss the two of them in detail.

1) It becomes evident that the VRN-1 gene product is the main regulator of the developmental transition to the less frost-tolerant reproductive phase. This hypothesis has recently been confirmed by Shitsukawa *et al.* (2007) who have prepared and further characterised a mutant *maintained vegetative phase* (*mvp*) of *T. monococcum* which does not flower as a consequence of a deletion of the promoter and coding regions of *VRN-1* gene. In contrast, Adam *et al.* (2007) have observed enhanced transition to flowering in transgenic *Arabidopsis* plants overexpressing the *VRN-1* gene from common wheat. Therefore, the regulation of the *VRN-1* gene expression seems to present a powerful tool how to affect the plant´s potential to induce a sufficiently high level of FrT under the conditions of CA and to maintain it for a sufficiently long period of time. Detailed regulation of *VRN-1* gene expression is still unknown. However, it becomes obvious that the VRN-2 gene product is not the only repressor of the *VRN-1* gene. Kane *et al.* (2005) have described *VRT-2* gene, a MADS-box gene distinct structurally from the *VRN-2* gene (a *ZCCT* gene) as another repressor of the *VRN-1* gene, whose expression is maintained by SDs in barley (in contrast to the *VRN-2* gene whose expression is down-regulated by SDs). It also becomes evident that the *VRN-1* gene is under positive

regulation of a LD-responsive pathway mediated by VRN-3 gene product.

Another mechanism, which regulates the transition to flowering in *Arabidopsis*, but has not experimentally been studied in *Triticeae* yet, presents the epigenetic regulation of gene activity *via* chromatin modification. It is well known from *Arabidopsis* that the silencing of the major flowering repressor *FLC* is mediated via histone methylation. In wheat and barley, no experimental evidence of chromatin modification at the vernalization loci has been found yet, but it can be expected that the changes in pattern of chromatin modification may play an important role in the changes of gene expression associated with the effect of LD photoperiod after the fulfillment of vernalization requirement. Trevaskis *et al.* (2007) have hypothesized that the activity of *VRN-1* gene can be regulated via chromatin modification and that the 'vernalization critical region' in the first intron of this gene might be a target of protein complexes that regulate chromatin modification. According to this model, the expression of the recessive *vrn-1* alleles would be repressed via chromatin methylation and LD pathway would activate protein complexes which would change pattern of chromatin modification after the fulfillment of vernalization requirement (down-regulation of *VRN-2* gene), thus leading to the activation of *VRN-1* gene. Three homologues of *A. thaliana VIL* (*Vernalization-Insensitive 3 Like*) genes, which regulate the epigenetic silencing of *FLC* after vernalization in *Arabidopsis*, have been recently found in *T. monococcum* by Fu *et al.* (2007). Experimental evidence which would confirm or reject this hypothesis is still lacking.

However, a creation and testing of a complex model of the expression of the *VRN-1* gene presents a great challenge for scientists studying the regulation of the developmental transition in *Triticeae*.

2) It has been confirmed by many researchers that the activity of the *VRN-1*/*Fr-1* locus is crucial for the *Triticeae´* ability to develop a sufficient level of FrT upon CA. However, the precise role of the *VRN-1*/*Fr-1* locus in this process remains to be elucidated. First, until now, the nature of the *VRN-1*/*Fr-1* locus itself is still not clear, *i.e.*, it is not clear whether the FrT QTL mapped to this locus is the effect of some yet unidentified gene or simply a pleiotropic effect of the *VRN-1* gene itself. Therefore, two possible hypotheses could be formulated:

a) The *VRN-1*/*Fr-1* locus contains two different genes – the *VRN-1* gene affecting the vernalization response and another, yet uncharacterised gene responsible for the induction of FrT and regulation of the *Fr-2* locus. A possible scenario could be the following: both genes are tightly linked together, *i.e.*, there are no recombination events between them. Thus, winter genotypes, which have only recessive *vrn-1* alleles, also have only winter-type *Fr-1* alleles, which strongly activate the *Fr-2* located genes (the *CBF* genes). In contrast, spring genotypes, which have at least one dominant *Vrn-1* allele, possess at least one spring-type *Fr-1* allele, which can activate the *Fr-2* genes only weakly. Therefore, the winter cultivars in the vegetative phase can induce a higher FrT than the spring ones. During developmental transition into the reproductive phase, the *VRN-1* gene expression is induced and the *VRN-1* gene product then represses the activity of *Fr-1*.

b) According to the latter scenario, the FrT QTL is simply one effect of the activity of the *VRN-1* gene. It can be proposed that the *VRN-1* gene product acts, in coordination with some other TFs, as a repressor of the *CBF* genes at the *Fr-2* locus. Winter cultivars, where the *vrn-1* allele is repressed under LT and SD conditions, can thus induce high activity of *Fr-2* genes via signalling pathways that respond directly to LT (*e.g.*, *via ICE-1* or *ZAT12* homologues). Since the activity of the *CBFs* is not repressed in these cultivars, they are able to induce a high level of FrT prior to the fulfillment of their vernalization requirement. In contrast, spring cultivars cannot repress the activity of *Vrn-1*, thus the *Vrn-1* acts as a repressor of *CBFs* at the *Fr-2* locus and the activity of *CBFs* at this locus under CA is a result of a positive regulation by some signalling pathways (maybe via *ICE-1* or *ZAT12* homologues) and a negative regulation by *Vrn-1*. Thus, the resulting activity of the *CBFs* at *Fr-2* in spring genotypes upon CA is weaker compared to the winter genotypes prior to the fulfillment of vernalization requirement; therefore, the spring genotypes can induce an enhanced FrT upon CA only transiently and at lower levels than the winter ones. A transient activity of CA-induced genes in a spring wheat cultivar compared to a winter one under cold has recently been found by Monroy *et al.* (2007) on a *T. aestivum*-specific microarray.

The characterization of the *VRN-1*/*Fr-1* locus thus remains a task for scientists who study the genetic basis of the impact of individual development on the ability to induce FrT in *Triticeae*. Recently, Stockinger *et al.* (2007) have confirmed the effect of the *VRN-1/Fr-1* region on the regulation of some *Fr-2* located *CBF* genes in the Nure × Tremois barley mapping population. These authors have also hypothesized that the QTL for FrT may be a pleiotropic effect of the *VRN-1* gene and that an allelic variation in *VRN-1* may exist which can cause that some *VRN-1* alleles have a strong effect on flowering, but no effect on *CBF* gene expression and development of FrT. The allelic variation in *VRN-1* could explain the QTL for FrT in the reproductive stage mapped to the *VRN-1/Fr-1* region by Reinheimer *et al.* (2004). However, other experiments need to be conducted to investigate the role of *VRN-1/Fr-1* region in *CBF* gene expression and development of FrT in *Triticeae*. A detailed comprehension to this regulatory pathway will certainly contribute to our better understanding of the mechanisms which regulate the development of FrT in *Triticeae* with respect to the plant individual development. Furthermore, the results of this research may be useful for the breeders in order to design new varieties of wheat and barley with known physiological characteristics which will be able to better respond to changing environments.

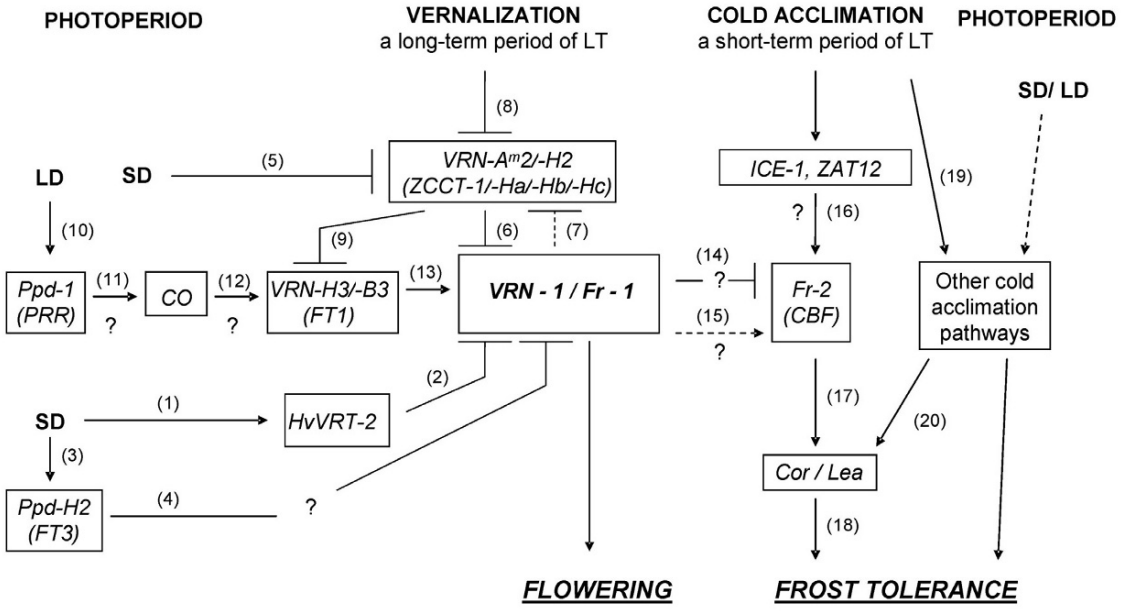

Fig. 1. A descriptive scheme of the relationships between vernalization- and photoperiodically-regulated loci (their candidate genes, respectively) which regulate the transition to flowering and loci which regulate the development of frost tolerance (FrT) upon cold acclimation (CA). (1) Kane *et al.* (2005); (2) Kane *et al.* (2007); (3) Laurie *et al.* (1995); (4) Faure *et al.* (2007); (5) Yan *et al.* (2004b); (6) when *vrn-1* is recessive and *Vrn-2* is dominant; Von Zitzewitz *et al.* (2005); Szücs *et al.* (2007); (7) possibly when *Vrn-1* is dominant or when *vrn-1* is recessive under prolonged LD treatment; Loukoianov *et al.* (2005); Trevaskis *et al.* (2006); (8) Dubcovsky *et al.* (2006); Trevaskis *et al.* (2006); (9) Yan *et al.* (2006); (10) Laurie *et al.* (1994); (11) Turner *et al.* (2005); Beales *et al.* (2007); (12) Griffiths *et al.* (2003); Turner *et al.* (2005); (13) Yan *et al.* (2006); (14) Danyluk *et al.* (2003); Kane *et al.* (2005); Kobayashi *et al.* (2005); Stockinger *et al.* (2007); (15) Vágújfalvi *et al.* (2000); Kobayashi *et al.* (2005); Stockinger *et al.* (2007); (16) Skinner *et al.* (2006); (17) Vágújfalvi *et al.* (2000); Choi *et al.* (2002); Kume *et al.* (2005); Skinner *et al.* (2005); Miller *et al.* (2006); (18) Houde *et al.* (1992b); Crosatti *et al.* (1995); Vágújfalvi *et al.* (2000); Vítámvás *et al.* (2007); Kosová *et al.* (2008); (19) Thomashow (1999); (20) Choi *et al.* (1999); Thomashow (1999). Question marks indicate uncertain or unknown components of the regulatory pathways, dashed lines indicate uncertain regulatory relationships.

References

Adam, H., Quellet, F., Kane, N.A., Agharbaoui, Z., Major, G., Tominaga, Y., Sarhan, F.: Overexpression of *TaVRN1* in *Arabidopsis* promotes early flowering and alters development. - Plant Cell Physiol. **48**: 1192-1206, 2007.

Allagulova, Ch.R., Gimalov, F.R., Shakirova, F.M., Vakhitov, V.A.: The plant dehydrins: structure and putative functions. - Biochemistry **68**: 945-951, 2003.

Badawi, M., Danyluk, J., Boucho, B., Houde, M., Sarhan, F.: The *CBF* gene family in hexaploid wheat and its relationship to the phylogenetic complexity of cereal *CBFs*. - Mol. Genet. Genomics **277**: 533-554, 2007.

Bastow, R., Mylne, J.S., Lister, C., Lippman, Z., Martienssen, R.A., Dean, C.: Vernalization requires epigenetic silencing of *FLC* by histone methylation. - Nature **427**: 164-167, 2004.

Beales, J., Turner, A., Griffiths, S., Snape, J.W., Laurie, D.A.: A *Pseudo-Response Regulator* is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). - Theor. appl. Genet. **115**: 721-733, 2007.

Ben-Naim O., Eshed, R., Parnis A., Teper-Bamnolker, P., Shalit, A., Coupland, G., Samach, A., Lifschitz, E.: The CCAAT binding factor can mediate interactions between CONSTANS-like proteins and DNA. - Plant J. **46**: 462-476, 2006.

Cattivelli, L., Baldi, P., Crosatti, C., Di Fonzo, N., Faccioli, P., Grossi, M., Mastrangelo, A.M., Pecchioni, N., Stanca, A.M.: Chromosome regions and stress-related sequences involved in resistance to abiotic stress in *Triticeae*. - Plant mol. Biol. **48**: 649-665, 2002.

Chauvin, L.-P., Houde, M., Fowler, D.B.: Nucleotide sequence of a new member of the freezing tolerance-associated protein family in wheat. - Plant Physiol. **105**: 1017-1018, 1994.

Chauvin, L.-P., Houde, M., Sarhan, F.: A leaf-specific gene stimulated by light during wheat acclimation to low temperature. - Plant mol. Biol. **23**: 255-265, 1993.

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

Choi, D.W., Rodriguez, E.M., Close, T.J.: Barley *Cbf3* gene identification, expression pattern, and map location. - Plant Physiol. **129**: 1781-1787, 2002.

Choi, D.W., Zhu, B., Close, T.J.: The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 *Dhn* genes of cv. Dicktoo. - Theor. appl. Genet. **98**: 1234-1247, 1999.

Chouard, P.: Vernalization and its relations to dormancy. - Annu. Rev. Plant Physiol. **11**: 191-238, 1960.

Close, T.J.: Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. - Physiol. Plant. **97**: 795-803, 1996.

Close, T.J.: Dehydrins: a commonalty in the response of plants to dehydration and low temperature.- Physiol. Plant. **100**: 291-296, 1997.

Close, T.J., Fenton, R.D., Moonan, F.: A view of plant dehydrins using antibodies specific to the carboxy terminal peptide. - Plant mol. Biol. **23**: 279-286, 1993.

Close, T.J., Meyer, N.C., Radik, J.: Nucleotide sequence of a gene encoding a 58.5-kilodalton barley dehydrin that lacks a serine tract. - Plant Gene Register. Plant Physiol. **107**: 289-290, 1995.

Cockram, J., Chiapparino, E., Taylor, S.A., Stamati, K., Donini, P., Laurie, D.A., O'Sullivan, D.M.: Haplotype analysis of vernalization loci in European barley germplasm reveals novel *VRN-H1* alleles and a predominant winter *VRN-H1*/*VRN-H2* multi-locus haplotype. - Theor. appl. Genet. **115**: 993-1001, 2007a.

Cockram, J., Jones, H., Leigh, F.J., O´Sullivan, D., Powell, W., Laurie, D.A., Greenland, A.J.: Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. - J. exp. Bot. **58**: 1231-1244, 2007b.

Crosatti, C., Soncini, C., Stanca, A.M., Cattivelli, L.: The accumulation of a cold-regulated chloroplastic protein is light-dependent. - Planta **196**: 458-463, 1995.

Dal Bosco, C., Busconi, M., Govoni, C., Baldi, P., Stanca, A.M., Crosatti, C., Bassi, R., Cattivelli, L.: *Cor* gene expression in barley mutants affected in chloroplast development and photosynthetic electron transport. - Plant Physiol. **131**: 793-802, 2003.

Danyluk, J., Houde, M., Rassart, E., Sarhan, F.: Differential expression of a gene encoding an acidic dehydrin in chilling sensitive and freezing tolerant *Gramineae* species. - FEBS Lett. **344**: 20-24, 1994.

Danyluk, J., Kane, N.A., Breton, G., Limin, A.E., Fowler, D.B., Sarhan, F.: *TaVRT-1*, a putative transcription factor associated with vegetative to reproductive transition in cereals. - Plant Physiol. **132**: 1849-1860, 2003.

Danyluk, J., Perron, A., Houde, M., Limin, A., Fowler, B., Benhamou, N., Sarhan, F.: Accumulation of an acidic dehydrin in the vicinity of the plasma membrane during cold acclimation of wheat. - Plant Cell **10**: 623-638, 1998.

Dubcovsky, J., Chen, C., Yan, L.: Molecular characterization of the allelic variation at the *VRN-H2* vernalization locus in barley. - Mol. Breed. **15**: 395-407, 2005.

Dubcovsky, J., Loukoianov, A., Fu, D., Valarik, M., Sanchez, A., Yan, L.: Effect of photoperiod on the regulation of wheat vernalization genes *VRN1* and *VRN2*. - Plant mol. Biol. **60**: 469-480, 2006.

Dure, L.: A repeating 11-mer amino acid motif and plant desiccation. - Plant J. **3**: 363-369, 1993.

Faure, S., Higgins, J., Turner, A., Laurie, D.A.: The flowering locus T-like gene family in barley (*Hordeum vulgare*). - Genetics **176**: 599-609, 2007.

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

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

Francia, E., Rizza, F., Cattivelli, L., Stanca, A.M., Galiba, G., Tóth, B., Hayes, P.M., Skinner, J.S., Pecchioni, N.: Two loci on chromosome 5H determine low-temperature tolerance in a 'Nure' (winter) × 'Tremois' (spring) barley map. - Theor. appl. Genet. **108**: 670-680, 2004.

Fu, D., Dunbar, M., Dubcovsky, J.: Wheat *VIN3*-like PHD finger genes are up-regulated by vernalization. - Mol. Genet. Genomics **277**: 301-313, 2007.

Fu, D., Szücs, P., Yan, L., Helguera, M., Skinner, J.S., Von Zitzewitz, J., Hayes, P.M., Dubcovsky, J.: Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. - Mol. Genet. Genomics **273**: 54-65, 2005.

Galiba, G., Quarrie, S.A., Sutka, J., Morgounov, A., Snape, J.W.: RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. - Theor. appl. Genet. **90**: 1174-1179, 1995.

González-Schain, N.D., Suárez-López, P.: CONSTANS delays flowering and affects tuber yield in potato. - Biol. Plant. **52**: 251-258, 2008.

Griffiths, S., Dunford, R.P., Coupland, G., Laurie, D.A.: The evolution of *CONSTANS*-like gene families in barley, rice, and *Arabidopsis*. - Plant Physiol. **131**: 1855-1867, 2003.

Guo, W.W., Ward, R.W., Thomashow, M.F.: Characterization of a cold-regulated wheat gene related to *Arabidopsis Cor47*. - Plant Physiol. **100**: 915-922, 1992.

Guy, C.L.: Cold acclimation and freezing stress tolerance: role of protein metabolism. - Annu. Rev. Plant Physiol. Plant mol. Biol. **41**: 187-223, 1990.

Houde, M., Daniel, C., Lachapelle, M., Allard, F., Laliberté, S., Sarhan, F.: Immunolocalization of freezing-tolerance-associated proteins in the cytoplasm and nucleoplasm of wheat crown tissues. - Plant J. **8**: 583-593, 1995.

Houde, M., Danyluk, J., Laliberté, J.-F., Rassart, E., Dhindsa, R.S., Sarhan, F.: Cloning, characterization, and expression of a cDNA encoding a 50 kilodalton protein specifically induced by cold acclimation in wheat. - Plant Physiol. **99**: 1381-1387, 1992a.

Houde, M., Dhindsa, R.S., Sarhan, F.: A molecular marker to select for freezing tolerance in *Gramineae*. - Mol. Gen. Genet. **234**: 43-48, 1992b.

Ingram, J., Bartels, D.: The molecular basis of dehydration tolerance in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **47**: 377-403, 1996.

Ishibashi, M., Kobayashi, F., Nakamura, J., Murai, K., Takumi, S.: Variation of freezing tolerance, *Cor*/*Lea* gene expression and vernalization requirement in Japanese common wheat. - Plant Breed. **126**: 464-469, 2007.

Jaglo-Ottosen, K.R., Gilmour, S.J., Zarka, D.G., Schabenberger, O., Thomashow, M.F.: *Arabidopsis CBF1* overexpression induces *COR* genes and enhances freezing tolerance. - Science **280**: 104-106, 1998.

Kane, N.A., Agharbaoui, Z., Diallo, A.O., Adam, H., Tominaga, Y., Quellet, F., Sarhan, F.: TaVRT2 represses transcription of the wheat vernalization gene *TaVRN1*. - Plant J. **51**: 670-680, 2007.

Kane, N.A., Danyluk, J., Tardif, G., Quellet, F., Laliberté, J.F., Limin, A.E., Fowler, D.B., Sarhan, F.: *TaVRT-2*, a member of the *St*-MADS-11 clade of flowering repressors, is regulated by vernalization and photoperiod in wheat. - Plant Physiol. **138**: 2354-2363, 2005.

Karsai, I., Mészáros, K., Hayes, P.M., Bedö, Z.: Effects of loci on chromosomes 2 (2H) and 7 (5H) on developmental patterns in barley (*Hordeum vulgare* L.) under different photoperiod regimes. - Theor. appl. Genet. **94**: 612-618,

1997.

Karsai, I., Szücs, P., Mészáros, K., Filichkina, T., Hayes, P.M., Skinner, J.S., Láng, L., Bedö, Z.: The *Vrn-H2* locus is a major determinant of flowering time in a facultative × winter growth habit barley (*Hordeum vulgare* L.) mapping population. - Theor. appl. Genet. **110**: 1458-1466, 2005.

Kobayashi, F., Takumi, S., Kume, S., Ishibashi, M., Ohno, R., Murai, K., Nakamura, C.: Regulation by *Vrn-1*/*Fr-1* chromosomal intervals of CBF-mediated *Cor*/*Lea* gene expression and freezing tolerance in common wheat. - J. exp. Bot. **56**: 887-895, 2005.

Kobayashi, F., Takumi, S., Nakata, M., Ohno, R., Nakamura, T., Nakamura, C.: Comparative study of the expression profiles of the *Cor/Lea* gene family in two wheat cultivars with contrasting levels of freezing tolerance. - Physiol. Plant. **120**: 585-594, 2004.

Kosová, K., Holková, L., Prášil, I.T., Prášilová, P., Bradáčová, M., Vítámvás, P., Čapková, V.: The expression of dehydrin 5 during the development of frost tolerance in barley (*Hordeum vulgare*). - J. Plant Physiol. 2008, DOI: 10.1016/j.jplph.2007.10.009

Kosová, K., Vítámvás, P., Prášil, I.T.: The role of dehydrins in plant response to cold. - Biol. Plant. **51**: 601-617, 2007.

Košner, J., Pánková, K.: The detection of allelic variants at the recessive *vrn* loci of winter wheat. - Euphytica **101**: 9-16, 1998.

Kóti, K., Karsai, I., Szücs, P., Horváth, Cs., Mészáros, K., Kiss, G.B., Bedö, Z., Hayes, P.M.: Validation of the two-gene epistatic model for vernalization response in a winter × spring barley cross. - Euphytica **152**: 17-24, 2006.

Krekule, J.: Vernalization in wheat. – In: Atherton, J.G. (ed.): Manipulation of flowering. Pp. 159-169. Buttersworths London - Boston - Durban - Singapure - Sydney - Toronto - Wellington 1987.

Kume, S., Kobayashi, F., Ishibashi, M., Ohno, R., Nakamura, C., Takumi, S.: Differential and coordinated expression of *Cbf* and *Cor/Lea* genes during long-term cold acclimation in two wheat cultivars showing distinct levels of freezing tolerance. - Genes genet. Syst. **80**: 185-197, 2005.

Laurie, D.A.: Comparative genetics of flowering time. - Plant mol. Biol. **35**: 167-177, 1997.

Laurie, D.A., Griffiths, S., Dunford, R.P., Christodoulou, V., Taylor, S.A., Cockram, J., Beales, J., Turner, A.: Comparative genetic approaches to the identification of flowering time genes in temperate cereals. - Field Crops Res. **90**: 87-99, 2004.

Laurie, D.A., Pratchett, N., Bezant, J.H., Snape, J.W.: Genetic analysis of a photoperiod response gene on the short arm of chromosome 2(2H) of barley (*Hordeum vulgare* L.). - Heredity **72**: 619-627, 1994.

Laurie, D.A., Pratchett, N., Bezant, J.H., Snape, J.W.: 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, 1995.

Limin, A.E., Corey, A., Hayes, P., Fowler, D.B.: Low-temperature acclimation of barley cultivars used as parents in mapping populations: response to photoperiod, vernalization and phenological development. - Planta **226**: 139-146, 2007.

Loukoianov, A., Yan, L., Blechl, A., Sanchez, A., Dubcovsky, J.: Regulation of *VRN-1* vernalization genes in normal and transgenic polyploid wheat. - Plant Physiol. **138**: 2364-2373, 2005.

McIntosh, R.A., Devos, K.M., Dubcovsky, J., Rogers, W.J.: Catalogue of gene symbols for wheat: 2004 supplement. Wheat Information Service. http://wheat.pw.usda.gov/ggpages/awn/50/Textfiles/WGC.html

Miller, A.K., Galiba, G., Dubcovsky, J.: A cluster of 11 *CBF* transcription factors is located at the frost tolerance locus *Fr-A^{m}2* in *Triticum monococcum*. - Mol. Genet. Genomics **275**: 193-203, 2006.

Monroy, A.F., Dryanova, A., Malette, B., Oren, D.H., Farajalla, M.R., Liu, W., Danyluk, J., Ubayasena, L.W.C., 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. **64**: 409-423, 2007.

Murai, K., Miyamae, M., Kato, H., Takumi, S., Ogihara, Y.: *WAP1*, a wheat *APETALA1* homolog, plays a central role in the phase transition from vegetative to reproductive growth. - Plant Cell Physiol. **44**: 1255-1265, 2003.

Nakamichi, N., Kita, M., Ito, S., Sato, E., Yamashino, T., Mizuno, T.: The *Arabidopsis* pseudo-response regulators, *PRR5* and *PRR7*, coordinately play essential roles for circadian clock function. - Plant Cell Physiol. **46**: 609-619, 2005.

NDong, C., Danyluk, J., Wilson, K.E., Pocock, T., Huner, N.P.A., Sarhan, F.: Cold-regulated cereal chloroplast late embryogenesis abundant-like proteins. Molecular characterization and functional analyses. - Plant Physiol. **129**: 1368-1381, 2002.

Ohno, R., Takumi, S., Nakamura, C.: Kinetics of transcript and protein accumulation of a low-molecular-weight wheat LEA D-11 dehydrin in response to low temperature. - J. Plant Physiol. **160**: 193-200, 2003.

Prášil, I.T., Prášilová, P., Pánková, K.: Relationships among vernalization, shoot apex development and frost tolerance in wheat. - Ann. Bot. **94**: 413-418, 2004.

Prášil, I.T., Prášilová, P., Pánková, K.: The relationship between vernalization requirement and frost tolerance in substitution lines of wheat. - Biol. Plant. **49**: 195-200, 2005.

Preston, J.C., Kellogg, E.A.: Conservation and divergence of *APETALA1*/*FRUITFULL*-like gene function in grasses: evidence from gene expression analyses. - Plant J. **52**: 69-81, 2007.

Putterill, J., Robson, F., Lee, K., Simon, R., Coupland, G.: The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. - Cell **80**: 847-857, 1995.

Quellet, F., Houde, M., Sarhan, F.: Purification, characterization and cDNA cloning of the 200 kDa protein induced by cold acclimation in wheat. - Plant Cell Physiol. **34**: 59-65, 1993.

Reinheimer, J.L., Barr, A.R., Eglinton, J.K.: QTL mapping of chromosomal regions conferring reproductive frost tolerance in barley (*Hordeum vulgare* L.). - Theor. appl. Genet. **109**: 1267-1274, 2004.

Robson, F., Costa, M.M.R., Hepworth, S.R., Vizir, I., Pineiro, M., Reeves, P.H., Putterill, J., Coupland, G.: Functional importance of conserved domains in the flowering-time gene *CONSTANS* demonstrated by analysis of mutant alleles and transgenic plants. - Plant J. **28**: 619-631, 2001.

Sakai, A., Larcher, W.: Frost Survival of Plants. Responses and Adaptation to Freezing Stress. - Springer-Verlag, Berlin - Heidelberg - New York - London - Paris - Tokyo 1985.

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

Schmitz, J., Franzen, R., Ngyuen, T.H., Garcia-Maroto, F., Pozzi, C., Salamini, F., Rohde, W.: Cloning, mapping and

expression analysis of barley MADS-box genes. - Plant mol. Biol. **42**: 899-913, 2000.

Shimamura, C., Ohno, R., Nakamura, C., Takumi, S.: Improvement of freezing tolerance in tobacco plants expressing a cold-responsive and chloroplast-targeting protein WCOR15 of wheat. - J. Plant Physiol. **163**: 213-219, 2006.

Shitsukawa, N., Ikari, C., Shimada, S., Kitagawa, S., Sakamoto, K., Saito, H., Ryuto, H., Fukunishi, N., Abe, T., Takumi, S., Nasuda, S., Murai, K.: The einkorn wheat (*Triticum monococcum*) mutant, *maintained vegetative phase*, is caused by a deletion in the *VRN1* gene. - Genes genet. Syst. **82**: 167-170, 2007.

Skinner, J.S., Szücs, P., Von Zitzewitz, J., Marquez-Cedillo, L., Filichkin, T., Stockinger, E.J., Thomashow, M.F., Chen, T.H.H., Hayes, P.M.: Mapping of barley homologs to genes that regulate low temperature tolerance in *Arabidopsis*. - Theor. appl. Genet. **112**: 832-842, 2006.

Skinner, J.S., Von Zitzewitz, J., Szücs, P., Marquez-Cedillo, L., Filichkin, T., Amundsen, K., Stockinger, E.J., Thomashow, M.F., Chen, T.H.H., Hayes, P.M.: Structural, functional, and phylogenetic characterization of a large *CBF* gene family in barley. - Plant mol. Biol. **59**: 533-551, 2005.

Snape, J.W., Semikhodskii, A., Sarma, R., Quarrie, S.A., Galiba, G., Sutka, J.: Mapping frost resistance loci in wheat and comparative mapping with other cereals. - Acta agron. hung. **45**: 265-270, 1997.

Stockinger, E.J., Skinner, J.S., Gardner, K.G., Francia, E., Pecchioni, N.: Expression levels of barley *Cbf* genes at the *Frost resistance-H2* locus are dependent upon alleles at *Fr-H1* and *Fr-H2*. - Plant J. **51**: 308-321, 2007.

Sung, S., Amasino, R.M.: Vernalization and epigenetics: how plants remember winter. - Curr. Opin. Plant Biol. **7**: 4-10, 2004.

Sung, S., Amasino, R.M.: Remembering winter: toward a molecular understanding of vernalization. - Annu. Rev. Plant Biol. **56**: 491-508, 2005.

Sutka, J., Galiba, G., Vágújfalvi, A., Gill, B.S., Snape, J.W.: Physical mapping of the *Vrn-A1* and *Fr1* genes on chromosome 5A of wheat using deletion lines. - Theor. appl. Genet. **99**: 199-202, 1999.

Szücs, P., Karsai, I., Von Zitzewitz, J., Mészáros, K., Cooper, L.L.D., Gu, Y.Q., Chen, T.H.H., Hayes, P.M., Skinner, J.S.: Positional relationships between photoperiod response QTL and photoreceptor and vernalization genes in barley. - Theor. appl. Genet. **112**: 1277-1285, 2006.

Szücs, P., Skinner, J.S., Karsai, I., Cuesta-Marcos, A., Haggard, K.G., Corey, A.E., Chen, T.H.H., Hayes, P.M.: Validation of the *VRN-H2*/*VRN-H1* epistatic model in barley reveals that intron length variation in *VRN-H1* may account for a continuum of vernalization sensitivity. - Mol. Genet. Genomics **277**: 249-261, 2007.

Takumi, S., Koike, A., Nakata, M., Kume, S., Ohno, R., Nakamura, C.: Cold-specific and light-stimulated expression of a wheat (*Triticum aestivum* L.) *Cor* gene *Wcor15* encoding a chloroplast-targeted protein. - J. exp. Bot. **54**: 2265-2274, 2003.

Takumi, S., Shimamura, C., Kobayashi, F.: Increased freezing tolerance through up-regulation of downstream genes via the wheat *CBF* gene in transgenic tobacco. - Plant Physiol. Biochem. **46**: 205-211, 2008.

Thomashow, M.F.: Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 571-599, 1999.

Tóth, B., Galiba, G., Fehér, E., Sutka, J., Snape, J.W.: Mapping genes affecting flowering time and frost resistance on chromosome 5B of wheat. - Theor. appl. Genet. **107**: 509-514, 2003.

Trevaskis, B., Hemming, M.N., Dennis, E.S., Peacock, W.J.: The molecular basis of vernlization-induced flowering in cereals. - Trends Plant Sci. **12**: 352-357, 2007.

Trevaskis, B., Hemming, M.N., Peacock, W.J., Dennis, E.S.: *HvVRN2* responds to daylength, whereas *HvVRN1* is regulated by vernalization and developmental status. - Plant Physiol. **140**: 1397-1405, 2006.

Tsuda, K., Tsvetanov, S., Takumi, S., Mori, N., Atanassov, A., Nakamura, C.: New members of a cold-responsive group-3 *Lea/Rab*-related *Cor* gene family from common wheat (*Triticum aestivum* L.). - Genes genet. Syst. **75**: 179-188, 2000.

Tsvetanov, S., Ohno, R., Tsuda, K., Takumi, S., Mori, N., Atanassov, A., Nakamura, C.: A cold-responsive wheat (*Triticum aestivum* L.) gene *wcor14* identified in a winter-hardy cultivar 'Mironovska 808'. - Genes genet. Syst. **75**: 49-57, 2000.

Turner, A., Beales, J., Faure, S., Dunford, R.P., Laurie, D.A.: The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. - Science **310**: 1031-1034, 2005.

Vágújfalvi, A., Aprile, A., Miller, A., Dubcovsky, J., Delugu, G., Galiba, G., Cattivelli, L.: The expression of several *Cbf* genes at the *Fr-A2* locus is linked to frost resistance in wheat. - Mol. Genet. Genomics **274**: 506-514, 2005.

Vágújfalvi, A., Crosatti, C., Galiba, G., Dubcovsky, J., Cattivelli, L.: Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the *cor14b* gene in frost-tolerant and frost-sensitive genotypes. - Mol. gen. Genet. **263**: 194-200, 2000.

Vágújfalvi, A., Galiba, G., Cattivelli, L., Dubcovsky, J.: The cold-regulated transcriptional activator *Cbf3* is linked to the frost-tolerance locus *Fr-A2* on wheat chromosome 5A. - Mol. Genet. Genomics **269**: 60-67, 2003.

Vítámvás, P., Saalbach, G., Prášil, I.T., Čapková, V., Opatrná, J., Jahoor, A.: WCS120 protein family and proteins soluble upon boiling in cold-acclimated winter wheat. - J. Plant Physiol. **164**: 1197-1207, 2007.

Vogel, J.T., Zarka, D.G., Van Buskirk, H.A., Fowler, S.G., Thomashow, M.F.: Roles of the *CBF2* and *ZAT12* transcription factors in configuring the low temperature transcriptome of *Arabidopsis*. - Plant J. **41**: 195-211, 2005.

Von Zitzewitz, J., Szücs, P., Dubcovsky, J., Yan, L., Francia, E., Pecchioni, N., Casas, A., Chen, T.H.H., Hayes, P.M., Skinner, J.S.: Molecular and structural characterization of barley vernalization genes. - Plant mol. Biol. **59**: 449-467, 2005.

Xue, G.P.: An AP2 domain transcription factor HvCBF1 activates expression of cold-responsive genes in barley through interaction with a (G/a)(C/t)CGAC motif. - Biochim. biophys. Acta **1577**: 63-72, 2002.

Xue, G.P.: The DNA-binding activity of an AP2 transcriptional activator *HvCBF2* involved in regulation of low-temperature responsive genes in barley is modulated by temperature. - Plant J. **33**: 373-383, 2003.

Yamaguchi-Shinozaki, K., Shinozaki, K.: Organization of *cis*-acting regulatory elements in osmotic- and cold-stress-responsive promoters. - Trends Plant Sci. **10**: 88-94, 2005.

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S., Dubcovsky, J.: The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. - Proc. nat. Acad. Sci. USA **103**: 19581-19586, 2006.

Yan, L., Helguera, M., Kato, K., Fukuyama, S., Sherman, J.,

Dubcovsky, J.: Allelic variation at the *VRN-1* promoter region in polyploid wheat. - Theor. appl. Genet. **109**: 1677-1686, 2004a.

Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., SanMiguel, P., Bennetzen, J.L., Echenique, V., Dubcovsky, J.: The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. - Science **303**: 1640-1644, 2004b.

Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T., Dubcovsky, J.: Positional cloning of the wheat vernalization gene *VRN1*. - Proc. nat. Acad. Sci. USA **100**: 6263-6268, 2003.

Zarka, D.G., Vogel, J.T., Cook, D., Thomashow, M.F.: Cold induction of *Arabidopsis CBF* genes involves multiple *ICE1* (inducer of *CBF* expression 1) promoter elements and a cold-regulatory circuit that is desenzitized by low temperature. - Plant Physiol. **133**: 910-918, 2003.