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# Semiquantitative RT-PCR Analysis to Assess the Expression Levels of *Wcor14* Transcripts in Winter-Type Wheat

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**Abstract:** The objective of this study was to determine the quantitative expression of COR gene (*Wcor14*) in different cold treatment in Iranian winter-type wheat, using semiquantitative RT-PCR analysis. Semiquantitative RT-PCR analysis showed that *Wcor14* specifically induced by low temperature. The transcripts of *Wcor14* were up-regulated within 3-6 h of cold acclimation at 4°C.

**Key words:** Semiquantitative RT-PCR, cold acclimation, wheat, *Wcor14*

## INTRODUCTION

Cold/freezing temperatures represent a significant abiotic stress limiting geographical distribution of plants and reducing crop quality and productivity. Plants in temperate regions have evolved varying degrees of ability to survive cold/freezing stress. One prominent adaptive mechanism to this stress is known as cold hardening or acclimation (Levitt, 1980), which is triggered by induction of a battery of *Cor* (cold responsive) gene after exposure of plants to low but non freezing temperatures (LT) for certain periods of time (Guy, 1990). The cold acclimation process in plants is primarily regulated through the signal transduction pathways that lead to the induction and enhancement of expression of different sets of cor gene. Cold acclimation requires recognition of low temperatures by cells and a signaling process to activate responsive genes that allow the plant to survive low temperatures. In signaling process ions such as calcium ($Ca^{2+}$) are released into the cell where they initiate the response to low temperatures by affecting the protein phosphorylation in cells (Sangwan *et al*., 2001). External and internal calcium are used by the cell during the cold response and increased availability of calcium enhances cold tolerance (Sangwan *et al*., 2002). Low temperatures will affect the membrane fluidity and cause the membrane to become rigid (Sangwan *et al*., 2002). According to Thomashow (1994, 1998), *Cor* (cold-responsive or cold-regulated) genes are groups of genes which encode extremely hydrophilic and boiling-stable COR proteins. Although, physiological function of these different classes of COR proteins remains largely unknown, some showed a positive correlation with the degree of freezing tolerance which is enhanced by cold acclimation (Monroy *et al*., 1993; Houde *et al*., 1992; Jaglo-Ottosen *et al*., 1998). Difference of the *Cor/LEA* gene expression patterns is related to cultivar difference of the cold/freezing tolerance in wheat (Kobayashi *et al*., 2004). In Arabidopsis, a transcriptional factor ICE1 (Inducer of CBF Expression) is activated by the low temperature signaling pathway and subsequently activates the CBF (C-repeat-Binding Factor) protein family (Chinnusamy *et al*., 2003). Three transcriptional activators, CBF1, CBF2 and CBF3, have been reported (Gilmour *et al*., 2000) to activate cold tolerance genes. *Wcs120* and *Wcor410* are examples of cold-regulated (*Cor*) genes (Danyluk *et al*., 1994; Kane *et al*., 2005) that are activated by the CBF protein family. This family of transcription factors called CRT-Binding Factors (CBFs) or DRE-Binding (DREBs) proteins is involved in the ABA-independent pathway and regulates the *Cor* gene expression through binding to the CRT/DRE cis elements. These transcription factors contain a DNA binding domain found in the Ethylene-responsive Element Binding Protein/APETALA2 (EREBP/AP2) family (Stockinger *et al*., 1997; Liu *et al*., 1998). Furthermore, *Wcs120* and *Wcor14* have C-repeat Binding Factors (CBF) in their promoter regions and are activated by the CBF transcription factors (Crosatti *et al*., 2003; Shen *et al*., 2003; Kobayashi *et al*., 2005). The CBF-mediated cold-response mechanism in Arabidopsis appears to be conserved in both dicotyledonous and monocotyledonous plants including rape, tomato, wheat and rye (Jaglo *et al*., 2001; Hsieh *et al*., 2002). Expression of the other wheat *Cor/Lea* genes including *Wcor14*, *Wcs19*, *Wlt10* and *Wcs120* is also ABA independent (Houde *et al*., 1992; Tsvetanov *et al*., 2000; Ohno *et al*., 2001; Ndong *et al*., 2002).

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The *Wcor14b* protein is located in the chloroplast of cells (Tsvetanov *et al*., 2000) and the amount of protein present in Cheyenne winter wheat increased over the first 28 days of cold acclimation (Vagujfalvi *et al*., 2000). *COR14b* is, like *Arabidopsis thaliana COR15a* (Lin and Thomashow, 1992; Thomashow, 1994), a leaf-specific protein and transported into the stromal compartment of the chloroplasts during cold acclimation (Crosatti *et al*., 1995, 2003).

To understand the molecular basis of cold acclimation and freezing tolerance and cultivar differences of some Iranian common wheat, we monitored the developmental time course of freezing tolerance and the expression profiles of recently isolated wheat *Wcor14* genes. The study was performed during the cold acclimation period extending for up to 30 days and using some Iranian wheat cultivars with distinct levels of freezing tolerance.

## MATERIALS AND METHODS

**Plant materials:** This research was conducted in spring of 2008 in Institute of Biotechnology, University of Shiraz. Three cultivars of Iranian common wheat (*Triticum aestivum* L.), winter-type Azare2, Sardari and Alamoot were used. Azare2 was reported as one of the hardiest cultivars among tetraploid and hexaploid common wheat tested for cold tolerance. Seeds from each of the cultivars were planted as separate groups in the same pots (20×12 cm in width and 12 cm in depth) with soil and incubated in a growth chamber under the following standard temperature and light conditions; 24±0.5°C with a 14 h photoperiod at a light intensity of 100-110 μm photons photons/$m^2$/sec provided by cool white fluorescent lamps. Twenty Three day old seedlings were cold-acclimated at 4±0.5°C for different periods (3, 6, 24 h, 4 and 35 days) under the standard light condition at intensity of 100-110 μm photons/$m^2$/sec provided by cool white fluorescent lumps with a 14 h photoperiod.

**Total RNA extraction and cDNA synthesis:** Total RNA was isolated from 100 mg of leaves with RNX-Plus™ kit according to the manufacturer's specifications. The concentration of total RNA was determined with NanoDrop by measuring absorbance at a wavelength of 260 nm (A260) and it's purity was assessed by the ratio of the absorbance values at 260 and 280 nm, wherein a ratio of about 2.0 was considered a good indication of purity. One microgram total RNA, 0.5 μg oligo dT18 primer were added to a microtube and incubated for 5 min at 70°C, cooled on ice. dNTP (1 mM), Tris buffer (10 mM), RNAse inhibitor (40 u/20 μL) were added and 5 min incubated at 37°C for 5 min. Finally, 200 u M-MuLV enzyme was added to cocktail and incubated for 60 min at 37°C. Then incubated for 10 min at 70°C in order to inactivate the enzyme. Synthesized cDNA stored in -20°C (Sambrook and Russell, 2001).

**Semi-quantitative PCR:** As a result, Northern blot methods usually require additional time consuming selective chromatography to isolate poly A mRNA and radioactive labeled probes to generate a measurable signal to quantify. Therefore, in order to reduce labor, a semi-quantitative RT-PCR method was developed. RT-PCR (Real Time-Polymerase Chain Reaction) is the most sensitive method for detecting gene transcription products; however, it can be highly variable and may not accurately reflect gene activity (Bustin, 2002). Relative semi-quantitative PCR was performed to measure gene expression of *Wcor14*. Each PCR reaction was performed in a total volume of 25 μL containing, Tris buffer (10 mM, pH 8), $MgCl_2$ (2 mM), dNTP mix (0.8 mM, each dATP, dTTP, dCTP, dGTP 0.2 mM), Taq DNA polymerase (1 u), cDNA (2 μL), sense primer; 5'-CTGCCTGC AAACCCCTCCTA -3′ and antisense primer 5'-CCTCCT CCGTCGCCTGCTTCGCCT-3' (each 0.5 μM), product size 215 bp. To insure that no false positive PCR fragments would be generated from pseudogenes in contaminating genomic DNA; primer sequences were designed to span intron regions. An initial denaturation step of 5 min at 94°C was followed by 30 cycles of: 45 sec 94°C, 45 sec 68°C, 2.3 min 72°C and end step 5 min 72°C. β-tubulin was used as the reference gene for *Wcor14* (Fig. 1).

**Gel electrophoresis:** The PCR products were loaded onto Ethidium bromide stained 1% agarose gels in TBE buffer.

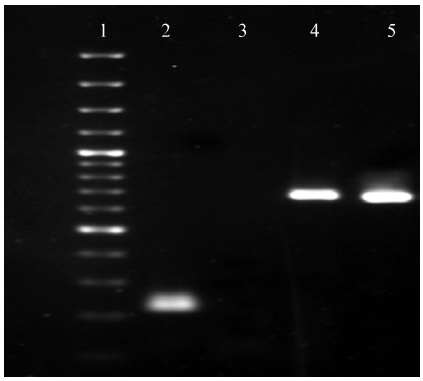


Fig. 1: Semiquantitative RT-PCR of *Wcor14* mRNA from acclimated plant (lane 2) and control plant (lane 3) and β-tubulin as the reference gene (lane 4, 5). Lane 1 is 100 bp molecular weight marker

Images of the RT-PCR Ethidium bromide stained agarose gel was acquired with a Cohu High Performance CCD camera (Cohu Inc. San Diego, CA) and quantification of amplified products was performed by Total lab software. The intensities of the *Wcor14* mRNA bands were normalized relative to that of β-tubulin bands by dividing the former by the β-tubulin specific PCR product densities. β-tubulin acted as a control for sample to sample variation in reverse transcription and PCR conditions and the extent of degradation and recovery of RNA.

**Protein isolation and analysis:** Protein extracts were prepared from 4 day cold-acclimated plant by homogenizing 500 mg of leaves in 1 mL of extract buffer (Tris-HCl 0.05 M, pH 8, 0.02% SDS, 30.3% urea and 1% 2-mercaptoethanol) was added to each micro tube and centrifuged at 13000 rpm for 10 min at 4°C. The supernatant contains dissolved extracted protein ready for experiment purposes. The protein concentration was determined by NanoDrop and 100 ng protein was used for the experiment. SDS-PAGE (Sodium Dodecy Sulphate-Polyacryloconide Gel Electrophorsis) gel preparation, running and staining were as standard procedures (Sambrook and Russell, 2001).

## RESULTS AND DISCUSSION

A differential display reverse transcriptase-PCR experiment was initially carried out to compare the mRNA profile of plants grown at 24°C with those of plants exposed at 4°C. The previous studies showed a much higher freezing tolerance of winter-hardy common wheat (*Triticum aestivum* L.) cv. Mironovska 808 compared with that of a spring-type common wheat cv. Chinese Spring by the simple one-point assay (Kume *et al*., 2005). It has been demonstrated that such cultivar difference of freezing tolerance could be at least partly controlled by the differential accumulation levels of *Cor/LEA* transcripts during cold acclimation (Vagujfalvi *et al*., 2000; Kobayashi *et al*., 2004). We studied *Wcor14* transcript accumulation under the low temperature condition by Semi-quantitative PCR using specific primer of *Wcor14*. We compared the expression profiles of three Iranian cultivars *Wcor14* genes and found that they were also induced early and at high levels in Azar2 and Sardari. Transcripts of *Wcor14* rapidly accumulated within 3-6 h after cold acclimation at 4°C. The expression patterns clearly showed a rapid response to LT in Azar2 and Sardari than Alamoot. There was a sharp increase in *Wcor14* transcripts in the leaves of all three genotypes after four days of LT acclimation (Fig. 2a, b). In the non-

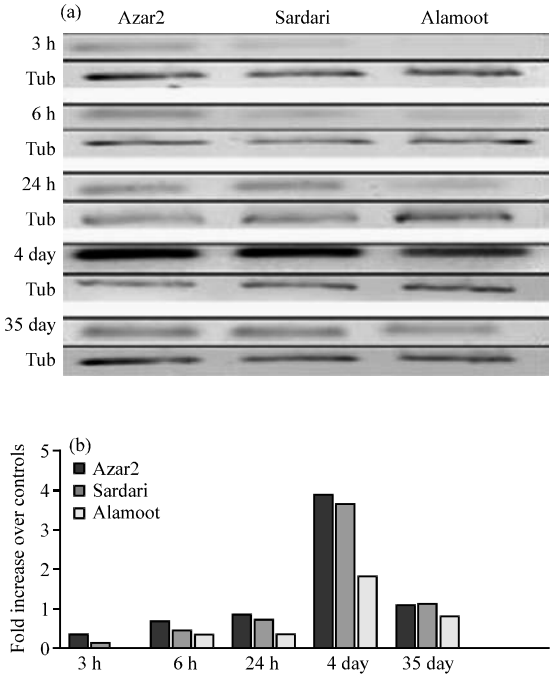


Fig. 2: (a) Transient enhancement of the *Wcor14* gene expression by cold stress. Total RNA was isolated from seedling leaves of Azar2, Alamoot and Sardari at the indicated time points. Semi-quantitative RT-PCR analysis was conducted to detect the *Wcor14* gene transcript. The β-tubulin gene was used as a control and (b) Quantification of the *Wcor14* transcripts in different time. Using the internal standard β-tubulin RT-PCR signal as the denominator as described earlier

acclimated control plants, no *Wcor14* transcripts were detected. To examine the effect of long-term acclimation on *Wcor14* transcript accumulation, 23 day old seedlings were placed under the LT condition and kept for 35 days. The amount of *Wcor14* transcript showed a gradual decrease under the long-term acclimation condition The amount of the major transcript reached at maximum at day 4 and thereafter leveled down under the long-term acclimation condition (Fig. 3). These observations are in contrast to the absence of *Wcor14* down-regulation under the long-term acclimation condition in previous study. Regulatory loci at the Vrn-A1/Fr1 loci on chromosome 5 A have been shown to control expression of *Wcor14* (Vagujfalvi *et al*., 2000; Kobayashi *et al*., 2005), more specifically regions associated with cbf-lik sequences (Crosatti *et al*., 2003; Kobayashi *et al*., 2005). Furthermore, Kume *et al*. (2005) demonstrated that a cbf homologue, *Wcbf2*, when activated by LT led to the induction and increase in accumulation of *Cor/Lea* genes such as

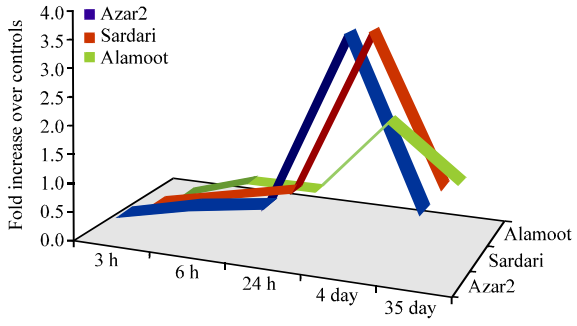


Fig. 3: The amplification curve for *Wcor14* for three genotype during the LT condition

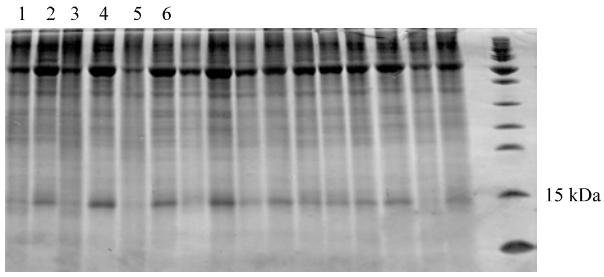


Fig. 4: Electrophoresis profiles of soluble proteins in three genotypes (Azar2, Alamoot and Sardari). Lane 1, 3, 5 profiles of Azare2, Alamoot and Sardari non-acclimated control plant, respectively. Lane 2, 4, 6 profiles of Azare2, Alamoot and Sardari 4 days cold-acclimated plant, respectively

*Wcor14* and *Wcor15*. Thus, the discrepancy in expression between Azare2, Sardari and Alamoot could be due to the influence of the Vrn-A1 locus.

*Wcor14* encodes a chloroplast-targeted polypeptide of 14 kDa with a putative N- terminal transit peptide. Interestingly, the N-terminal 51 amino acid residues were absent in COR14a purified from the chloroplast fraction. This sequence was thus suggested to serve as a chloroplast transit peptide (Crosatti *et al*., 1995). The electrophoresis profiles of soluble proteins in three genotypes (Azar2, Alamoot and Sardari) of contrasting freezing tolerance are shown in Fig. 4. Comparison of profiles of proteins extracted from 4 days acclimated plants indicates that polypeptides of 14 kDa were the major proteins that accumulated during LT condition. The 14 kDa band was present at a very high intensity in the acclimated plants than control plants. Dörffling *et al*. (1998) reported the existence of a close relationship between cold-induced Pro accumulation and cold adaptation in wheat. Differential accumulation of cold-regulated proteins has been reported for members of the Poacea differing in freezing tolerance (Houde *et al*., 1992; Ouellet *et al*., 1993). Immunoblot analysis of the *Wcor14* proteins with using polyclonal antibodies showed that accumulation of *Wcor14* protein increases severely during cold acclimation condition (Kume *et al*., 2005).

One of the important conclusions to emerge from recent studies is that cold acclimation includes the expression of certain cold-induced genes encoding proteins involved in membrane stabilization during freezing stress. Based on the present results, we conclude that the expression of wheat *Wcor14* is low temperature specific. Further, its turnover appeared to be rapid and the expression remains at a high steady-state level during the cold acclimation period present results strongly suggest that cold acclimation induced protein accumulation plays a determinant role in freezing tolerance of these genotype. However, subsequent studies have suggested that LT tolerance is affected by other interacting regulatory circuitries (Chinnusamy *et al*., 2007), such as low temperature, genetic potential and the factors determining developmental stage, such as vernalization and photoperiod (Mahfoozi *et al*., 2001).These observations emphasize the need for additional research aimed at achieving a more complete understanding of the genetic cascade that determines LT tolerance in wheat. They also identify the need to pay close attention to experimental conditions and genotype and tissue selection in experiments designed to identify the critical genetic components of the highly integrated systems for LT adaptation that are regulated by environmentally-induced complex pathways (Fowler and Limin, 2007).

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## REFERENCES

Bustin, S.A., 2002. Invited review: Quanitification of mRNA using real-time reverese transcription PCR (RT-PCR): Trends and problems. J. Mol. Endocrinol., 29: 23-39.

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

Chinnusamy, V., J. Zhu and J.K. Zhu, 2007. Cold stress regulation of gene expression in plants. Trends Plant Sci., 12: 444-451.

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

Crosatti, C., C. Mare, E. Mazzucotelli, S. Selioni and S. Barilli *et al*., 2003. Genetic analysis of the expression of the cold-regulated gene cor14b: A way toward the identi?cation of components of the cold response signal transduction in Triticeae. Can. J. Bot., 81: 1162-1167.

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

Dörffling, K., M. Abromeit, U. Bradersen, H. Dörffling and G. Melz, 1998. Involvement of Abscisic Acid and Proline in Cold Acclimation of Winter Wheat. In: Plant Cold Hardinesss, Li and Chen (Eds.). Pergamon Press, New York, pp: 283-292.

Fowler, D.B. and A.E. Limin, 2007. Progress in Breeding Wheat with Tolerance to Low Temperature in Different Phenological Developmental Stages. In: Wheat Production in Stressed Environments, Buck, H.T., J.E. Nisi and N. Salomon (Eds.). Vol. 17, Springer, Dordrecht, The Netherlands, pp: 301-314.

Gilmour, S.J., A.M. Sebolt, M.P. Salazar, J.D. Everard and M.F. Thomashow, 2000. Over expression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiol., 124: 1854-1865.

Guy, L., 1990. Cold acclimation and freezing stress tolerance: Role of protein metabolism. Annu. Rev. Plant Physiol., 41: 187-223.

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

Hsieh, T.H., L.T. Lee, P.T. Yang, L.H. Chiu, Y.Y. Charng, Y.C. Wang and M.T. Chan, 2002. Heterology expression of the Arabidopsis C-repeat/dehydration response element binding factor 1 gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. Plant Physiol., 129: 1086-1094.

Jaglo, K.R., S. Kleff, K.L. Amundsen, X. Zhang and V. Haake *et al*., 2001. Components of the Arabidopsis C-repeat/dehydration responsive element binding factor cold-responsive pathway are conserved in *Brassica napus* and other plant species. Plant Physiol., 127: 910-917.

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

Kane, N.A., J. Danyluk, G. Tardif, F. Ouellet and J.F. Laliberte *et al*., 2005. TaVRT-2, a member of the StMADS-11 clade of flowering repressors, is regulated by vernalization and photoperiod in wheat. Plant Physiol., 138: 2354-2363.

Kobayashi, F., S. Takumi, M. Nakata, R. Ohno, T. Nakamura and C. Nakamura, 2004. 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.

Kobayashi, F., S. Takumi, S. Kume, M. Ishibashi, R. Ohno, K. Murai and C. Nakamura, 2005. 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.

Kume, S., F. Kobayashi, M. Ishibashi, R. Ohno, C. Nakamura and S. Takumi, 2005. 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.

Levitt, J., 1980. Responses of Plants to Environmental Stresses. 2nd Edn., Vol. 1, Academic Press, New York, pp: 166-222.

Lin, C. and M.F. Thomashow, 1992. DNA sequence analysis of a complementary DNA for cold-regulated Arabidopsis gene cor15 and characterization of the COR15 polypeptide. Plant Physiol., 99: 519-525.

Liu, Q., Y. Sakuma, M. Kasuga, H. Abe and M. Kasuga *et al*., 1998. Two transcription factors, DREB1 an DREB2, with an EREBP/AP2 DNA binding domain, separate two cellular signal transduction pathways in drought and low temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell, 10: 1391-1406.

Mahfoozi, S., A.E. Limin and D.B. Fowler, 2001. Influence of vernalization and photoperiod responses on cold hardiness in winter cereals. Crop Sci., 41: 1006-1011.

Monroy, A.F., Y. Castonguay, S. Laberge, F. Sarhan, L.P. Vezina and R.S. Dhindsa, 1993. A new cold-induced alfalfa gene is associated with enhanced hardening at subzero temperature. Plant Physiol., 102: 873-879.

Ndong, C., J. Danyluk, K.E. Wilson, T. Pocock, N.P.A. Huner and F. Sarhan, 2002. Cold-regulated cereal chloroplastlate embryogenesis abundanlike proteins. Molecular characterization and functional analysis. Plant Physiol., 129: 1368-1381.

Ohno, R., S. Takumi and C. Nakamura, 2001. Expression of a cold-responsive Lt-Cor gene and development of freezing tolerance during cold acclimation in wheat (*Triticum aestivum* L.). J. Exp. Bot., 52: 2367-2374.

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

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning a Laboratory Manual. 3rd Edn., Cold Spring Harbor Laboratory Press, New York, ISBN: 978-087969577-4.

Sangwan, V., I. Foulds, J. Singh and R.S. Dhindsa, 2001. Cold-activation of *Brassica napus* BN115 promoter is mediated by structural changes in membranes and cytoskeleton and requires $Ca^{2+}$ influx. Plant J., 27: 1-12.

Sangwan, V., B.L. Orvar and R.S. Dhindsa, 2002. Early Events During Low Temperature Signaling. In: Plant Cold Hardiness, Li and Palva (Eds.). Kluwer Academic/Plenum Publishers, USA., pp: 43-53.

Shen, Y.G., W.K. Zhang, S.J. He, J.S. Zhang, Q. Liu and S.Y. Chen, 2003. An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration and ABA stress. Theor. Applied Geneti., 106: 923-930.

Stockinger, E.J., S.J. Gilmour and M.F. Thomashow, 1997. *Arabidopsis thaliana* CBF1 encodes an AP2 domain containing transcription activator that binds to the Crepeat/DRE, a cis acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc. Natl. Acad. Sci. USA., 94: 1035-1040.

Thomashow, M.F., 1994. *Arabidopsis thaliana* as a Model for Studying Mechanisms of Plant Cold Tolerance. In: Arabidopsis, Meyerowitz, E. and C. Somerville (Eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY., pp: 807-834.

Thomashow, M.F., 1998. Role of cold-responsive genes in plant freezing tolerance. Plant Physiol., 118: 1-8.

Tsvetanov, S., R. Ohno, K. Tsuda, S. Takumi, N. Mori, A. Atanassov and C. Nakamura, 2000. A cold-responsive wheat (*Triticum aestivum* L.) gene Wcor14 identified in a winter-hardy cultivar Mironovska 808. Genes Genet. Syst., 75: 49-57.

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: 194-200.