# International Journal of Botany

ISSN: 1811-9700

science alert

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International Journal of Botany 4 (3): 315-320, 2008
ISSN 1811-9700


# PCR Screening of Barley Genotypes in Relation to Cold Hardiness

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**Abstract:** Several molecular methods were tested to distinguish 24 barley genotypes based on cold hardiness. We used a convenient, efficient and reliable PCR based method for barley to determine the cold tolerant and sensitive genotypes. Cold hardiness was strongly correlated with the existence of cold-induced genes. Significant differences were found based on Field Survival Index (FSI) of genotypes. Based on combination of FSI values and PCR detection pattern of cold-induced genes, PCR analysis can be used in plant breeding programs in order to shorten selection period at environmental stress studies and get exact results.

**Key words:** Barley, cold-induced genes, field survival index, environmental stress

## INTRODUCTION

Cold temperature is one of the most severe environmental stresses, limiting the distribution of native flora and reducing agricultural productivity. Major challenge to plant breeders has been to improve plant tolerance to cold. The primary winter stress is low temperature (Fowler *et al*., 1981). The main limitation of field survival experiments in the determination of the cold-hardiness potential of cultivars is that results are often inconclusive, due either to complete winterkill, or a lack of it (Limin and Fowler, 1991). Furthermore, variation in factors such as soil moisture, soil fertility, disease, ice encasement and smothering can have an indirect effect on survival by limiting the level of cold hardiness that a plant can attain (Limin and Fowler, 1991). To prevent the above limitations it is necessary to carry out repeated experiments in diverse locations. They are, however, rather expensive and the experimental errors made on such occasions are usually large (Fowler and Carles, 1979). The limitations mentioned above have led to a search for rapid and efficient laboratory methods for evaluating cold hardiness.

In many overwintering temperate plant species a period of low positive temperature will increase tolerance to a subsequent subzero (freezing) temperature. This process is known as cold or frost acclimation and is acknowledged to be complex, involving a number of biochemical and physiological changes. Cellular and metabolic changes that occur during cold acclimation include increased levels of sugars, soluble proteins, proline and organic acids as well as the appearance of new isoforms of proteins and altered lipid membrane composition (Hughes and Dunn, 1990). There is an increasing body of evidence to show that many of these biochemical and physiological changes are regulated by low temperature through changes in gene expression and in recent years a number of low temperature-responsive (LTR) genes have been cloned from a range of both dicotyledon and monocotyledon species (Hughes and Dunn, 1996).

It was reported that many genes were induced in cold hardiness barley during cold acclimation (Roger *et al*., 1998). They were involved in different cellular functions and in the contest of cold resistant; some of them may protect chloroplast and cell functions under cold (Dunn *et al*., 1991), while others may be involved in the metabolic adjustment to cold (White *et al*., 1994). Testing of existence of cold-induced genes in barley genotypes would be quick method to identify cold tolerant barley genotypes.

In this study, we looked for some correlation evidences between selection of cold resistant genotypes and gen existences to make that kind of plant breeding programs shorter. For this reason, we describe a comparative study of the existence of the genes induced by cold in 24 genotypes and assessment the relationship between molecular cold hardiness evaluation and field evaluations of barley genotypes. From a plant breeders' point of view, detection of cold-induced genes by PCR can be used in marker-assisted selection in plant breeding program.

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Table 1: Forward and reverse primer sequences, product size and references of 6 cold-induced genes in barley

| Gene | Forward | Reverse | Product size | References |
|---|---|---|---|---|
| Blt 4.9 | GCCATGCTCATCGTAGCTACC | AGAGCAGTCCACGGAAGCA | 287 | White *et al.* (1994) |
| pAF 93 | GAGGATGAGAGGAGCACCCA | TCTTCTCCTCCTCGGGCACT | 504 | Grossi *et al.* (1995) |
| pAO 29 | GGGAGGGAGCTGAACCAGAA | TGAGCAGGGATCCCAGGAA | 166 | Grossi *et al.* (1998) |
| PA 986 | GGATCCAAGTCCGCTCGTCT | AATCATGGGTGAGCAGGGAC | 429 | Cattivelli and Bartels (1990) |
| Cor 14b | ACTGGGTGGTGGCCAACAT | AGAAGACGGCCGTGAGCTCT | 290 | Cattivelli (2000) |
| Blt 14 | ATGGCAAAGAGTCTCGCCG | GCTTCTTGTAGCGCCAGCAC | 263 | Dunn *et al.* (1990) |

## MATERIALS AND METHODS

**Plant material:** The experiments were performed using 24 barley genotypes representing the spectrum from cold tolerance to cold sensitive genotypes from their documented contrasting cold tolerance. Genotypes were Plaisant/RadicalBiooin1093, Star/3/Perga/Sw/Wa1094-67, Icb-100104/3/Mal/Owb753328-5h//11840-76, Ste/Antares//Viringa's' 1667, Ste/Antares//Viringa's' 1670, Ste/Antares//Viringa's' 1676, Ste/Antares//Viringa's' 1678, Viringa's2/Zdm 8307, Xemus/Zdm3485//Plaisant, Debut/3/B67-1623/Ne69293//Robur, Ste/Antares// Viringa's'1666, Wb763126-Vip3/Owb773248//72ab89/ Wa1245-68, Antares/Ky63-1294//Marageh, Narcis, Tokak, Bülbül-89, Tarm-92, Yesevi-93, Orza-96, Ankara Sulu-1, Obruk-86, Anadolu-86, Krusevac, Hudson.

**Field survival index:** Plants were sown in the experimental field of Ataturk University, Faculty of Agriculture at optimal term (between September 1 and October 1), where the mean temperatures were -4.9°C/month in years 2000/01 and -3.2°C/month in years 2000/02 between December and March and the mean of number of days covered by snow of two years was 22.2 day/month between December and March (Anonymous, 2006). Altitude of experimental field is 1850 m. Every genotype was sown in six rows; 6 m in length with rows spaced at 20 cm. Fertilization of N and P was based on soil test recommendations. The experiment was analyzed as a randomized complete block design, with 3 replications. Analyses of variance and LSD test were computed by SAS/PC statistical programs (SAS, 1996). Field Survival Index (FSI) was calculated for each barley genotype by dividing number of germinated plants after 40 days of sowing by number of plants survived in the spring following the winters of 2000/01 and 2001/02. Counting of plants was made in two rows of 50 cm interior of plots for each genotype.

**PCR analysis:** Genomic DNA was extracted from barley genotypes using the extraction procedure of Dellaporta *et al.* (1983). Primers were designed based on cold-induced genes in barley and summarized in Table 1. Cycling parameters were; an initial template denaturation at 94°C for 5 min (1 cycle) followed by 30 cycles of 1) 94°C for 1 min, 2) 55°C for 1 min and 3) 72°C for 2 min. Thirty cycles were followed by a final elongation step at 72°C for 5 min. Following PCR amplification; 10 μL PCR product was loaded into a 1% agarose gel and subjected to electrophoresis for one hour at 90V followed by visualization under UV light.

## RESULTS

**Field survival index:** Barley genotypes were investigated to determine their cold tolerance in field conditions for two years based on their Field Survival Index (FSI) values and results were summarized in Table 2. There were significant differences among genotypes in respect to cold tolerance. Average cold tolerance was 33.06% in the first year, whereas, it was decreased to 28.53% in the second year. None of the genotypes fell into same group based on LSD test (Table 2). In the first year, Tokak, Krusevac and Hudson took first three places in terms of having higher cold tolerance, as they were 53.64, 50.0 and 46.88%, respectively. In addition, Tokak took the first place as 48.16% FSI in the second year, followed by Orza-96 (42.77%) and Hudson (40.06%). On the other hand, Narcis showed the lowest cold tolerance as of 8.06 and 7.12% FSI in the first and second year, respectively. Based on average FSI of two years, genotypes having the highest cold tolerance based on FSI were Tokak (50.9%), Orza-96 (44.72%), Hudson (43.92%) and Krusevac (42.92%). In addition, Narcis showed the lowest cold tolerance (7.59%) in the average of two years based on FSI.

**PCR analysis:** Cold hardiness was strongly correlated with the existence of cold-induced genes. There was significant relationship between FSI of genotypes and existence of cold-induce genes in barley (Table 2). Therefore, the lower existence of cold-induce genes, the lower the cold hardiness of barley. With this finding, barley genotypes can be screened by PCR based on existence of cold-induced genes to determine cold tolerant barley genotypes.

Table 2 shows existence of 6 cold-induced genes in 24 barley genotypes. In this experiment, Cor14b gene was found at most in barley genotypes. Eighteen out of 24

Table 2: Band patterns of 6 cold-induced genes and field survival index (%) in 24 Barley genotypes

| | Band Patterns of 6 cold-induced genes | | | | | | | Field survival index (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Blt 4.9 | pAF 93 | pAO 29 | PA 986 | Cor 14b | Blt14 | Total | 1st year | 2nd year | Mean |
| 1 | + | + | + | + | + | + | 7 | 53.64a[1] | 48.16a | 50.90a |
| 2 | + | + | + | + | + | + | 7 | 46.67d | 42.77b | 44.72b |
| 3 | + | + | + | + | + | + | 7 | 46.88c | 40.96c | 43.92c |
| 4 | + | + | + | + | + | + | 7 | 50.00b | 35.83f | 42.92d |
| 5 | + | + | + | + | + | + | 6 | 43.85f | 40.19d | 42.02e |
| 6 | + | | + | | + | + | 5 | 40.88g | 37.05e | 38.97f |
| 7 | | + | | + | + | + | 5 | 45.68e | 31.09k | 38.39g |
| 8 | | + | | + | + | + | 5 | 39.13i | 35.63g | 37.38h |
| 9 | + | + | | + | + | + | 5 | 39.86h | 34.05h | 36.96i |
| 10 | + | + | | | + | + | 4 | 36.22l | 33.19i | 34.71j |
| 11 | | | + | + | | + | 3 | 36.50m | 33.04j | 34.55k |
| 12 | + | | + | | + | | 3 | 37.95j | 30.31l | 34.13l |
| 13 | | | | + | + | + | 3 | 37.25k | 30.14m | 33.70m |
| 14 | + | | | | + | | 2 | 34.85n | 30.09n | 32.47m |
| 15 | | | | | + | | 2 | 32.05o | 29.38o | 30.72o |
| 16 | | | + | | | + | 2 | 29.13p | 26.17p | 27.65p |
| 17 | | | | + | | + | 2 | 26.89q | 24.65q | 25.77q |
| 18 | | | | + | | + | 2 | 26.77r | 24.54r | 25.66r |
| 19 | + | | | | + | | 2 | 19.23s | 17.25s | 18.24s |
| 20 | | + | | | + | | 2 | 18.59t | 17.04t | 17.82t |
| 21 | | + | | | | | 1 | 15.23v | 13.96u | 14.60u |
| 22 | | | | | + | | 1 | 15.79u | 11.47v | 13.63v |
| 23 | | | | | | + | 1 | 12.82w | 11.01w | 11.92w |
| 24 | | | | | + | | 1 | 8.06x | 7.12x | 7.59x |
| Total | 12 | 11 | 9 | 12 | 18 | 16 | Average | 33.06 | 28.53 | 30.81 |
| | | | | | | | LSD | 0.06938 | 0.006938 | 0.06788 |
| | | | | | | | F | 684637.80** | 760654.90** | 1386964.10** |

+: Bands seen on the gel, [1]: The same letter(s) within the same column are not significantly different at $\alpha = 0.01$

genotypes carry Cor14b gene. Blt14 gene took second place as the existence of cold-induced genes. Sixteen out of 24 genotypes that were formed 263 bp bands for blt14 gene.

Four genotypes, Tokak, Orza-96, Krusevac and Hudson which took first four` places in terms of having the highest cold tolerance are assented by PCR analysis as having all cold-induced genes tested. This indicates that PCR analysis can replace the field test to asses the cold hardiness in barley.

In the present study, the variation between the species from the cold resistance point of view and the genetic basis of this variation were examined and for this purpose PCR technique was used. PCR technique was used to determine presence or existence of the genes which are acting on cold resistance mechanism in the species used in the present study (Dunn *et al*., 1990; White *et al*., 1994; Grossi *et al*., 1995, 1998; Cattivelli, 2000). PCR and DNA analysis of 6 genes (blt 14, blt 4.9, Cor14b, pA986, paf 93 and poA 029) which were inducing the cold resistance, were done in the plant samples which were belonging to 24 genotypes used in this study.

When the comparison was made between the results of field cold resistance study and the results of DNA analysis; it has been noticed that all the studied genes (6 genes) formed bands. From the winter resistance point of view, the 15, 19, 23 and 24 numbered genotypes located in first 4 rows. Because these genotypes formed band, this considered as these genotypes were carrying the gene that we used in the study and these genes are effective on the cold resistance. At the same time, among all the genes only one of them could formed bend in 4 genotypes (5, 8, 11 and 14) and these genotypes located in last rows according to their winter resistance rate.

## DISCUSSION

In the studies concerning the determination of resistant genes in plants and differentiation of resistant and nonresistant plants; DNA analysis gives exact results in a short time (Muramoto *et al*., 1999). Similar to our findings, Choi *et al*. (1999) stated that PCR technique can be used to select cold resistant genotype in barley.

It has been stated that PCR analysis together with specific primers to determine genetic differences in resistant specie selection, is the exact way (Tanno *et al*., 2002). Similarly, Domon *et al*. (2002) said that, PCR studies shortens the selection period in breeding programs and exactly determines the genetic differences between the species.

Cold resistance is a quantitative characteristic and directed by many genes. In recent days too many studies

are being carried on in order to determine these genes, explanation of DNA sequences and determine their functions on cold resistance. Vagujfalvi *et al.* (2000) stated that COR 14b gene is effective on determination of cold resistance. These researchers said that resistant and nonresistant species can be differentiated from each other according to their electrophoretic band design. Other investigators who studied with same gene found similar results in barley plant (Crosatti *et al*., 1996; Giorni *et al*., 1999).

Mastrangelo *et al.* (2000) described the cold controlled proteins as COR proteins and they concluded that the accumulation of these proteins under cold conditions is the part of resistance event. Investigators recommended DNA analysis in order to differentiate cold resistant species and cold sensitive species.

*In vitro* translation analyses of mRNA isolated from crowns of unhardined and hardened plants have been used to establish a potential relationship between the expressions of specific cold-regulated (COR) genes and the freezing tolerance. Under environmentally-controlled conditions it has been documented the occurrence of numerous changes in the populations of translatable mRNAs in crowns of cold acclimated plants. It has been founded out that COR translation products were more highly expressed in the hardy plants than in the cold-sensitive plants. Also, it has been proved that increasing freezing tolerance is associated with an enhancement of COR polypeptides and with appearance of new translatable mRNAs. *In vitro* translation using [$^3$H] glycine as the label revealed that many COR translation products are glycine-rich. High glycine content must have a special relevance to either the confirmation, stability, or function of COR polypeptides. The link between the accumulation of COR gene products and cold tolerance is currently being verified through bidirectional selection for these traits within populations of alfalfa (Laberge *et al*., 1993; Montroy *et al*., 1993; Castonguay and Nadeau, 1998).

Grossi *et al.* (1995) explained DNA sequences of paf 93 gene in barley which we also used in our study. Researchers, by the help of band designs of genes, found relation between gene and cold. Similarly, different researchers (Stanca *et al*., 1996; Giorni *et al*., 1999) also stated that this gene (paf 93) is effective on cold resistance.

Blt 4.9 and blt 14 genes used in this study, are synthesizing the proteins which are acting in cold resistance mechanism in barley (Pearce *et al*., 1996, 1998; Phillips *et al*., 1997). Studies conducted with use of synthetic oligonucleotides and blt 4.9, can be used as a criterion in the differentiation of resistant and non resistant barley plants (Dunn *et al*., 1991).

In view of the fact that changes in membrane lipid composition are known to be important in the acquisition of tolerance to low temperatures (Hughes and Dunn, 1996), the production of new Lipid-Transfer Proteins (LTPs) during frost acclimation in barley, suggests a possible role of these proteins in modifying plasma membranes. Keresztessy and Hughes (1998) used the technique of molecular modeling to compare the ligand binding characteristics of a seedling LTP from the chill-sensitive plant, maize, whose tertiary structure has been resolved by X-ray crystallography, with a low-temperature responsive LTP (blt4.9) from barley shoot meristems which have been acclimated for freezing tolerance. Their analysis shows that potential differences between the ligand binding properties of the two proteins may exist, particularly with respect to di-unsaturated lipids and this finding supports the hypothesis that novel LTPs, which have a role in altering the plasma membrane lipid composition, are produced during frost acclimation.

Election of cold resistant plants by using traditional selection methods in field conditions, is very difficult and takes long time. Because in field, environmental factors can not be controlled and climate changes year by year. So the studies can not be continued in long term. This condition necessitates longer time period and more work force. In this case laboratory techniques that can be used without field conditions and gives fast and exact results are important.

The mechanism acting on cold resistance are controlled by DNA and many genes are also effective. By the determination of presence of these genes in plants; cold resistance potential of plants can be determined. The success of this study will increased by the follow up of the gene expression. In this study, it has been determined that the 6 genes (Blt 49, paf 93, pAO 29, PA 986, Cor 14b and Blt 14) were acting on cold resistance and they are great in number in the winter resistant barley genotypes. This shows these genotypes are carrying the genes that were used in this study and these genes are effective on cold resistance. Furthermore, the genotypes which were located in last rows are the genotypes which forms less number of bands, support this conclusion. PCR analysis can be used in plant breeding programs in order to shorten selection period of environmental stress studies and get exact results.

## REFERENCES

Anonymous, 2006. Web page of Turkish Meteorological Service, Republic of Turkey Ministry of Environment and Forestry, http://www.meteor.gov.tr/2006/english/eng-climateofturkey.aspx.

Castonguay, Y. and P. Nadeau, 1998. Enzymatic control of carbohydrate accumulation in cold-acclimated crowns of alfalfa. Crop Sci., 38 (5): 1183-1189.

Cattivelli, L. and D. Bartels, 1990. Molecular cloning and characterization of cold-regulated genes in barley. Plant Physiol., 93 (4): 1504-1510.

Cattivelli, L., 2000. Plastid development and plastoquinone redox state control the low temperature specific accumulation of COR14b protein in barley. GenBank, GI "10799809".

Choi, D.W., B. Zhu and T.J. Close, 1999. The barley (*Hordeum vulgare* L.) dehydrin multigene family: Sequences, allele types, chromosome assignments and expression characteristics of 11 Dhn genes of cv. Dicktoo. Theor. Applied Genet., 98 (8): 1234-1247.

Crosatti, C., E. Nevo, A.M. Stanca and L. Cattivelli, 1996. Genetic analysis of the accumulation of COR14 proteins in wild (*Hordeum spontaneum*) and cultivated (*Hordeum vulgare*) barley. Theor. Applied Genet., 93 (5-6): 975-981.

Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation: Version 11. Plant Mol. Biol. Rep., 1 (4): 19-21.

Domon, E., M. Fuijita and N. Ishikawa, 2002. The insertion/deletion polymorphisms in the waxy gene of barley genetic resources from East Asia. Theor. Applied Genet., 104 (1): 132-138.

Dunn, A.M., M.A. Hughes, R.S. Pearce and P.L. Jack, 1990. Molecular characterization of a barley gene induced by cold treatment. J. Exp. Bot., 41 (11): 1405-1413.

Dunn, M.A., M.A. Hughes, L. Zhang, R.S. Pearce, A.S. Quigley and P.L. Jack, 1991. Nucleotide sequence and molecular analysis of the low temperature induced cereal gene, BLT4. Mol. Gen. Genet., 229 (3): 389-394.

Fowler, D.B. and R.J. Carles, 1979. Growth, development and cold tolerance of fall-acclimated cereal grains. Crop Sci., 19 (1): 915-922.

Fowler, D.B., L.V. Gusta and N.J. Tyler, 1981. Selection for winter hardiness in wheat, III. Screening methods. Crop Sci., 21 (6): 896-901.

Giorni, E., C. Crosatti, P. Baldi, M. Grossi, C. Marè, A.M. Stanca and L. Cattivelli, 1999. Cold-regulated gene expression during winter in frost tolerant and frost susceptible barley cultivars grown under field conditions. Euphytica, 106 (2): 149-157.

Grossi, M., M. Gulli, A.M. Stanca and L. Cattivelli, 1995. Characterization of two barley genes that respond rapidly to dehydration stress. Plant Sci., 105 (1): 71-80.

Grossi, M., E. Giorni, F. Rizza, A.M. Stanca and L. Cattivelli, 1998. Wild and cultivated barleys show differences in the expression pattern of a cold-regulated gene family under different light and temperature conditions. Plant Mol. Biol., 38 (6): 1061-1069.

Hughes, M.A. and M.A. Dunn, 1990. The effect of temperature on plant growth and development. Biotechnol. Gen. Eng. Rev., 8 (1): 161-188.

Hughes, M.A. and M.A. Dunn, 1996. The molecular biology of plant acclimation to low temperature. J. Exp. Bot., 47 (3): 291-305.

Keresztessy, Z. and M.A. Hughes, 1998. Homology modelling and molecular dynamics aided analysis of ligand complexes demonstrates functional properties of lipid-transfer proteins encoded by the barley low-temperature-inducible gene family, blt4. Plant J., 14 (5): 523-533.

Laberge, S., Y. Costonguay and L.P. Vézina, 1993. New cold and drought-regulated gene from *Medicago sativa*. Plant Physiol., 101 (4): 1411-1412.

Limin, A.E. and D.B. Fowler, 1991. Breeding for cold hardiness in winter wheat-problems, progress and alien gene expression. Field Crops Res., 16 (1): 190-107.

Mastrangelo, M.A., P. Baldi, C. Mare, V. Terzi, G. Galiba, L. Cattivelli and N. Di Fonzo, 2000. The cold dependent accumulation of COR TMC-AP3 in cereals with contrasting, frost tolerance is regulated by different mRNA expression and protein turnover. Plant Sci., 156 (1): 47-54.

Montroy, A.F., F. Sarhan and R.S. Dhindsa, 1993. Cold-induced changes in freezing tolerance, protein phosphorylation and gene expression. Evidence for a role for calcium. Plant Physiol., 102 (1): 1227-1235.

Muramoto, Y., A. Watanabe, T. Nakamura and T. Takabe, 1999. Enhanced expression of a nuclease gene in leaves of barley plants under salt stress. Gene, 234 (2): 315-321.

Pearce, R.S., M.A. Dunn, J.E. Rixon, P. Harrison and M.A. Hughes, 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 (3): 275-290.

Pearce, R.S., C.E. Houlston, K.M. Atherton, J.E. Rixon, P. Harrison, M.A. Hughes and M.A. Dunn, 1998. Localization of expression of three cold-induced genes, blt101, blt4.9 and blt14, in different tissues of The crown and developing leaves of cold-acclimated cultivated barley. Plant Physiol., 117 (3): 787-795.

Phillips, J.R., M.A. Dunn and M.A. Hughes, 1997. mRNA stability and localization of the low-temperature-responsive barley gene family blt14. Plant Mol. Biol., 33 (1): 1013-1023.

Roger, S.P., C.E. Houlston, K.M. Atherton, J.E. Rixon, P. Harrison, M.A. Hughes and A. Dunn, 1998. Localization of expression of three cold-induced genes, blt101, blt4.9 and blt12, in different tissues of the crown and developing leaves of cold-acclimated cultivated barley. Plant Physiol., 117 (1): 787-795.

SAS, 1996. SAS/STAT Software. Changes and Enhancements Through Release 6.11. SAS Institute, Cary, NC.

Stanca, A.M., C. Crosatti, M. Grossi, N.G. Lacerenza, F. Rizza and L. Cattivelli, 1996. Molecular adaptation of barley to cold and drought conditions. Euphytica, 92 (1-2): 215-219.

Tanno, K., S. Taketa, K. Takeda and T. Komatsuda, 2002. A DNA marker closely linked to the vrs1locus (row-type gene) indicates multiple origins of six-rowed cultivated barley (*Hordeum vulgare* L.). Theor. Applied Genet., 104 (1): 54-60.

Vagujfalvi, A., C. Crosatti, G. Galiba, J. Dubcovsky and L. Cattivelli, 2000. Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the cor14b gene in frost tolerant and sensitive genotypes. Mol. Gen. Genet., 263 (2): 194-200.

White, A.J., M.A. Dunn, K. Brown and M.A. Hughes, 1994. Comparative analysis of genomic sequence and expression of a lipid transfer protein gene family in winter barley. J. Exp. Bot., 45 (12): 1885-1892.