UC Davis

UC Davis Previously Published Works

Title

Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the cor14b gene in frost-tolerant and frost-sensitive genotypes.

Permalink

<https://escholarship.org/uc/item/3f67d94m>

Journal

Molecular & general genetics : MGG, 263(2)

ISSN

0026-8925

Authors

Vágújfalvi, A
Crosatti, C
Galiba, G
et al.

Publication Date

2000-03-01

DOI

10.1007/s004380051160

Peer reviewed

ORIGINAL PAPER

A. Vágújfalvi · C. Crosatti · G. Galiba · J. Dubcovsky
L. Cattivelli

Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the *cor14b* gene in frost-tolerant and frost-sensitive genotypes

Received: 20 July 1999 / Accepted: 15 November 1999

Abstract Although cold acclimation in cereals involves the expression of many cold-regulated genes, genetic studies have shown that only very few chromosomal regions carry loci that play an important role in frost tolerance. To investigate the genetic relationship between frost tolerance and the expression of cold-regulated genes, the expression and regulation of the wheat homolog of the barley cold-regulated gene *cor14b* was studied at various temperatures in frost-sensitive and frost-tolerant wheat genotypes. At 18/15 °C (day/night temperatures) frost-tolerant plants accumulated *cor14b* mRNAs and expressed COR14b proteins, whereas the sensitive plants did not. This result indicates that the threshold temperature for induction of the wheat *cor14b* homolog is higher in frost-resistant plants, and allowed us to use this polymorphism in a mapping approach. Studies made with chromosome substitution lines showed that the polymorphism for the threshold induction temperature of the wheat *cor14b* homolog is controlled by a locus(*i*) located on chromosome 5A of wheat, while the *cor14b* gene was mapped in *Triticum monococcum* on the long arm of chromosome 2A^m. The analysis of single chromosome recombinant lines derived from a cross between Chinese Spring/*Triticum spelta* 5A and Chinese Spring/Cheyenne 5A identified two loci with additive effects that are involved in the genetic

control of *cor14b* mRNA accumulation. The first locus was tightly linked to the marker *psr911*, while the second one was located between the marker *Xpsr2021* and *Frost resistance 1* (*Fr1*).

Key words Wheat · Frost resistance · Cold-regulated gene · Mapping

Introduction

Cold is an important abiotic stress with which plants must cope. Frost-tolerant plants can survive exposure to low temperatures without serious damage. To achieve the full genetic potential of frost tolerance, tolerant plants must be hardened to cold. Under natural conditions, hardening takes place when the temperature gradually decreases to about 0 °C over several weeks. Under controlled environmental conditions temperature slightly above 0 °C and photoperiods of 8–12 h are considered to be optimal for cold hardening (Fowler et al. 1983).

Although frost tolerance in wheat is a polygenic trait, certain chromosomes are known to carry genes that play an important role. Thus, when the 5A chromosome of the frost-sensitive variety Chinese Spring (CS) is replaced by the corresponding chromosome of the frost-resistant variety Cheyenne (CNN), the frost tolerance of CS is greatly increased (Sutka 1981; Veisz and Sutka 1989). Conversely, when the 5A chromosome of CS was replaced by the corresponding chromosome from a highly frost-sensitive *Triticum spelta* (TSP) accession, the frost resistance of the recipient CS decreased (Galiba et al. 1995). Major genes influencing frost tolerance (*Fr1*) and vernalization requirement (*Vrn-A1*) have been mapped on the long arm of chromosome 5A using single-chromosome recombinant lines developed from a cross between the substitution lines CS/CNN 5A and CS/TSP 5A (Galiba et al. 1995). The *Vrn-A1* locus has been found to form a homoeologous series with *Vrn-B1* (formerly *Vrn2*) on chromosome 5B, *Vrn-D1* (formerly

Communicated by H. Saedler

A. Vágújfalvi (✉) · G. Galiba
Agricultural Institute of the Hungarian Academy of Sciences,
2462 Martonvásár, Hungary
E-mail: vagujfal@fsnew.mgki.hu
Tel.: +36-22-569500; Fax: +36-22-460213

L. Cattivelli
Experimental Institute for Cereal Research,
29017 Fiorenzuola d'Arda, Italy

J. Dubcovsky
Department of Agronomy and Range Science,
University of California, Davis, CA 95616, USA

Vrn3) on chromosome 5D (Snape et al. 1997) and *Vrn-A^m1* on the long arm of chromosome 5A^mL in the diploid wheat *Triticum monococcum* (Dubcovsky et al. 1998).

Messenger RNAs corresponding to the cold-regulated gene *cor14b* (formerly *pt59*) accumulate in barley leaves when plants are exposed to low temperatures. The expression of *cor14b* is strictly regulated by cold (Cattivelli and Bartels 1990), although it is enhanced by light-dependent factor(s) (Crosatti et al. 1995, 1999). A polyclonal antibody raised against the COR14b protein expressed in vitro cross-reacts with two barley polypeptides with slightly different relative molecular weights, referred to as COR14a and COR14b, both of which are cold-regulated and imported into the chloroplasts (Crosatti et al. 1996). Further studies have demonstrated that the *cor14b* gene codes for the COR14b protein, while COR14a represents an immunologically related polypeptide encoded by a homologous gene (Crosatti et al. 1999). Several lines of evidence suggest a relationship between the accumulation of the COR14 proteins and frost resistance. In particular, it has been demonstrated in barley that the threshold temperature for induction of COR14a is lower in frost-sensitive than in frost-tolerant cultivars (Crosatti et al. 1995, 1996), and, when evaluated under field conditions, winter barley accumulated more COR14a and COR14b than spring varieties (Giorni et al. 1999).

A gene homologous to *cor14b* is expressed in response to low temperature in other monocots including wheat (Cattivelli and Bartels 1990). In this work we report on the regulation of the wheat *cor14b* homolog, the localization of the *cor14b* gene, and the approximate map positions of two genes that regulate the temperature-dependent expression of wheat *cor14b*.

Materials and methods

Plant material

Four frost-resistant wheat (*Triticum aestivum*) genotypes (Cheyenne, Mironovskaya 808, Albidum 11 and Ulyanovka), three frost-sensitive wheat genotypes (Chinese Spring, Saratovskaya 29 and Sakha 8), and a *Triticum spelta* accession were tested. Two chromosome substitution lines (Chinese Spring/Cheyenne 5A and Chinese Spring/*Triticum spelta* 5A), as well as nine single-chromosome recombinant lines, developed from the cross between Chinese Spring/*Triticum spelta* 5A and Chinese Spring/Cheyenne 5A (Galiba et al. 1995), were used to investigate the regulation of the wheat *cor14b* homolog. The *cor14b* gene was mapped using an F₂ population obtained from a cross between a cultivated *T. monococcum* (DV92) and a wild *T. monococcum* ssp. *aegilopoides* (G3116) (Dubcovsky et al. 1996). *T. monococcum* was selected for mapping instead of *T. aestivum* because of its higher level of polymorphism compared to bread wheat.

Plant growth and development

Seeds were germinated on moist filter paper at room temperature for 2 days. The young seedlings were transferred into perforated

plastic boxes containing modified Hoagland solution (Nagy and Galiba 1995). The seedlings were grown in half-strength nutrient solution in a plant growth chamber (Convion, Ontario, Canada). Plants were grown for 2 weeks with 16 h light (260 µmol/s m²) at 18 °C and 8 h dark at 13 °C, and subsequently cold hardened at 2 °C on a 16 h light (260 µmol/s m²)/8 h dark regime. Alternatively, plants were grown with 16 h light (as above) at 25 °C and 8 h dark at 20 °C for 6 days. For the kinetic analysis of the induction of COR14 protein, plants were hardened for 1, 2, 3, and 4 weeks. Treatment with abscisic acid (ABA) was carried out at 18 °C/13 °C, using 1.5 × 10⁻⁴ M ABA. Osmotic stress was induced in plants grown at 18 °C/13 °C by treatment with 21% (w/v) PEG (MW 3350).

Protein extraction and Western analysis

Frozen leaves were ground to a fine powder in liquid nitrogen. Proteins were precipitated with 10% (w/v) trichloroacetic acid containing 0.07% (v/v) β-mercaptoethanol, dissolved in acetone, and washed three times with 0.07% (v/v) β-mercaptoethanol dissolved in acetone. Exactly 5 mg of dried powder was solubilized using 280 µl of loading buffer [4% (w/v) SDS, 12% (v/v) glycerol, 50 mM TRIS-HCl pH 6.8, 2% (v/v) β-mercaptoethanol, 0.01% (w/v) bromophenol blue]. The samples were boiled for 2 min, centrifuged for 5 min at 10,000 ×g, and 30 µl aliquots of the supernatant were loaded onto a 10% Tricine-SDS polyacrylamide gel overlaid with a 4% stacking gel according to Schagger and von Jagow (1987). Proteins were electroblotted onto a nitrocellulose membrane (BA83, Schleicher and Schuell) according to Szewczyk and Kozloff (1985) and probed with the polyclonal COR14 antibody (Crosatti et al. 1995). The Amersham ECL plus kit was used for detection after Crosatti et al. (1999). Besides the COR14 proteins, the COR14 antibody recognizes an additional polypeptide of about 29 kDa, whose expression was not affected by cold. This anonymous protein was used as a loading control as described by Crosatti et al. (1999).

Northern analysis and RFLP mapping

Frozen shoots were ground to a fine powder in liquid nitrogen, and re-suspended in 0.05 M TRIS buffer (pH 9.00) containing 0.1 M NaCl, 0.01 M EDTA, and 2% (w/v) SDS. Homogenates were extracted three times with phenol-chloroform and the poly(A)⁺ RNAs were isolated on oligodT-cellulose columns. Equal amounts of the isolated poly(A)⁺ RNAs were fractionated by electrophoresis on formaldehyde-agarose gels and then blotted to nylon membranes (Bio-Rad Zeta-probe). A cDNA fragment corresponding to the whole *cor14b* gene (Cattivelli and Bartels 1990; Accession No. M60732) were radioactively labelled with [α-³²P]dCTP by the oligo-labelling method of Feinberg and Vogelstein (1984) and used for hybridisation. Filters were hybridised in a solution containing 6× SSC, 5× Denhardt's solution, 10% SDS and 100 µg/ml salmon sperm DNA (Sambrook et al. 1989). Hybridization was carried out overnight at 65 °C. Filters were washed three times at 65 °C in 2× SSC, 0.1% SDS, and exposed to Kodak Scientific films at -70 °C. To control for the integrity and the amount of poly(A)⁺ RNA loaded in each lane, the filter were hybridized with α-³²P-labeled oligo dT (20mer, Capel et al. 1997). Hybridization signals were evaluated by densitometric scanning of the films and plotted with Bio-Rad Molecular Analyst software (ver. 1.5).

The *cor14b* cDNA clone was used as a probe to screen for polymorphism between the parental lines of the *T. monococcum* mapping population. An RFLP polymorphism was detected with the restriction enzyme *Xba*I and analyzed in 74 F₂ individuals. The locus was incorporated in the most likely location on existing maps (Dubcovsky et al. 1996) using the TRY command of the computer program Mapmaker/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992).

Results

Threshold induction temperature for accumulation of *cor14b* mRNA

The accumulation of *cor14b* mRNAs is temperature dependent, although a strong genotype $\times$ temperature interaction was observed. When four highly frost-resistant and four frost-sensitive wheat genotypes were grown at 18/13 °C, all the frost-resistant cultivars accumulated *cor14b*-homologous mRNAs to some extent. Cheyenne and Mironovskaya accumulated very similar amounts of *cor14b* mRNAs, while plants of the cultivars Albidum and Ulyanovka grown under identical conditions accumulated, respectively, two and three times more mRNA than Cheyenne (Fig. 1A). Three frost-sensitive genotypes (Chinese Spring, *T. spelta* and Saratovskaya) accumulated *cor14b*-homologous mRNAs at very low levels (detectable only after long exposure) at 18/13 °C; however, the susceptible genotype Sakha 8 accumulated *cor14b* mRNA to the same level as Cheyenne. All genotypes studied showed up-regulation of *cor14b* mRNAs when hardened at 2 °C for 6 days. All the resistant genotypes expressed about the same amount of mRNA, although variations were detected between the sensitive varieties. In particular, the amount of *cor14b*-homologous mRNAs detected in *T. spelta* was 40 to 50% lower (evaluated after normalization on the basis of three independent experiments) than in other samples grown at 2 °C. No gene activation was detected in any of the genotypes when the plants were grown at 25/20 °C. The wheat *cor14b* homolog can therefore be considered a cold-regulated gene, although the threshold temperature for gene activation differs among wheat cultivars.

A Western analysis was carried out with a resistant (Cheyenne) and a susceptible (Chinese Spring) genotype grown at 18/13 °C and then cold hardened at 2 °C for 1, 2, 3 or 4 weeks to detect the accumulation of the COR14b protein (Fig. 2). The expression of *cor14b*

mRNA at 18/13 °C in the resistant genotype is followed by accumulation of the corresponding protein (control samples in Fig. 2). When plants were subsequently hardened at 2 °C, the protein in the resistant cultivar Cheyenne accumulated faster and to a higher level than in the susceptible variety Chinese Spring. No signal was detected in protein samples from wheat plants grown at 25/20 °C (data not shown). Due to the polyclonal origin of the antibody some additional bands are detected; the presence of these polypeptides was found not to be related to cold acclimation, and they can therefore be used as internal standards in the experiment.

Localization of regulatory loci controlling the accumulation of *cor14b*-related mRNAs

The difference in threshold induction temperature observed between Cheyenne and Chinese Spring allowed us to map this polymorphism using CS/CNN substitution lines. The outcome of the previous experiment, as well as published data obtained with barley (Crosatti et al. 1996), suggested a general association between threshold induction temperature polymorphism and frost resistance. Because previous studies have shown that loci on the long arm of the chromosome 5A play a major role in frost resistance (Veisz and Sutka 1989; Galiba et al. 1995), the CS/CNN 5A substitution line was considered. The accumulation of *cor14b*-homologous mRNA was compared in the parental and the substitution lines under various conditions of temperature and stress. When the chromosome 5A from the frost-tolerant cultivar Cheyenne was introduced into the Chinese Spring background, the accumulation of *cor14b* mRNAs at 18/13 °C reached 25–35% of the level found in Cheyenne (Fig. 3). During hardening at 2 °C, Cheyenne, Chinese Spring and CS/CNN 5A plants expressed about the same amounts of the mRNA; while the levels of *cor14b* accumulation in *T. spelta* and CS/TSP 5A at 2 °C were much lower (about 20–30%, and 40–50%

Fig. 1A Accumulation of *cor14b* mRNA in frost-resistant (**R**) and frost-sensitive (**S**) wheat genotypes grown at different temperatures. The resistant genotypes were: R1, Cheyenne; R2, Mironovskaya 808; R3, Albidum 11; R4, Ulyanovka. The sensitive genotypes were: S1, Chinese Spring; S2, *Triticum spelta*; S3, Saratovskaya 29; S4, Sakha 8. **B** The integrity and the amount of poly(A)⁺ RNA loaded in each lane was assessed by hybridization with α -³²P-labeled oligo dT (20mer)

Fig. 2 Accumulation kinetics of the COR14b protein in Cheyenne (frost resistant) and Chinese Spring (frost susceptible). Total protein extracts were isolated from plants grown at 18/13 °C (control) and subsequently cold hardened at 2 °C for 1, 2, 3 or 4 weeks, fractionated by Tricine-SDS-PAGE and probed with an anti-COR14 antibody. Besides the COR14b protein, the antibody cross-reacts with an additional polypeptide of about 29 kDa whose expression was not affected by cold. This anonymous protein served as an internal standard

respectively, as determined on the basis of three independent experiments) than the amount found in Cheyenne under the same conditions.

Plants grown at 18/13 °C were treated with ABA or PEG to evaluate the effects of the hormone and of osmotic stress on the expression of the wheat *cor14b* homolog. Although treated plants showed a severe reduction in growth rate compared with untreated ones, no accumulation of *cor14b* mRNA was detected in any of the tested genotypes. On the contrary, Cheyenne and CS/CNN 5A plants exposed to PEG or ABA showed a level of *cor14b* mRNA expression lower than that found in untreated samples (Fig. 3). These results suggest that the accumulation of *cor14b* mRNAs is dependent on low temperature and not on ABA accumulation or osmotic stress. It is noteworthy that the ability to activate the low-temperature-dependent signal-transduction pathway differs among Cheyenne, Chinese Spring and *T. spelta*, and a large part of the polymorphism for the accumulation of *cor14b* homologous mRNA is controlled by gene(s) on chromosome 5A.

The difference between CS/CNN 5A and CS/TSP 5A prompted us to use the 5A single-chromosome recombinant lines for the mapping of the locus(*i*) controlling the accumulation of *cor14b* homologous mRNA. A selection of nine single-recombinant lines with different allele compositions in the region surrounding the loci *Vrn-A1* and *Fr1* on the long arm of chromosome 5A, plus the corresponding parents, were grown at 18/13 °C and tested for the accumulation of *cor14b* mRNA (Fig. 4). Only three recombinant lines showed a phenotype equivalent to the parental plants. Genotypes 56.1 and 46.1 accumulated as much *cor14b* mRNA as CS/CNN 5A, while the genotype 34.3, like CS/TSP 5A, gave no signal. All the other single-chromosome recombinant lines accumulated *cor14b* mRNAs to a level intermediate between those of the two parents. Quantification of the hybridization signals was carried out on the basis of three independent experiments. The expression of *cor14b* mRNA in the genotypes 56.1 and 58.3 ranged between 90 and 100% of the level found in CS/CNN 5A, while the signals detected in the genotypes 7-3, 32-5, 4-4, 12-4,

Fig. 3A, B Northern analysis of parental and 5A substitution lines grown under different conditions. Treatments with ABA and PEG were carried out as described in Materials and methods. CNN, Cheyenne; CS, Chinese Spring; TSP, *Triticum spelta*. **A** Poly(A)⁺ RNAs were fractionated on a formaldehyde-agarose gel and probed with radioactively labeled *cor14b* cDNA. **B** The integrity and amounts of poly(A)⁺ RNA transferred to the filter were assessed by hybridization with α -³²P-labeled oligo dT (20mer)

Fig. 4A, B Northern analysis of the parental and single-chromosome recombinant lines grown at 18/13 °C. CNN, Cheyenne; CS, Chinese Spring; TSP, *Triticum spelta*. The genomic constitution of the each recombinant line is given in the table, according to Galiba et al. (1995, 1997). The RFLP probes representing TSP and CNN alleles are indicated by T and C, respectively; nd, allele not determined. A two-locus model (*Rcg1* and *Rcg2*, upper case letters indicate the alleles deriving from CS/CNN 5A) describing the regulation of *cor14b* is shown in parentheses (see the text for details). **A** Poly(A)⁺ RNAs were fractionated on agarose gels and probed with radioactively labeled *cor14b* cDNA. **B** The integrity and amounts of poly(A)⁺ RNA loaded on the filter were assessed by hybridization with α -32P-labeled oligo dT

58-3 and 38-6 varied between 15 and 40% of the amount found in the resistant parent.

The data on *cor14b* mRNA accumulation were analyzed on the basis of the molecular map available for the cross between CS/CNN 5A and CS/TSP 5A (Galiba et al. 1995, 1997; Snape et al. 1997) and shown in Fig. 4. Genotypes 46.1 and 56.1, which showed the same level of mRNA accumulation as the frost-resistant parent, shared CS/CNN 5A alleles in the genomic region encompassed by *psrB89* and *Fr1*. The genotype 34.3 – with only TSP alleles between *psrB89* and *Fr1*, did not reveal any signal, like CS/TSP 5A. Genotypes with recombination events in the *psrB89*–*Fr1* region (i.e. the recombinant lines 7.3, 32.5, 4.4, 12.4, 58.3, and 38.6 in Fig. 4) showed an intermediate phenotype. This suggests that two loci (*Regulator for cor* genes, *Rcg*) with additive effect are involved in the genetic control of the polymorphism detected between the two parents. The first locus (*Rcg1*) is tightly linked to the marker *psr911*, while the second one (*Rcg2*) can be located between the marker *Xpsr2021* and the *Fr1* locus. A two-locus model describing the regulation of *cor14b* is shown in Fig. 4. This hypothesis is compatible with the results obtained from all single-chromosome recombinant lines.

The difference in accumulation of *cor14b*-homologous mRNA could be due either to polymorphism in the sequence of the gene itself (i.e. different *cis*-active elements or different mRNA stabilisation signals) or to a regulatory locus (i.e. a *trans*-acting factor controlling gene expression or a protein controlling mRNA stability) (Dunn et al. 1994). In order to distinguish between these two types of hypothesis the wheat *cor14b* homolog was mapped in the *Triticum* A genome. A single *Xba* I RFLP band was detected by Southern analysis using the *cor14b* cDNA as probe; the corresponding locus was localised on the long arm of chromosome 2A^m of *Triticum monococcum* within the 15-cM interval between the RFLP markers *XksuE3* and *Xmwg649*. This interval is approximately 10 cM from the centromere (Dubcovsky et al. 1996). Based on these results, the polymorphism affecting the accumulation of *cor14b*-homologous mRNA can be attributed to different alleles of a regulatory locus(*i*) on chromosome 5A.

Discussion

In barley the expression of the nuclear gene *cor14b* (formerly *pt59*), which codes for a protein localised in

the chloroplast, is controlled by temperature and light through an ABA-independent pathway (Crosatti et al. 1999). Based on Southern analysis, a single *cor14b*-homologous sequence exists in wheat. A single RFLP fragment was detected in the *Triticum* A genome using *cor14b* as probe; moreover, the corresponding wheat gene has recently been cloned and it was found to share 87% sequence homology at the amino acid level with the barley homolog (Tsvetanov et al. 1998).

The data here reported demonstrate that the wheat *cor14b*-homologous mRNAs accumulate in response to low temperature, but not to treatment with exogenous ABA or to osmotic stress induced by PEG. Neither the *cor14b*-homologous mRNA nor the corresponding protein were present at 25/20 °C, while at 18/13 °C a clear polymorphism was detected within the genotypes tested, such that only some of them (mainly the frost-resistant varieties) accumulated *cor14b* mRNA.

The introduction of the 5A chromosome from the frost-tolerant variety Cheyenne into the frost-sensitive Chinese Spring resulted in an increase in the threshold temperature for induction of the wheat *cor14b* gene. On the other hand, when the 5A chromosome of Chinese Spring was replaced by the corresponding one from *T. spelta* – an extremely cold sensitive genotype – no mRNA accumulation was detected at 18/13 °C and lower amounts of *cor14b* mRNA were found at 2 °C (Fig. 3). Since the wheat *cor14b* gene was mapped on the long arm of chromosome 2A^m, these findings indicate that chromosome 5A carries a locus(*i*) involved in the regulation of the *cor14b* gene. Allelic differences at the *cor14b* regulatory locus(*i*) lead to different kinetics of accumulation of the corresponding protein when plants are exposed to cold. The level of this cold-regulated protein increased more slowly in the sensitive genotypes than in the tolerant ones during the course of the hardening process (Fig. 2). By testing the frost tolerance of these genotypes at different times, we found that Cheyenne becomes frost tolerant earlier in the hardening process and reached maximum tolerance after 11 days of cold hardening. The frost-sensitive Chinese Spring started to increase its frost tolerance after 11 days and reached the maximum only around the 40th day of cold hardening (Vágújfalvi et al. 1999). These differences in the kinetics of cold hardening paralleled the accumulation of the protein encoded by the wheat *cor14b* gene.

Several characters related to frost resistance in wheat have been mapped on the long arm of chromosome 5A (Galiba et al. 1994, 1997), together with other stress-related genes (Dubcovsky et al. 1995). Single-chromosome recombinant lines with different allelic compositions in this region were therefore investigated. The results suggest the presence of two regulatory loci (*Rcg*). The *Rcg1* locus was found to be linked to the *psr911* marker and is thus rather distant from *Fr1*, while *Rcg2* was mapped between *Xpsr2021* and *Fr1* (Fig. 4). The genetic linkage between *Rcg2* and *Fr1* could explain the general association between frost resistance and accumulation of *cor* genes/proteins described in wheat

(Houde et al. 1992; Fowler et al. 1996), as well as in barley (Crosatti et al. 1996; Pearce et al. 1996). Nevertheless, recombination could also take place between *Fr1* and one of the two *Rcg* loci. The cultivar Sakha 8 could be considered a possible example of such recombination. This is an Egyptian spring variety (Schmalz and Al Najres 1994), with high salt tolerance (Trivedi et al. 1991), which showed some accumulation of *cor14b* mRNAs when grown at 18/13 °C (Fig. 1A).

The fact that the single-chromosome recombinant genotype 46-1 has both the loci mediating the high-level expression of the *cor14b* gene at 18/13 °C associated with the *T. spelta* allele at the *Fr1* locus (Fig. 4) demonstrates that *Fr1* is not the regulatory gene that controls the expression of *cor14b*. On the other hand, increasing experimental evidence suggests that the expression of *cor* genes is required for the acquisition of frost resistance. Transgenic *Arabidopsis* plants that over-express a transcription factor (*CBF1*) which controls the expression of an array of *cor* genes show enhanced frost tolerance (Jaglo-Ottosen et al. 1998); while a barley albino mutant that cannot acclimate to cold showed a ten-fold reduction in expression of many *cor* genes including *cor14b* (Grossi et al. 1998; Crosatti et al. 1999).

A genetic analysis of cold tolerance in wheat, published by Roberts (1990), suggested that two loci on the chromosome 5A are involved in cold hardening, one of them is tightly linked to *Vrn-A1* (and could therefore correspond to *Fr1*), while the other locus is associated with a gene controlling the length of the leaves of hardened plants. The map position of this latter locus is not known; nevertheless, the results of the present work demonstrate that loci on chromosome 5A, other than *Fr1*, are involved in the control of the molecular basis of cold acclimation. Our previous results showed that the threshold induction temperature of *cor14b* differs between frost-tolerant and frost-sensitive genotypes in barley also (Crosatti et al. 1996). Because of the high degree of synteny among the Triticeae homeologous group 5 in the *Vrn-A1-Fr1* region (Galiba et al. 1995; Dubcovsky et al. 1996), orthologous loci controlling the expression of *cor* genes are likely to be present in all Triticeae genomes.

Acknowledgements This work was supported by grants from the Hungarian National Research Fund (Nos. F023856 and T016972); from the National Committee for Technological Development (Nos. 06518/97.08.15), the National Research Council of Italy (CNR-MTA Bilateral Project), and the Italian Ministry of Agriculture (Project Biotecnologie).

References

- Capel J, Jarillo JA, Salinas J, Martinez-Zapater JM (1997) Two homologous low temperature-inducible genes from *Arabidopsis* encode highly hydrophobic proteins. *Plant Physiol* 115: 569–576
- Cattivelli L, Bartels D (1990) Molecular cloning and characterization of cold-regulated genes in barley. *Plant Physiol* 93: 1504–1510

- Crosatti C, Soncini C, Stanca AM, Cattivelli L (1995) The accumulation of a cold-regulated chloroplastic protein is light-dependent. *Planta* 196: 458–463
- Crosatti C, Nevo E, Stanca AM, Cattivelli L (1996) Genetic analysis of the accumulation of COR14 proteins in wild (*Hordeum spontaneum*) and cultivated (*Hordeum vulgare*) barley. *Theor Appl Genet* 93: 975–981
- Crosatti C, Polverino de Laureto P, Bassi R, Cattivelli L (1999) The interaction between cold and light controls the expression of the cold-regulated barley gene *cor14b* and the accumulation of the corresponding protein. *Plant Physiol* 119: 671–680
- Dubcovsky J, Luo MC, Dvorak J (1995) Linkage relationships among stress-induced genes in wheat. *Theor Appl Genet* 91: 795–801
- Dubcovsky J, Lou MC, Zhong GY, Bransteitter R, Desai A, Kilian A, Kleinhofs A, Dvorak J (1996) Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. *Genetics* 143: 983–999
- Dubcovsky J, Ljajevetzky D, Appendino L, Tranquilli G (1998) Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. *Theor Appl Genet* 97: 968–975
- Dunn MA, Goddard NJ, Zhang L, Pearce RS, Hughes MA (1994) Low-temperature-responsive barley genes have different control mechanisms. *Plant Mol Biol* 24: 879–888
- Feinberg AP, Vogelstein B (1984) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 137: 266–267
- Fowler DB, Limin AE, Gusta LV (1983) Breeding for winter hardiness in wheat. In: Fowler DB, Gusta LV, Sinlard AE, Hobin BA (eds) *New frontiers of winter wheat production*. University of Saskatchewan, Saskatoon, Canada, pp 136–184
- Fowler DB, Chauvin LP, Limin AE, Sarhan F (1996) The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. *Theor Appl Genet* 93: 554–559
- Galiba G, Sutka J, Snape JW, Tuberosa R, Quarrie SA, Sarkadi L, Veisz O (1994) The association of frost tolerance gene *Fr1* with stress induced osmolyte and abscisic acid accumulation in wheat. In: Dorffling K, Bretschneider B, Tantau H, Pithan K (eds) *Crop adaptation to cool climates*. COST 814 Workshop, Hamburg, Germany. European Commission, Brussels, pp 389–401
- Galiba G, Quarrie SA, Sutka J, Morgounov A, Snape JW (1995) RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. *Theor Appl Genet* 90: 1174–1179
- Galiba G, Kerepesi I, Snape JW, Sutka J (1997) Location of a gene regulating cold-induced carbohydrate production on chromosome 5A of wheat. *Theor Appl Genet* 95: 265–270
- Giorni E, Crosatti C, Baldi P, Grossi M, Marè C, Stanca AM, Cattivelli L (1999) Cold-regulated genes expression during winter in frost tolerant and frost susceptible barley cultivars grown under field conditions. *Euphytica* 106: 149–157
- Grossi M, Giorni E, Rizza F, Stanca AM, Cattivelli L (1998) Wild and cultivated barleys show differences in the expression of a cold-regulated gene family under different light and temperature conditions. *Plant Mol Biol* 38: 1061–1069
- Houde M, Dhindsa RS, Sarhan F (1992) A molecular marker to select for freezing tolerance in Gramineae. *Mol Gen Genet* 234: 43–48
- Jaglo-Ottosen KR, Sarah J, Gilmour J, Zarka DG, Schabenberger O, Thomashow MF (1998) *Arabidopsis CBF1* overexpression induces *COR* genes and enhances freezing tolerance. *Science* 280: 104–106
- Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174–181
- Lincoln S, Daly M, Lander E (1992) Mapping genes controlling traits with MAPMAKER/QTL 1.1. Whitehead Institute Press, Cambridge, Mass
- Nagy Z, Galiba G (1995) Drought and salt tolerance are not necessarily linked: a study on wheat varieties differing in drought tolerance under consecutive water and salinity stresses. *J Plant Physiol* 145: 168–174
- Pearce RS, Dunn MA, Rixon J, Harrison P, Hughes MA (1996) Expression of cold-inducible genes and frost hardiness in the crown meristem of young barley (*Hordeum vulgare* L. cv. Igri) plants grown in different environments. *Plant Cell Environ* 19: 275–290
- Roberts DWA (1990) Identification of loci on chromosome 5A of wheat involved in control of cold hardiness, vernalization, leaf length, rosette growth habit, and height of hardened plants. *Genome* 33: 247–259
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual* (2nd edn). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Schagger H, von Jagow G (1987) Tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 166: 368–379
- Schmalz H, Al Najres KM (1994) Investigation into the breeding of high-yielding spring wheats for the region Syria/Egypt through introgression of German winter wheats in a Syrian and two Egyptian spring wheat varieties in Hohenthurm. *Kuhn Archive* 88: 154–164
- Snape JW, Semikhodskii A, Fish L, Sarma RN, Quarrie SA, Galiba G, Sutka J (1997) Mapping frost tolerance loci in wheat and comparative mapping with other cereals. *Acta Agron Hung* 45: 265–270
- Sutka J (1981) Genetic studies of frost resistance in wheat. *Theor Appl Genet* 59: 145–152
- Szewczyk B, Kozloff LM (1985) A method for the efficient blotting of strongly basic proteins from sodium dodecyl sulfate-polyacrylamide gels to nitrocellulose. *Anal Biochem* 148: 403–407
- Trivedi S, Galiba G, Sankhla N, Erdei L (1991) Responses to osmotic and NaCl stress of wheat varieties differing in drought and salt tolerance in callus cultures. *Plant Sci* 73: 227–232
- Tsvetanov S, Etoh K, Tsuda K, Takumi S, Mori N, Atanassov A, Nakamura C (1998) Cloning and characterization of several members of a cold-responsive gene family, *Wcor*, and an alternative oxidase gene, *Waox*, in wheat. In: Slinkard AE (ed) *Proceedings of the Ninth International Wheat Genetics Symposium*, vol 1. University Extension Press, Saskatoon, Saskatchewan, pp 225–228
- Vágújfalvi A, Kerepesi I, Galiba G, Tischner T, Sutka J (1999) Frost hardiness depending carbohydrate changes during cold acclimation in wheat. *Plant Sci* 144: 85–92
- Veisz O, Sutka J (1989) The relationships of hardening period and the expression of frost resistance in chromosome substitution lines of wheat. *Euphytica* 43: 41–45