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COLD STRESS-INDUCED REGULATION OF DIFFERENTIALLY EXPRESSED GENES IN BARLEY (*HORDEUM VULGARE*L.) LEAVES

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ABSTRACT

Cold stress adversely affects the growth and development of plant. To identify cold stress-induced genes in barley leaves an annealing control primer (ACP)-based approach was applied. In this study, we identified cold induced-14 genes which were differentially expressed genes (DEG) in barley leaves. Total ten genes were up-regulated such as alcohol dehydrogenase gene (*Adh2*), cryptochrome 2 (*Cry2*) gene, SnRK1-type protein kinase(*SnRK1*),CHS gene for chalcone synthase (*CHS*), glyceraldehyde-3-phosphate dehydrogenase mRNA (*GAPDH*), dehydrin 9 (*dhn9*) gene, LHC II type I protein(*LHCII*), 17 kDa class I small heat shock protein (*HSP17*), and myo-inositol 1-phosphate synthase mRNA (MIPS). Two genes, alpha-tubulin 4 (*atub4*), metallothioneine type2 (*mt2b*) were down-regulated. Interestingly, cold-regulated another two homologues LHC IItype I protein (*LHC II*), and clone cortma-*ap3*were newly induced under cold stress. The identified genes are mostly involved in several biological functions including plant growth, photosynthesis, glycolysis, abiotic stress tolerance, and stress homeostasis in plants. The identified candidate genes would be suitable target for molecular breeding toward enhanced cold stress tolerance in plants.

Key words: Cold stress, differentially expressed genes, Barley, Annealing control primer, Gene Fishing.

INTRODUCTION

Cold stress negatively impacts plant physiology such as photosynthesis, growth, and antioxidant system (Zhou *et al*., 2017). Genetic regulation is occurred in response to cold stress either chilling (<20 °C) or freezing (<0 °C) in plants (Chinnusamy *et al*., 2007). Metabolic reactions get slow down, also inhibited by cold stress that leads to induce osmotic stress in plants. As a consequence, plants have developed some molecular and physiological mechanisms that enhance low temperature acclimation in plants. For understanding the mechanism of cold acclimation, various studies have performed on freezing tolerance in *Arabidopsis* that considerably helped to understand the mechanisms are associated with cold acclimatization (Wanner *et al*., 1999). A series of molecular and metabolically changes have been occurred in response in low temperature and its acclimation in plants (Thomashow, 1990; Viswanathan and Zhu, 2002).

Different morphological and physiological alterations in plants help to adapt in cold stress. Plants exploit different methods for survival in cold stress condition. Some plants are reported to be induced antifreeze protein (Guy *et al.,* 1985), along with involved in the alterations of lipids and carbohydrate compositions that support to enhance cold stress tolerance in plants (Guy, 1990). Extensive studies have been conducted in model plant *Arabidopsis* along with other plant species in response to cold stress (Wanner *et al*., 1999; Chinnusamy *et al*., 2007; Yadav, 2010; Zhou *et al*., 2017; Lee *et al*., 2018a).

Gene identification is a fundamental approach, by which response of genes with specific mechanisms, and functions are easily clarified. Modern genomic tools support to understand the global gene expression in an organism. Several molecular tools such as suppression subtractive hybridization (SSH; Zhang *et al*., 2002), serial analysis of gene expression (SAGE; Lee and Lee, 2003), microarray approach (Galbraith and Edwards, 2010) are used for genomic based profiling and crop improvement. Recently, annealing control primer (ACP)-based Gene Fishing™ an efferent and simple approach is being used for gene expression analysis in plants under several abiotic stresses (Lee *et al*., 2018b; Rahman *et al*., 2017). In this molecular approach only few amount of RNA are needed by which multiple stress responsive candidates are easily identified. The objective of this study was to identify cold-induced genes in barley leaves, consequently to clarify some new candidates with specific molecular function that would be useful for enhancing cold tolerance in plants.

MATERIALS AND METHODS

Growing of barly and cold treatment: Barley (*Hordeumvulgare* L.) was considered as experimental plant material. Plant seeds were surface sterilized using 70%ethanol for 2 min followed by 30% sodium

hypochlorite (NaClO) solution and washed three times using deionized water. Seeds were placed to tissue paper for 3-5 min for air drying then moved to horticulture soil containing germination pots (13 cm in diameter).Plants were raised for 2 weeks in a controlled growth chamber at 25°C,with a photoperiod 16/8 h light/dark with intensity of 350 µmol m^{-2} s^{-1} irradiance, and humidity was maintained at 60-70%.Two-week-old seedlings were exposed to cold (4°C)stress for 48 h. Another set of plants were kept at normal temperature (25 °C) was considered as control. After the termination of cold stress leaves samples were harvested separately and store quickly at -80°C for further molecular experiments.

RNA isolation, preparation, and cDNAs synthesis: Total RNA was extracted from the cold -treated and control leaf samples using RNA isolation kit (Qiagen, USA) following manufacturer's instruction. RNA quality was checked by staining rRNA bands with EtBr in 1.5% agarose gel. Further, it was maintained the OD ration using spectrophotometer. Subsequently, the RNA was used for reversed-transcriptase (RT) mediated cDNA synthesis (first-strand). The whole process was carried out following the protocol of Lee *et al*. (2009).

cDNA synthesis, and PCR amplification: The process of cDNA synthesis and subsequent amplification were performed using a tube containing Gene Fishing^{TM} DEG premix (See gene, Seoul, South Korea). Briefly, for second-strand cDNA synthesis, low stringency (50°C) was maintained during one cycle of first-stage PCR where the final reaction volume consisted of 49.5 µL. Briefly, 5 µL of diluted cDNA (first stand) was prepared. In order to second-strand synthesis, the following PCR conditions were programmed: i) one cycle incubated at 94°C for 1 min, ii) next step programmed at 50°C for 3 min, ii) final step set at 72°C for 1 min. After termination of second-strand DNA synthesis, 40 cycles of amplifications were performed according to Lee *et al*. (2017). Finally, the separation of amplified PCR product was performed using 100 V gel migration setup containing2% agarose gel with 0.5×TBE buffer. DEG was assessed with corresponding time interval with treated samples.

Cloning and sequencing of cDNA: Selected expressed PCR bands were processed using agel extraction kit (GENCLEAN II, USA). The targeted bands were excised carefully by sharp blade. Lysis buffer were calculated as 6X formula, where X= weight of gel piece as mg basis. The pieces of excised gels were warmed at 45-50°C for 8-10 min, subsequently overtaxes were performed to melt the gel fragments properly. The milted gel segments were moved to DNA-binding column with a collection tube (2 µl), centrifuged at 12000 ×g for 2 min. DNA binding column was rinsed using a washing buffer then centrifuged for 2 min followed by DNA was collected with elution buffer. The eluted cDNAs were ligated into a TOPO TA vector (Invitrogen, USA),and used to transform into TOP10 competent cells following the cloning protocol. The sequencing of cloned were performed using the M13 forward primer and reverse primer according the protocol of Lee *et al*.,2009. All sequences were analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Northern blot: In order to evaluate a temporal expression of a specific gene a Northern blot technique was performed. Briefly, barley plants were exposed to cold stress for 48 h, where leaf samples collectedat0, 6, 12, 24, 36, and 48 h of cold exposures. Total RNA was extracted from the leaf tissue using extraction kit (Qiagen, USA). Briefly, RNA (10 µg) was loaded in each lane and separated by 1.2% agarose gel electrophoresis, subsequently transferred to a nylon membrane. In order to RNA cross linking membrane was exposed to UV-light then fixed membrane was hybridized with label probe. Gene-specific (selected DEG) probes were labeled with [α^{-32P}] dATP according to method described earlier by Lee *et al.*(2018a).

RESULTS AND DISCUSSION

Cold-induced DEGs: In order to identify of cold-responsive DEGs, Gene Fishing PCR technology based on ACP technique was applied where 120 arbitrary primers were used (Figure1). Among the total of 120 Gene Fishing^{TM} primers (GPs), DNA bands were differentially expressed. In this study, we identified 14 DEG in barley leaves under cold stress wherein10 DEG were up-regulated,2 were down –regulated, and two genes were newly induced. DEGs 3, 89, 14, 15, 17, 20, 21, 32 and 39 showed increased intensity of bands in the treated samples as compared to the control sample (Figure 2). In contrast, decreased expressions were observed in case of DEGs 38 and 40, whereas new bands were found to be induced for DEGs 31 and 33(Figure2). These two genes were newly induced in barley leaves under cold stress. One newly induced gene was encoded for LHC II type I protein, and the other was encoded for *clone cortma-ap3* protein that act in a cold regulation in plants. The temporal expressions of all of identified cold-induced genes were further confirmed using a northern blot analysis. In figures 2 and 3, the cold-induced genes were expressed differently. This is not surprising because it may occur due to the variable strength of the signal stimulation on a membrane, and/or differential half-lives of the same isoform during different conditions (Kong *et al*., 2014). In this study, the possible roles of cold-induced 14 DEGs are discussed in following sections.

Cold-induced up-regulated genes: DEG 3 and 21 were identified as alcohol dehydrogenase gene *Adh2* (*Adh2*) (Table 1). In higher plants *Adh2* found to be activated in

abiotic stress conditions in plants (Baisakh *et al.,* 2008). At mRNA level alcohol dehydrogenase (*Adh*) genes respond to drought-stress. In transgenic *Arabidopsis*, over expression of *Adh* gene enhanced abiotic stress tolerance (Sunkar *et al*., 2003). DEG8 was identified as a partial cds of cryptochrome 2 (*Cry* 2) gene (Table 1); it was found to be induced at 36 h and 48h, respectively under cold treatment. This transcript encodes the biosynthesis of cryptochrome. Cryptochromes are present in animals, bacteria and plants and act as photoreceptors for blue-light. They act as DNA photolyases. These enzymes are class of flavoproeins that identified in several plant species. In tomato and *Arabidopsis, Cry1*reported to be involved in photo-morphogenesis (Ahmad and Cashmore, 1993; Ninu *et al.,*1999; Weller *et al.,*2001). DEG9 was screenedasSn-RK1 type protein kinase (Table 1). The central role of this protein kinase Sn-RK1 in energy metabolism, plant growth and stress response (Bechtold and Field, 2018). Moreover, Sn-RK1 was involved in antagonistic signaling pathway in plant where it was activated in low-energy condition under stress exposure.

Chalcone synthase (*CHS*; DEG14) gene up-regulated in response to cold stress in barley leaf. The *CHS* is a core candidate of isoflavonoid biosynthesis pathway, it was reported to be induced under multiple environmental stresses and functioned in plant resistance (Dao *et al*., 2011). However, our result was supported by this study, and the high expression of *CHS* gene suggests that this gene may provide abiotic stress tolerance in barley. DEG15 recognized as glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The *GAPDH* is a key enzyme involved in glycolysis pathway. In wheat, the high expression of *GAPDH* conferred abiotic stress tolerance (Zeng *et al*., 2016). DEG17 was identified as a gene encoding dehydrin 9(*DHD9*; Table1). *DHD9*is a member of the group II embryogenesis abundant (II ELA) proteins, play a vital role in multiple environmental stress tolerance in plants (Liu *et al*., 2017). In this study, *DHD9* highly expressed at mRNA level where the abundance of this candidate increased with increasing the time of treatment (Figure 3). DEG20 was recognized as a gene encoding light-harvesting complexes (LHC II type I protein) (Table 1). LHC II is a membrane protein contributes in plant photosynthesis by converting the energy from sunlight into chemical energy (Barros *et al*., 2009).

DEG-32 was recognized as a gene encoding the small class 1 heat-shock protein (*HSP17 kDa*) in barley leaves under cold stress (Table 1). The role of several *HSPs* and molecular chaperones were well documented in plants under several abiotic stresses (Rahman *et al*., 2015; Lee *et al*., 2017). In this study, up-regulation of *HSP17 kDa* under cold stress suggests that this group of proteins can induce by cold stress also.DEG-39 was identified as a gene encoding for Myo-inositol 1-phosphate synthase (*MIPS*). *MIPS* is a core enzyme involved in Myo-inositol biosysthesis, *MIPS* enhanced biotic and abiotic stresses in sweet potato (Zhai *et al*., 2016). In this study, we checked the mRNA level of this gene where *MIPS* was highly expressed at 48 h of cold treatment. However, our result supported by the findings of Zhai et al. 2016, and suggests that *MIPS* would be useful candidate for cold stress tolerance in plants.

Cold-induced down-regulated genes: In our study we also found down regulated genes including alpha-tubulin 4 (*atub4* DEG38) and metallothioneins type 2(*mt2b*; DEG40) in barley leaves (Table 1; Figure 2). Gene *atub4* is a member of reference genes in plants. According to previous study α-tubulin shown to be increased in rice under salt and heat stresses (Damaris *et al*., 2016). Moreover, α-tubulin found to be induced in response to hyperosmotic stress treatment in rice (Ban *et al*.,2013). In our study, *atub4*found to be down-regulated in barley leaves under cold stress. These, above results suggest that alpha-tubulin may response diversely based on stress exposure. We have identified DEG40 as metallothioneins type 2(*mt2b* in barley leaves. MTs are Cys-rich small proteins present in plant and animals, function as heavy-metal homeostasis (Shukla *et al*., 2016).*MT2* gene was down-regulated under cold stress(Table 1; Figure 2). Regulation of *MT2* depend on plant's age, organs and exposure of metal(loid)s (Kohler *et al*., 2004). In our study, *MT2* was down-regulated under cold stress in barley leaves. Similar expression has been obtained in brassica in exposure of Cu (Schäfer *et al*.,1997). In contrast, *MT2* found to be up-regulated in presence of excess B in tomato (Huanca-Mamani *et al*., 2018). However, the above investigations suggest that the *MT2* may involve in abiotic stress response along with heavy metal homeostasis in plants.

Newly induced genes under cold stress: DEG 31were was identified as a gene encoding for *light-harvesting complexes II type I*protein (*LHCII type I*). *LHCIItypeI* is also known as chlorophyll a-b binding protein. The *LHCII type I* protein plays a role as a light receptor that captures and transfers energy in photosynthesis process. In *Arabidopsis*, it has been explored that *LHCII* complexes are consisted of *Lhcb1, 2,*and *3* proteins, where *Lhcb1,* and *2* showed a complementary role in state transecting (Pietrzykowska *et al*.,2014). Hence, it is still to be explored the clear function of *LHCII type I* protein in response to abiotic stress. In this study, DEG33 discovered as *clone cortma-ap3*that is a cold-regulated (cor) protein (Table 1). This is a novel gene, the specific function of *clone cortma-ap3* is still unknown in plant. Though some other *cor* homologues (*cor14b*, *tmc-ap3*, and *blt14*) in plants were reported to be regulated during the development of chloroplast (Dal Bosco *et al*., 2003).

In summary, we identified cold-induced 14 DEG in barley leaves using an ACP-based RT-PCR method. In fact, it has been clarified the gene specific temporal

expression in barley leaves. These identified genes were mostly involved in several biological functions including plant growth, photosynthesis, glycolysis, abiotic stress tolerance, stress homeostasis in plants. In addition, two genes were newly identified. The identified candidate genes would be suitable breeding target to enhance cold stress tolerance in plants.

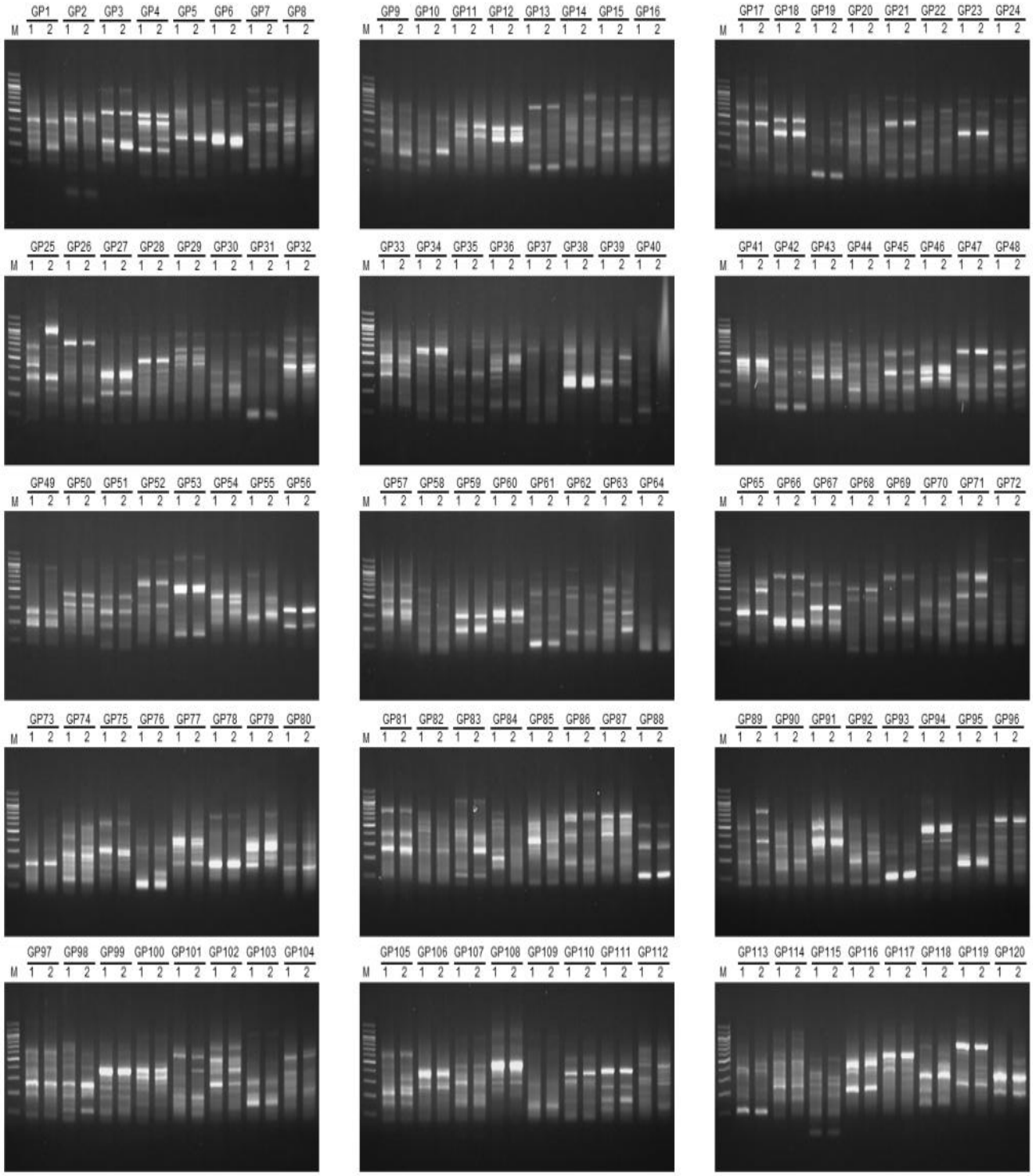

Figure1.Agarose gel images with expressed genes in barely leaves. Gel image shows different PCR bands where 120 arbitrary primers are used, left side of the each gel image shows molecular marker.

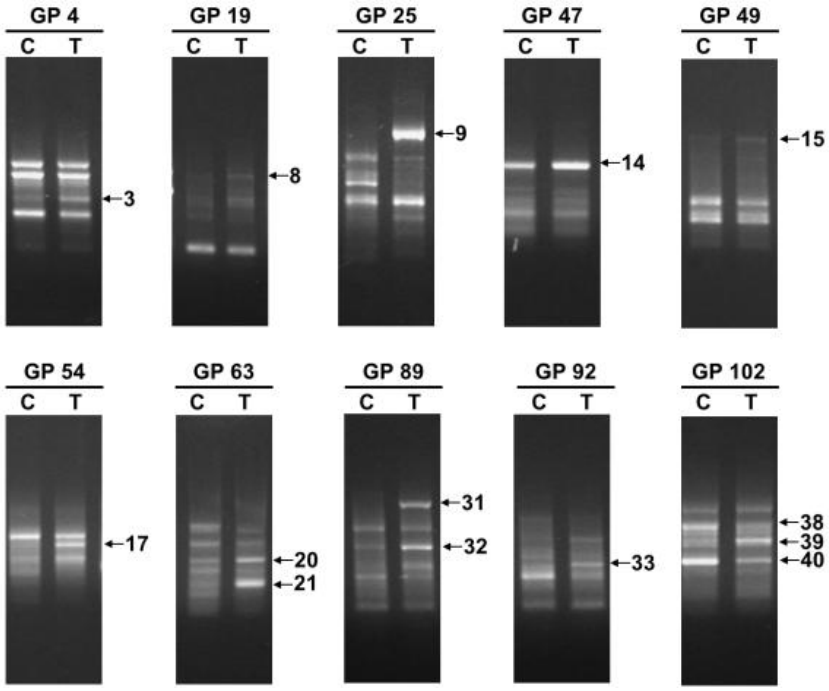

Figure2. Agarose gel image shows 14 differentially expressed genes (DEGs) specific bands in barley leaves in response to cold stress. An annealing control primer (ACP) based RT-PCR approach applied for the identification of genes induced by cold stress in barley leaves. The numeric mentioned at right side of each gel image indicates serial number of a specific gene. DEG 3, 8, 9, 14, 15, 17, 20, 21, 32 and 39 showed up-regulation whereas DEG 38 and 40 showed down regulation, andDEG31 and 33 were newly induced in barley leaves under cold stress. The lanes of treated (T) and control (C) samples were mentioned on gel image, respectively.

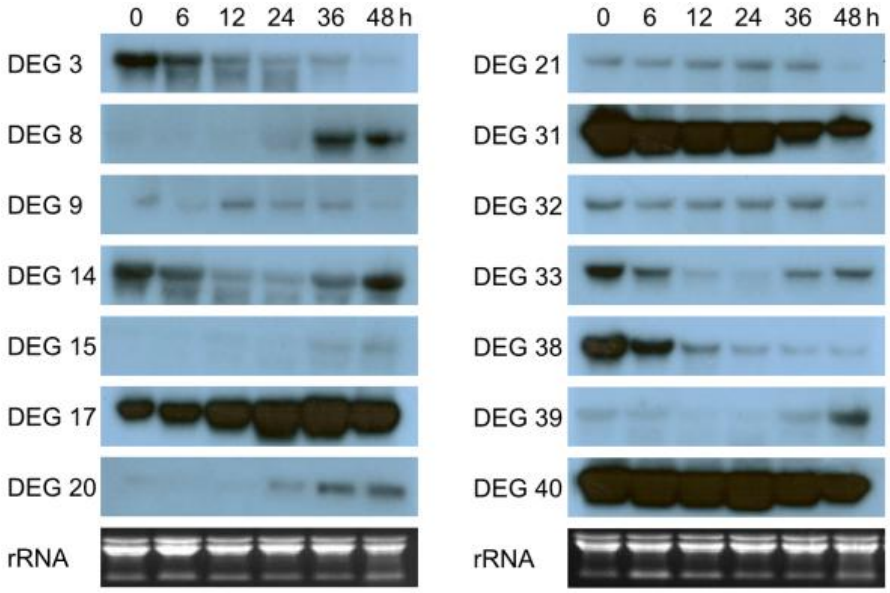

Figure 3. Temporal expression of cold-induced genes in barley leaves. A northern blot approach was applied to explore temporal gene expressions in a time course analysis. Leaf sample was exposed to cold treatment (4°C) for 48 h, leaf samples were collected at 0, 6, 12, 24, 36, and 48 h. Ten microgram (10 µg) of total RNA was loaded in each lane and separated by 1.2% agarose gel electrophoresis, subsequently transferred to a nylon membrane. Gene specific probe was labeled with [32^P], rRNA was used as an internal control.

Table 1. Cold-stress genes in barely leaves identified by gene sequencing approach where sequences were analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

DEG No.	Accession No.	Identity	Score	e-value
DEG 3↑	X12733	Alcohol dehydrogenase gene Adh2	36	0.028
DEG 8↑	AF348460	Cryptochrome 2 (Cry2) gene, partial cds.	30	1.4
DEG 9↑	AJ007990	SnRK1-type protein kinase, partial	32	0.99
DEG 14↑	X58339	CHS gene for chalcone synthase	1013	0
DEG 15↑	M36650	Glyceraldehyde-3-phosphate dehydrogenase mRNA, 3'end	44	2.00E-04
DEG 17↑	AF043094	Dehydrin 9 (dhn9) gene, complete cds	500	e-142
DEG 20↑	X89023	LHC II type I protein	100	1.00E-21
DEG 21↑	X12733	Alcohol dehydrogenase gene Adh2	30	0.38
DEG 31new	X89023	LHC II type I protein	521	e-148
DEG 32↑	Y07844	17 kDa class I small heat shock protein	32	0.26
DEG 33new	AJ291295	Cold-regulated protein, clone cortma-ap3.	353	4.00E-98
DEG 38↓	AJ276012	Alpha-tubulin 4 (atub4 gene).	32	0.48
DEG39↑	AF056325	Myo-inositol 1-phosphate synthase mRNA, complete cds	34	0.084
DEG 40↓	AJ511346	Metallothioneine type2 (mt2b gene).	367	e-102

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REFERENCES

Ahmad, M. and A.R. Cashmore (1993). HY4 gene of *Arabidopsis thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature. 366: 162-166.

Baisakh, N., P. K. Subudhi and P. Varadwaj (2008). Primary responses to salt stress in a halophyte, smooth cordgrass (*Spartinaalterniflora* Loisel.). Funct. Integr. Genomics. 8:287-300.

Ban Y., Y. Kobayashi, T. Hara, T. Hamada, T. Hashimoto, S. Takeda, and T. Hattori (2013). α-tubulin is rapidly phosphorylated in response to hyperosmotic stress in rice and *Arabidopsis*.Plant Cell Physiol. 54(6):848-58.

Barros, T., and W. Kühlbrandt (2009). Crystallisation, structure and function of plant light-harvesting complex II. Biochim. Biophys. Acta. 1787(6): 753-772.

Bechtold, U., and B. Field (2018). Molecular mechanisms controlling plant growth during abiotic stress. J. Exp. Bot. 69(11):2753-2758.

Chinnusamy, V., J. Zhu, and J. K. Zhu (2007). Cold stress regulation of gene expression in plants. Trends Plant Sci.12(10): 444-451.

Dal Bosco, C., M. Busconi, C. Govoni, P. Baldi, A.M. Stanca, C. Crosatti, R. Bassi, and L. Cattivelli (2003). *Cor* Gene expression in barley mutants affected in chloroplast development and photosynthetic electron transport. Plant Physiol. 131(2):793-802.

Damaris, R.N., M. Li, Y. Liu, X. Chen, H. Murage, and P. A. Yang (2016). Proteomic analysis of salt stress response in seedlings of two African rice cultivars. Biochim. Biophys. Acta. 1864(11):1570-8.

Dao, T. T. H., H. J. M. Linthorst, and R. Verpoorte (2011). Chalcone synthase and its functions in plant resistance. Phytochem. Rev. 10(3): 397-412.

Galbraith, D.W., and J. Edwards (2010). Applications of microarrays for crop improvement: here, there, and everywhere. BioScience. 60(5):337-348.

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

Guy, C.L., K.J. Niemi, and R. Brambl (1985). Altered gene expres-sion during cold acclimation of spinach. Proc. Natl. Acad. Sci USA. 82:3673-3677.

Huanca-Mamani, W., S. Cárdenas-Ninasivincha, and G. Acosta-García (2018). Expression analysis of three stress-related genes in response to excess of boron in *Solanumlycopersicum*cv Poncho Negro. IDESIA(Chile).36(1): 35-40.

Kohler, A., D. Blaudez, M. Chalot, and F. Martin (2004). Cloning and expression of multiple metallothioneins from hybrid poplar. New Phytol.164(1): 83-93.

Kong, F., Y. Deng, G. Wang, J. Wang, X. Liang, and Q. Meng (2014). *LeCDJ1*, a chloroplast DnaJ protein, facilitates heat tolerance in transgenic tomatoes. J. Integr. Plant Biol.56: 63–74.

Lee, J.Y., and D.H. Lee (2003). Use of serial analysis of gene expression technology to reveal changes in

gene expression in *Arabidopsis* pollen undergoing cold stress. Plant Physiol. 132(2):517-529.

Lee, K.-W, M.A. Rahman, K.Y. Kim, G.J. Choi, J.Y. Cha, M.S. Cheong, A.M. Shohael, C. Jones, and S.H. Lee (2018a). Overexpression of the alfalfa DnaJ-like protein (*MsDJLP*) gene enhances tolerance to chilling and heat stresses in transgenic tobacco plants. Turk. J. Biol. 42(1):12-22.

Lee, K.W., M.A. Rahman, G.J. Choi, H.C. Ji, T.Y. Hwang, and S. H. Lee (2018b). Identification of differentially expressed abiotic stress-induced genes in teff grass (*Eragrostistef*) leaves. The J. Anim. Plant Sci., 28(4):1189-93.

Lee, K.W., S.H. Lee, G.J. Choi, H.J. Ji, T.Y. Hwang, W.H. Kim, and M. A. Rahman (2017). Salt-induced differential gene expression in italian ryegrass (*Loliummultiflorum* Lam.) revealed by annealing control primer based gene fishing approach. J. Korean Soc. Grassl. Sci.37(3):231-236.

Lee, S, H., K.W. Lee, K.Y. Kim, G.J. Choi, S.H. Yoon, H.C. Ji, S. Seo, Y.C. Lim and A.Nagib (2009). Identification of salt-stress induced differentially expressed genes in barley leaves using the annealing control-primer-based Gene Fishing technique. Afr. J. Biotechnol. 8(7): 1326-1331.

Liu, Y., Q. Song, D. Li, X. Yang, andD.Li(2017). Multifunctional roles of plant dehydrins in response to environmental stresses. Front. Plant Sci. 8:1018.

Ninu, L., M. Ahmad, C.Miarelli, A.R. Cashmore and G. Giuliano (1999).Cryptochrome 1 controls tomato development in response to blue light. Plant J. 18:551-556.

Pietrzykowska, M., M. Suorsa, D.A. Semchonok, M. Tikkanen, E.J. Boekema, E.M. Aro, and S. Jansson (2014). The light-harvesting chlorophyll a/b binding proteins *Lhcb1* and *Lhcb2* play complementary roles during state transitions in *Arabidopsis*. Plant Cell. 26(9):3646-3660.

Rahman, M. A., I. Alam, Y. G. Kim, N.Y. Ahn, S.H. Heo, D. G. Lee, and B.H. Lee (2015). Screening for salt-responsive proteins in two contrasting alfalfa cultivars using a comparative proteome approach. Plant Physiol.Biochem.89: 112-122.

Rahman, M.A., S.H. Lee, G.J. Choi, H.J. Ji, W.H. Kim, and K.W. Lee (2017).Isolation and identification of short term drought-induced genes in *Zea mays* L. leaves. J. Korean Soc. Grassl. Sci. 37(3):237-241.

Schäfer, H. J., S. Greiner, T. Rausch, and A. Haag-Kerwer (1997). In seedlings of the heavy metal accumulator *Brassica juncea* Cu^{2+} differentially affects transcript amounts for γ-glutamyl cysteine synthetase (γ-ECS) and metallothionein (MT2). FEBS let.404(2-3): 216-220.

Shukla, D., P. K. Trivedi, P. Nath, and N. Tuteja (2016). Metallothioneins and phytochelatins: role and perspectives in heavy metal(loid)s stress tolerance in crop plants. Abiotic Stress Response In Plants, pp.237-264.

Sunkar, R., D. Bartels, and H. Kirch (2003). Overexpression of a stress-inducible aldehyde dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance. Plant J. 35: 452-464.

Thomashow, M.F. (1990). Molecular genetics of cold acclimation in higher plants. Adv. Genet. 28: 99-131.

Viswanathan, C. and J.K. Zhu (2002). Molecular genetic analysis of cold-regulated gene transcription. Philos. Trans. R.Soc.Lond B. 357:877-886.

Wanner, L. A., and O. Junttila (1999). Cold-induced freezing tolerance in *Arabidopsis.*Plant physiol.120(2): 391-400.

Weller, J.L., G. Perrotta, M. E. Schreuder, van A. Tuinen, M. Koornneef, G. Giuliano, and R.E. Kendrick (2001). Genetic dissection of blue-light sensing in tomato using mutants deficient in phytochromes A, B1, B2 and cryptochrome 1. Plant J. 25:427-440.

Yadav, S. K. (2010). Cold stress tolerance mechanisms in plants. A review. Agron. Sustain. Dev. 30: 515–527.

Zeng, L., R. Deng, Z. Guo, S. Yang, and X. Deng (2016). Genome-wide identification and characterization of glyceraldehyde-3-phosphate dehydrogenase genes family in wheat (*Triticumaestivum*). BMC Genomics. 17:240.

Zhai, H., F. Wang, Z. Si, J. Huo,L. Xing, Y. An,S.He, and Q. Liu (2016). A *myo*-inositol-1-phosphate synthase gene, *IbMIPS1*, enhances salt and drought tolerance and stem nematode resistance in transgenic sweet potato. Plant Biotechnol. J. 14: 592-602.

Zhang, X.N., Z.C. Qu, Y.Z. Wan, H. W. Zhang, and D. L. Shen (2002). Application of suppression subtractive hybridization (SSH) to cloning differentially expressed cDNA in *Dunaliellasalina* (chlorophyta) under hyperosmotic shock. Plant Mol. Biol. Rep. 20(1):49-57.

Zhou, X., S. Chen, H. Wu, and H.Xu(2017). Effects of cold stress on the photosynthesis and antioxidant system of *Rhododendron chrysanthum* Pall. pp. 1-10.