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Identification of Differentially Expressed Genes in Chilling-Induced Potato (*Solanum tuberosum* L.); a Data Analysis Study

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Abstract Cold stress, as chilling (<20 °C) or freezing (<0 °C), is one of the frequently exposed stresses in cultivated plants like potato. Under cold stress, plants differentially modulate their gene expression to develop a cold tolerance/acclimation. In the present study, we aimed to identify the overall gene expression profile of chilling-stressed (+4 °C) potato at four time points (4, 8, 12, and 48 h), with a particular emphasis on the genes related with transcription factors (TFs), phytohormones, lipid metabolism, signaling pathway, and photosynthesis. A total of 3504 differentially expressed genes (DEGs) were identified at four time points of chilling-induced potato, of which 1397 were found to be up-regulated while 2107 were down-regulated. Heatmap showed that genes were mainly up-regulated at 4-, 8-, and 12-h time points; however, at 48-h time point, they inclined to down-regulate. Seventy five up-regulated TF genes were identified from 37 different families/groups, including mainly from *bHLH*, *WRKY*, *CCAAT-binding*, *HAP3*, and *bZIP* families. Protein kinases and calcium were major signaling molecules in cold-induced signaling pathway. A collaborated regulation of phytohormones was observed in chilling-stressed potato. Lipid metabolisms were regulated in a way, highly probably, to change membrane composition to avoid cold damage and render in signaling. A down-regulated gene expression profile was observed in photosynthesis pathway, probably resulting from chilling-induced reduced enzyme activity or light-triggered ROSs damage. The findings of this study will be a valuable theoretical

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knowledge in terms of understanding the chilling-induced tolerance mechanisms in cultivated potato plants as well as in other *Solanum* species.

Keywords Cold acclimation · *COR* genes · Phytohormone · Microarray · Signal transduction

Introduction

Potato (*S. tuberosum*) is an economically important world's fourth largest food crop from Solanaceae family [1]. The potato, like other cultivated plants, can suffer from a number of abiotic stresses. Its bare and shallow root system also makes it more susceptible to these stresses [2]. Environmental abiotic constraints such as cold, heat, salinity, drought, flooding, radiation, heavy metal toxicity, etc. significantly inhibit the plant growth and cause to the serious crop losses. Among these constraints, cold stress, as chilling (<20 °C) or freezing (<0 °C), is one of the frequently exposed stresses in plants [3]. To overcome this, plants differentially regulate their genes to adapt their physiology for cold stress and to develop a cold tolerance/acclimation [4]. By using microarray technology, a large number of differentially expressed genes have been identified in *Arabidopsis* [5], potato [6], rice [7], wheat [8], barley [9], and oat [10] under cold acclimation/stress. DNA microarray analysis of *Arabidopsis* showed that cold stress (4 °C) causes the differentially expression of hundreds to thousands of cold-inducible (*COR*) genes, that are associated with accumulation of cryoprotectants, osmolytes, chaperones, transporters, dehydrins, and late embryogenesis abundant (LEA) proteins, and many enzymes involved in antioxidant detoxification, carbohydrate, lipid and secondary metabolite metabolism, and in biosynthesis of abscisic acid (ABA) and jasmonic acid (JA) [11, 12]. Elevated cytosolic Ca^{2+} is an early response to the cold stress and an important second messenger in cold stress transduction pathway, thereby, in cold acclimation development [13] (Fig. 1).

Cold stress changes the membrane composition, fluidity, and protein and nucleic acids conformations and causes to accumulate small molecules such as amino acids, soluble sugars, organic acids, polyamines, and lipids [14, 15]. These molecules function as osmolytes to reduce the cellular dehydration, as compatible solutes to stabilize the membrane or as chelators to involve in detoxification [16]. Verslues et al. [17] also reported that decreased water availability, changes of accumulated molecules and cell wall properties, and induction of stress proteins are the common metabolic responses to the abiotic stresses in plants. Moreover, molecular studies have identified many TF families such as *WRKY*, *DREB*, *ZF*, *ERF*, *MYB*, *bHLH*, *bZIP*, etc. that extensively involving in regulation of stress-inducible gene network [18, 19].

About 30 % of *Arabidopsis* transcriptome was regulated by abiotic stress, in which 2409 genes have been reported to have great importance in cold, salt, and drought tolerance [20]. Over 2 % of wheat genome demonstrated more than 2-fold changes upon cold stress [21]. In chilling-stressed rice genotypes, 8484 genes have been reported to be differentially regulated [22]. In cold/drought-treated barley, 3.8 % of genes (158) were chilling specific, 2.8 % (119) were freeze-thaw specific, and 34.1 % were freeze-thaw and drought responsive [23]. In cold/salt-stressed potato, 2599 genes significantly regulated, of which 1938 were cold-induced genes [12]. Thus, identification and characterization of differentially expressed genes (DEGs) in plants constitute the basis of understanding the cold stress pathway [24]. The aim of this study was to identify the overall expression profile of genes at four time points (4, 8, 12, and

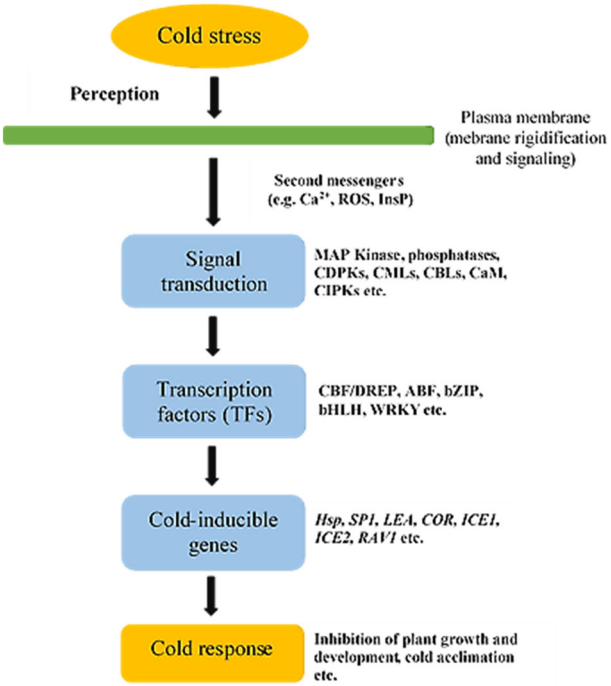

Fig. 1. Generic signal transduction pathway of cold stress in plants. Upon perception of cold stress by membrane receptors, a complex intracellurlar signaling cascade is activated involving secondary molecules, protein kinases, TFs etc. to develope a cold response

48 h) of cold-exposed (+4 °C) potato plant, with a particular emphasis on the genes related with TFs, phytohormones, lipid metabolism, signaling pathway, and photosynthesis.

Materials and Methods

The gene expression data of potato showing differential expression under different time periods of cold stress was retrieved from GEO (http://www.ncbi.nlm.nih.gov/geo/) database of NCBI. The expression data of cold stress was obtained from the series id GSE8203 by using MATLAB program. In the experiment, the TIGR10 K potato microarrays containing 15.264 complementary DNAs (cDNAs) (http://www.jcvi.org/potato/sol_ma_microarrays.shtml) were used. The data was already normalized. The boxplots displaying normalized data were provided in Supplementary Fig. 1. Researchers grown plants at 25 °C for 4–6 weeks after which cold stress was initiated by exposing the plants to 4 °C for 4, 8, 12, and 48 h. We retrieved gene expression values, which are in the form of log2, for four time points (4, 8, 12, and 4 8h). We did not include 24 h of cold treatment, since 24 h of cold treatment was performed one biological repeat. Statistically significant DEGs among sample treatments, each with two biological replicates, were identified using *t* test ($p < 0.05$) and false discovery rate of < %5. As confidence threshold for DEGs, we used a log2-fold change ≥0.6 and ≤−0.6. The ratios obtained from the microarray analysis were imported into MapMan Software [25]. Annotation and functional characterization was assigned using Stu_TIGR.m02 August07 [26].

Annotation for selected genes was confirmed by BLAST searches of the potato cDNA sequences against the TIGR potato EST database (http://www.tigr.org/) using BLAST.

Results

Identification of Chilling-Induced Genes

Log2-fold changes of ≥0.6 (up-regulated) and ≤−0.6 (down-regulated) ratios were used as confidence threshold to identify DEGs. We have identified a total of 3504 DEGs at four time points (4, 8, 12, and 48 h) (Supplementary Table S1). Of these, 1397 genes were up-regulated while 2107 were down-regulated. These genes include 419 up-regulated and 189 down-regulated genes for 4 h chilling stress, 360 up-regulated and 366 down-regulated genes for 8 h chilling stress, 356 up-regulated and 412 down-regulated genes for 12 h chilling stress, and 556 up-regulated and 1140 down-regulated genes for 48 h chilling stress. The common up- and down-regulated genes were found as 1 and 2, respectively (Supplementary Table S1) (Fig. 2).

Functional Annotation of DEGs

Annotation and functional characterization of identified 3504 (1397 up; 2107 down) DEGs at four time points (4, 8, 12, and 48 h) of chilling-stressed potato plant have been done by using MapMan tool (Supplementary Table S1) (Fig. 3). MapMan allows to hierarchically organizing the list of genes into functionally similar individual BINs and subBINs to understand the biological significance of DEGs [26]. Nine hundred ninety-two up-regulated genes were annotated in 31 major BINs while 1268 down-regulated genes annotated in 32 BINs, and the remaining up- and down-regulated genes could not be assigned to any ontology.

Although large numbers of genes have been identified to be differentially expressed, the main focus was on the genes involved in photosynthesis (BIN 1), lipid metabolism (BIN 11), hormones (BIN 17), RNA (BIN 27), and signaling (BIN 30) pathways. In photosynthesis pathway (BIN 1), some light reaction (9 genes up; 15 genes down), Calvin cycle (5 genes up; 7 genes down), and photorespiration (1 gene up; 3 genes down) genes were differentially expressed (Fig. 4a). Lipid metabolism (BIN 11) contained fatty acid (FA) synthesis and elongation (6 genes up; 10 genes down), lipid degradation (10 genes up; 11 genes down), FA desaturation (2 genes up; 3 genes down), phospholipid synthesis (1 gene up; 3 genes

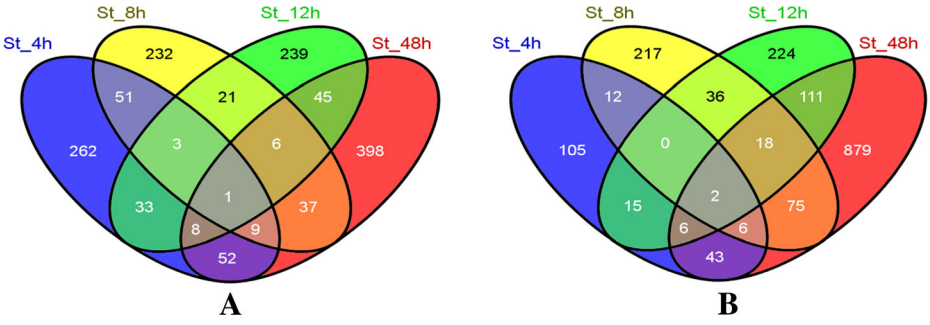

Fig. 2 Number of differentially expressed genes at four time points (4, 8, 12, and 48 h) of chilling-stressed potato (*S. tuberosum*) plant. Venn diagram **a** and **b** show the up- and down-regulated genes, respectively

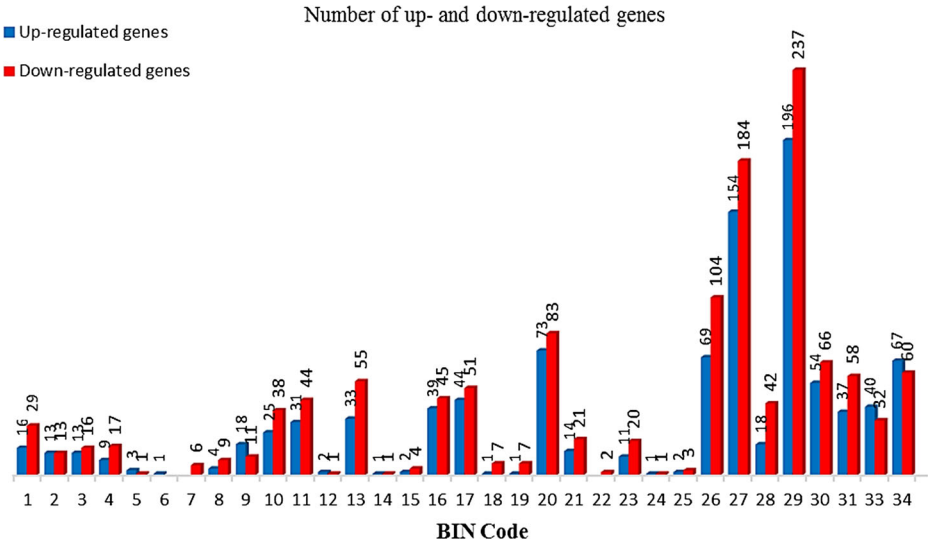

Fig. 3 Distribution of chilling-stressed potato up- and down-regulated genes in MapMan BINs. *Blue* and *red chart bars* show the up- and down-regulated genes, respectively. Each *number* at *x*-axis corresponds to an individual BIN code. *BIN 1*, photosynthesis, *BIN 2* major carbohydrates, *BIN 3* minor carbohydrates, *BIN 4* glycolysis, *BIN 5* fermentation, *BIN 6* gluconeogenesis/glyoxylate cycle, *BIN 7* oxidative pentose phosphate pathway, *BIN 8* TCA cycle/organic acid transformations, *BIN 9* mitochondrial electron transport/ATP synthesis, *BIN 10* cell wall, *BIN 11* lipid metabolism, *BIN 12* nitrogen assimilation, *BIN 13* amino acid metabolism, *BIN 14* S-assimilation, *BIN 15* metal handling, *BIN 16* secondary metabolism, *BIN 17* hormones, *BIN 18* cofactor and vitamin synthesis, *BIN 19* tetrapyrrole synthesis, *BIN 20* stress, *BIN 21* redox, *BIN 22* polyamine synthesis, *BIN 23* nucleotide metabolism, *BIN 24* biodegradation of xenobiotics, *BIN 25* C1-metabolism, *BIN 26* miscellaneous enzyme families, *BIN 27* RNA, *BIN 28* DNA, *BIN 29* protein, *BIN 30* signaling, *BIN 31* cell, *BIN 33* development, *BIN 34* transport

down), exotics (steroids, squalene etc.) (6 genes up; 8 genes down), and lipid transfer protein (1 gene up) genes (Fig. 4b). In RNA pathway (BIN 27), transcription (4 genes up; 8 genes down), RNA binding (9 genes up; 10 genes down), processing (16 genes up; 13 genes down), and a large number of TF (75 genes up; 128 genes down) genes have been identified (Table 1 and Fig. 4c).

Hormone metabolism (BIN 17) included abscisic acid (2 genes up; 5 genes down), auxin (12 genes up; 11 genes down), brassinosteroid (3 genes up; 10 genes down), cytokinin (4 genes up; 3 genes down), ethylene (5 genes up; 7 genes down), jasmonate (2 genes up; 3 genes down), gibberellin (4 genes up; 5 genes down), and salicylic acid (1 gene up) related genes (Fig. 4d). Signaling pathway (BIN 30) contained a wide range of subgroups such as receptor kinases (11 genes up; 17 genes down), MAP kinases (5 genes up; 2 genes down), calcium (12 genes up; 19 genes down), light (4 genes up; 8 genes down), G-proteins (6 genes up; 9 genes down), sugar and nutrient physiology (2 genes up; 2 genes down), phosphoinositides (1 gene up; 2 genes down), and 14-3-3 proteins (1 gene down) (Fig. 4e).

Heatmap of chilling-induced up- and down-regulated genes at four time points (4, 8, 12, and 48 h) in potato demonstrated that gene expression levels at 4, 8, and 12 h represented more similar pattern than that of 48 h. The genes have been observed to be mainly up-regulated at time points of 4, 8, and 12 h while at 48-h time point, genes inclined to down-regulate (Fig. 5 and Table 1).

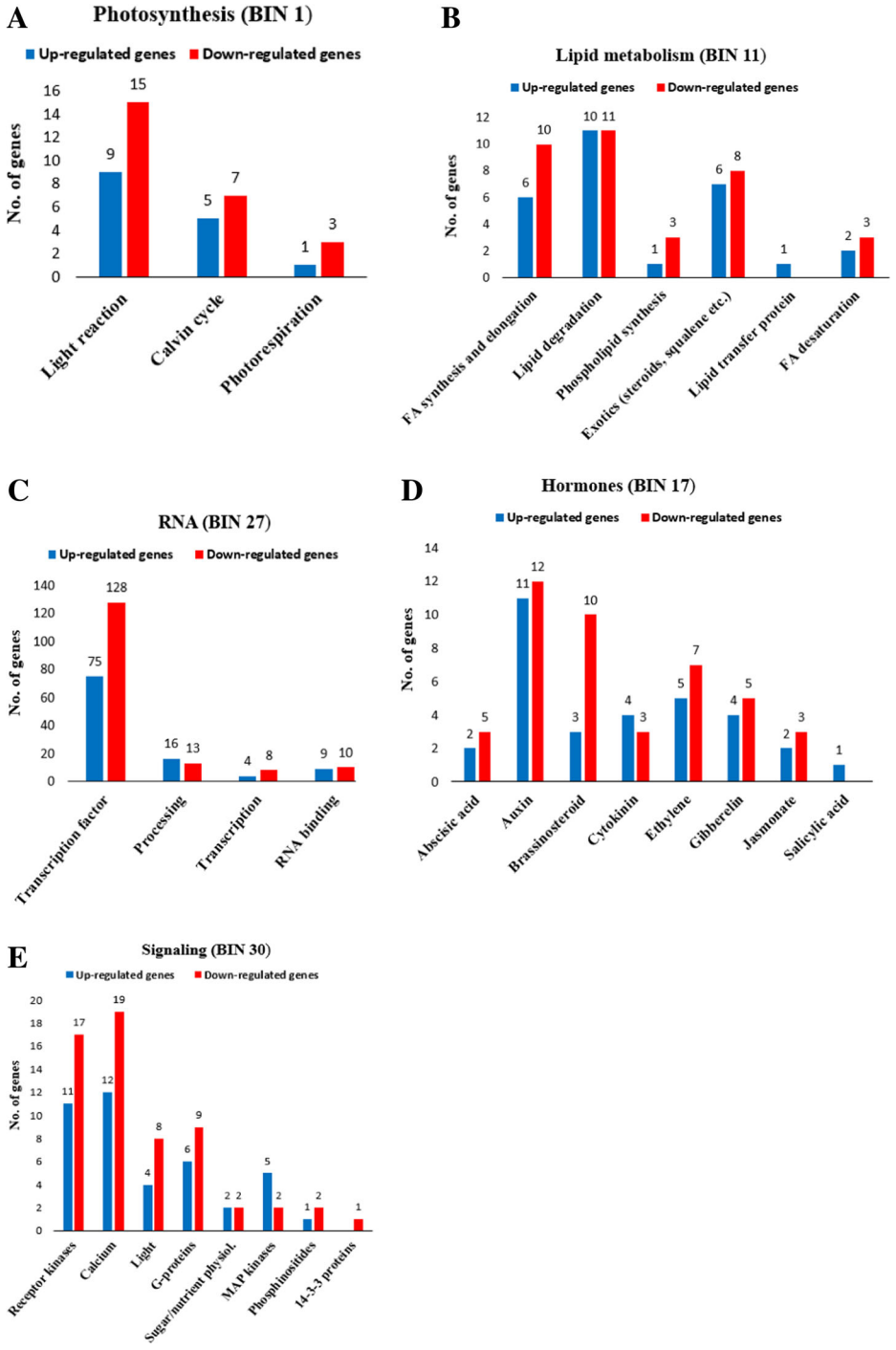

Fig. 4 Differentially expressed genes (DEGs) involved in photosynthesis (**a**), lipid metabolism (**b**), RNA (**c**), hormone (**d**), and signaling (**e**) pathways in chilling-stressed potato. Up- and down-regulated genes indicated *blue* and *red* colors, respectively. Annotations of DEGs have been made by using MapMan

Table 1 Expressed TF-related genes in chilling-stressed potato and their fold changes at four time points (4, 8, 12, and 48 h)

Clone name	BIN code	Annotation	4 h vs. control	8 h vs. control	12 h vs. control	48 h vs. control
STMHJ91	27.3.6	bHLH, basic helix-loop-helix family	**3.76** ↑	**0.81** ↑	–	**2.50** ↑
STMEK79	27.3.6	bHLH, basic helix-loop-helix family	**1.65** ↑	–	–	**1.61** ↑
STMDC31	27.3.6	bHLH, basic helix-loop-helix family	–	**1.70** ↑	–	–
STMDE79	27.3.6	bHLH, basic helix-loop-helix family	–	**1.82** ↑	–	–
STMDV67	27.3.6	bHLH, basic helix-loop-helix family	–	**0.80** ↑	–	–
STMER91	27.3.6	bHLH, basic helix-loop-helix family	–	**2.13** ↑	–	**1.84** ↑
STMHZ85	27.3.6	bHLH, basic helix-loop-helix family	–	–	**1.79** ↑	–
STMCU25	27.3.6	bHLH, basic helix-loop-helix family	–	–	–	**1.69** ↑
STMID25	27.3.39	AtSR transcription factor family	**3.75** ↑	**2.81** ↑	–	–
STMCA91	27.3.39	AtSR transcription factor family	**3.03** ↑	**1.23** ↑	–	–
STMID61	27.3.59	Methyl binding domain proteins	**3.13** ↑	–	–	–
STMHP31	27.3.7	Constans-like zinc finger family	**3.12** ↑	–	–	–
STMEG07	27.3.32	WRKY domain transcription factor family	**2.69** ↑	–	–	–
STMGO55	27.3.32	WRKY domain transcription factor family	**1.58** ↑	**1.27** ↑	–	–
STMCN79	27.3.32	WRKY domain transcription factor family	–	**0.91** ↑	–	–
STMIY73	27.3.32	WRKY domain transcription factor family	–	–	–	**1.18** ↑
STMFB31	27.3.11	C2H2 zinc finger family	**2.47** ↑	–	–	–
STMFA43	27.3.11	C2H2 zinc finger family	**1.18** ↑	–	–	**0.64** ↑
STMGT55	27.3.11	C2H2 zinc finger family	–	–	**1.11** ↑	–
STMHU85	27.3.73	Zn-finger(CCHC)	**2.34** ↑	–	**1.71** ↑	–
STMEO01	27.3.35	bZIP transcription factor family	**2.33** ↑	–	–	–
STMGP13	27.3.35	bZIP transcription factor family	–	–	**1.20** ↑	–
STMES62	27.3.35	bZIP transcription factor family	–	–	–	**1.11** ↑
STMJO92	27.3.35	bZIP transcription factor family	–	–	–	**0.75** ↑
STMJK91	27.3.25	MYB domain transcription factor family	**2.25** ↑	**0.70** ↑	–	–
STMCE49	27.3.70	Silencing group	**2.15** ↑	–	–	**1.25** ↑
STMGT91	27.3.22	HB, homeobox transcription factor family	**2.13** ↑	–	–	–
STMGX85	27.3.22	HB, homeobox transcription factor family	–	–	**2.07** ↑	–
STMCB07	27.3.4	ARF, auxin response factor family	**2.02** ↑	**1.83** ↑	–	–
STMCI37	27.3.4	ARF, auxin response factor family	–	**0.90** ↑	–	–
STMDM43	27.3.8	C2C2(Zn) DOF zinc finger family	**2.02** ↑	–	–	–
STMHJ68	27.3.42	Bromodomain proteins	**1.81** ↑	–	–	–
STMHK13	27.3.67	Putative DNA-binding protein	**1.65** ↑	–	–	–
STMGX91	27.3.67	Putative DNA-binding protein	–	**1.43** ↑	–	**0.77** ↑
STMIQ37	27.3.67	Putative DNA-binding protein	–	**1.17** ↑	–	–
STMDV61	27.3.67	Putative DNA-binding protein	–	–	**1.17** ↑	–
STMEA73	27.3.67	Putative DNA-binding protein	–	–	**0.94** ↑	**1.72** ↑
STMEF79	27.3.67	Putative DNA-binding protein	–	–	**0.82** ↑	–
STMDU38	27.3.67	Putative DNA-binding protein	–	–	–	**0.98** ↑
STMEY37	27.3.67	Putative DNA-binding protein	–	–	–	**1.13** ↑
STMHK14	27.3.67	Putative DNA-binding protein	–	–	–	**1.00** ↑
STMJH25	27.3.67	Putative DNA-binding protein	–	–	–	**1.12** ↑

Table 1 (continued)

Clone name	BIN code	Annotation	4 h vs. control	8 h vs. control	12 h vs. control	48 h vs. control
STMES86	27.3.34	Orphan family	**1.08** ↑	–	**1.51** ↑	–
STMHS67	27.3.23	HSF, heat-shock transcription factor family	**1.01** ↑	–	–	–
STMGD85	27.3.23	HSF, heat-shock transcription factor family	**0.71** ↑	–	–	–
STMHW61	27.3.3	AP2/EREBP transcription factor family	**0.89** ↑	–	–	–
STMIW13	27.3.3	AP2/EREBP transcription factor family	–	**0.60** ↑	–	–
STMHH49	27.3.69	SET-domain transcription regulator family	**0.64** ↑	–	–	–
STMCZ19	27.3.5	ARR	–	**0.80** ↑	–	–
STMDB79	27.3.12	C3H zinc finger family	–	–	**1.00** ↑	**0.86** ↑
STMDM49	27.3.12	C3H zinc finger family	–	–	–	**4.30** ↑
STMEM25	27.3.1	ABI3/VP1-related B3-domain-containing transcription factor family	–	**0.92** ↑	–	–
STMEN19	27.3.40	Aux/IAA family	–	–	**1.15** ↑	–
STMCF91	27.3.40	Aux/IAA family	–	**1.53** ↑	–	–
STMFB49	27.3.41	B3 transcription factor family	–	–	–	**0.88** ↑
STMII73	27.3.41	B3 transcription factor family	–	**0.66** ↑	–	–
STMHL13	27.3.15	CCAAT box binding factor family, HAP3	–	**2.43** ↑	–	–
STMIN14	27.3.15	CCAAT box binding factor family, HAP3	–	–	**2.86** ↑	–
STMGF80	27.3.15	CCAAT box binding factor family, HAP3	–	–	–	**2.17** ↑
STMII37	27.3.15	CCAAT box binding factor family, HAP3	–	–	–	**1.92** ↑
STMJB67	27.3.27	NAC domain transcription factor family	–	**0.63** ↑	–	**1.40** ↑
STMDI67	27.3.10	C2C2(Zn) YABBY family	–	**2.19** ↑	–	–
STMIC38	27.3.28	Squamosa promoter binding protein family	–	**0.97** ↑	–	**1.43** ↑
STMCL61	27.3.44	Chromatin remodeling factors	–	**0.78** ↑	–	–
STMGI31	27.3.44	Chromatin remodeling factors	–	–	**1.87** ↑	–
STMFB02	27.3.58	LUG	–	**3.50** ↑	–	**4.01** ↑
STMGB19	27.3.58	LUG	–	–	–	**1.36** ↑
STMHA79	27.3.71	SNF7	–	**0.76** ↑	**0.79** ↑	–
STMGY86	27.3.19	EIN3-like(EIL) transcription factor family	–	–	–	**0.70** ↑
STMIW67	27.3.26	MYB-related transcription factor family	–	–	**1.82** ↑	–
STMGH07	27.3.37	AS2	–	–	**1.17** ↑	–
STMID14	27.3.37	AS2	–	–	–	**1.30** ↑
STMHA37	27.3.50	General transcription	–	–	**2.65** ↑	–
STMCK55	27.3.62	Nucleosome/chromatin assembly factor group	–	–	**2.15** ↑	–
STMDG74	27.3.66	Pseudo ARR transcription factor family	–	–	–	**2.24** ↑

Arrow ↑ indicates up-regulated genes. Log2 fold changes of ≥0.6 (up-regulated) was used as confidence threshold to identify DEGs.

Thus, expression levels of up- and down-regulated genes at 48-h time point were visualized by using "Metabolism overview" pathway with their putative metabolic functions (Fig. 6). At 48-h time point, plant demonstrated an inclination towards to down-regulate the genes

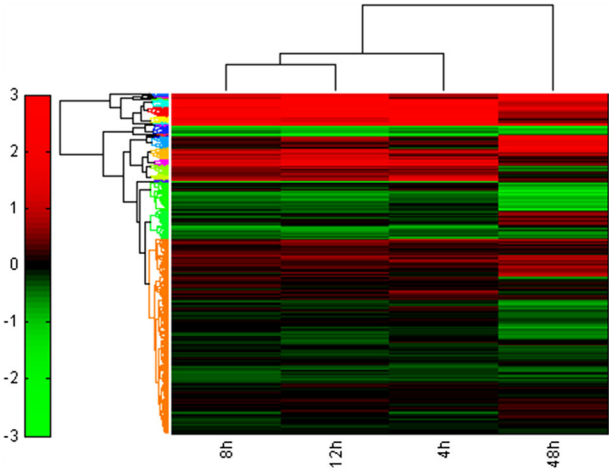

Fig. 5 Heatmap representing the expression levels of genes at four time points (4, 8, 12, and 48 h) in chilling-stressed potato. *Red* and *green* colors indicate the up- and down-regulated genes, respectively. Gene expression levels at 4, 8, and 12 h represent more similar pattern than that of 48 h

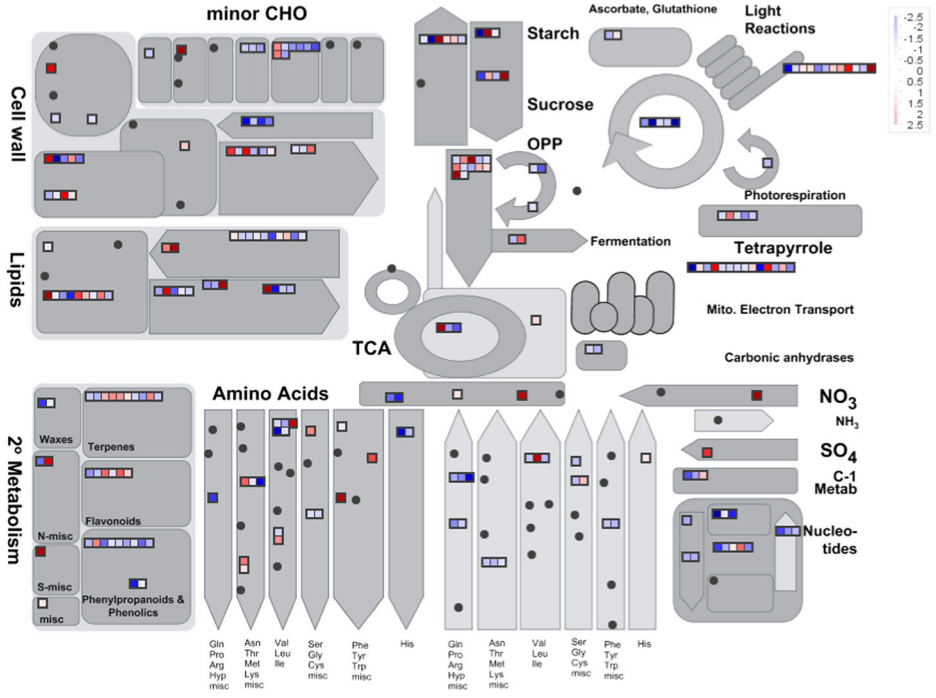

Fig. 6 Metabolic overview of DEGs in 48-h chilling-stressed potato. Pathway was visualized by using "Metabolism overview" pathway of MapMan. *Red* and *blue* colors indicate the up- and down-regulated genes, respectively. For better visualization, we set the threshold 2.5 and −2.5. *TCA* tricarboxylic acid, *OPP* oxidative pentose phosphate

involved in cell wall, lipid, minor CHO, light reaction, tricarboxylic acid (TCA) cycle, secondary metabolites, amino acids, and other metabolite pathways.

Discussion

Analysis of Differentially Expressed Genes

Cold stress affects various metabolic pathways leading to the differential expression of hundreds to thousands of genes. Transcriptome analysis of three wheat varieties such as Harnesk, Paragon, and Solstice has demonstrated 1711 up-regulated and 1402 down-regulated genes upon cold stress with significant increase in expression profile [21]. In cold/drought-treated barley, 158 genes (3.8 %) were identified to be chilling specific [23]. In cold-stressed *Populus simonii*, 5267 genes were up-regulated while 6359 genes were down-regulated [27]. In flowering plant *Anthurium andraeanum*, a total of 4363 genes were reported to be significantly expressed under cold stress [28]. In tea plant, 1168 genes were up-regulated and 602 genes were down-regulated under cold treatment [29]. In this study, we have identified a total of 3504 DEGs at four time points (4, 8, 12, and 48 h) of chilling-stressed potato plant. Of these genes, 1397 were up-regulated and 2107 were down-regulated. In addition, one gene (clone name: STMGH01) was commonly up-regulated and two genes (clone names: STMGV67 and STMJF91) were commonly down-regulated for four time points. However, they are not argued in this study due to either lack of annotation or weakly annotated data. In early exposure to chilling stress, potato gives a response by mainly up-regulating its genes, and then, it demonstrates an inclination towards to the down-regulation of its genome. Overall, it may be reported that short-term chilling stress (4, 8 and 12 h) could lead the potato to up-regulate its genes related with increased synthesis of a number of cold-induced molecules or/and a shock state-related increased gene activities. However, in long-term cold stress (48 h), it lowers its metabolic activities by down-regulating its genes, highly probably to avoid further damage.

Chilling Stress-Induced Transcriptional Regulation

It is well-known that specific sets of genes, collectively known as cold-regulated (*COR*) genes, are differentially expressed upon cold stress. Furthermore, expression of *COR* genes are modulated by a number of transcription factors (TFs) [3, 14]. In this study, 75 up-regulated genes encoding TFs have been identified in chilling-stressed potato plant (Table 1). Although we have identified 75 up-regulated TF genes from 37 different families/groups, we mainly focused on the four most abundantly expressed TF families such as basic helix-loop-helix (*bHLH*), *WRKY*, *CCAAT*-binding (*HAP3*), and *bZIP* transcription factor families. The *bHLH* transcription factor family plays very diverse roles in modulation of various physiological and stress-adaptive networks in plants. Two grapevine basic *bHLH* transcription factors, *VabHLH1* and *VvbHLH1*, positively modulated the CBF (C-repeat binding factor) pathway, which has an important role in cold acclimation and contributed the cold stress tolerance in *Arabidopsis* [30]. In a different study, six grapevine *bHLH* genes, including *VlCEbHLH1-6* demonstrated the differential expression pattern as response to various cold temperatures [31]. The up-regulation of *PtrbHLH* gene enhanced the cold stress tolerance in tobacco and lemon plants while down-regulation caused to the elevated cold sensitivity in trifoliate orange [32]. The

overexpression of apple *MdClbHLH1* gene conferred the cold tolerance in transgenic apple and *Arabidopsis* through MdCBF2 and AtCBF3 promoter, respectively. Additionally, ectopic expression of *MdClbHLH1* in transgenic tobacco enhanced the chilling tolerance [33]. Low temperatures were reported to up-regulate the *bHLH148* and *bHLH1* genes in rice [34, 35]. A transcription factor of *ICE2* in *bHLH* family enhanced the tolerance to deep freezing cold in transgenic *Arabidopsis* [36]. Banana *bHLH1/2/4* promoters were induced by cold stress and methyl jasmonate in tobacco BY2 protoplasts [37]. Furthermore, cold-related expressions of *bHLH* genes have been also reported in various other species. In the view of above-mentioned studies, *bHLH* TFs seem to play important functions in modulation of cold acclimation process in plants. In this study, we have identified the eight members of *bHLH* TFs with clone names such as STMHJ91, STMEK79, STMDC31, STMDE79, STMDV67, STMER91, STMHZ85, and STMCU25 at different expression folds in different time points of chilling-induced potato (Table 1). The clone STMHJ91, which showed the expression fold with 3.76, 0.81, and 2.50 at 4-, 8-, and 48-h time points, respectively, was similar to *Arabidopsis AT1G69010* (*AtbHLH102*) gene, whose product was reported to show DNA-binding TF and protein dimerization activities in various plant tissues. The clone STMEK79 demonstrated an expression fold with 1.65 (4 h) and 1.61 (48 h) and showed similarity to *Arabidopsis AT2G27230* (*AtbHLH156*) gene with DNA-binding TF and protein dimerization activities in guard and plant sperm cells and in primary root tips. The clone STMDC31 and STMDE79 expressed at 8-h time period with 1.70- and 1.82-folds, respectively, and showed similarity to *Arabidopsis AT5G64340* (*AtbHLH142*) gene. The clone STMDV67 expressed at 8-h period with 0.80-folds and showed similarity to *Arabidopsis AT1G72210* (*AtbHLH96*) gene with DNA TF and amino acid binding and protein dimerization activities in guard cells. The clone STMER91 expressed at 8- and 48-h time periods with 2.13- and 1.84-folds, respectively, and showed similarity to *Arabidopsis AT5G46690* (*AtbHLH71*) gene. The clone STMHZ85 expressed at 12-h time period with 1.79-fold (enrichment not available). The clone STMCU25 expressed at 48-h time period with 1.69-fold and identical to *Arabidopsis AT1G32640* (*AtbHLH6*) gene. Therefore, 8- and 48-h time periods were the points in which most of *bHLH* genes have been up-regulated. This may indicate that 8- and 48-h points could be critical times in induction and development of cold stress mechanisms.

In addition to *bHLH* family, *WRKY*, CCAAT-binding (*HAP3*) and *bZIP* transcription factor families have been most abundantly expressed. *WRKY* TF genes have been demonstrated to involve in regulation of plant growth and development and in stress responses [38]. In *Arabidopsis* genome, four *WRKY* family cDNAs have been identified as drought, cold, or high-salinity inducible genes [39]. Soybean *GmWRKY21* gene conferred the cold tolerance in transgenic *Arabidopsis* plants [40]. Barley *HvWRKY38* gene has been demonstrated to be expressed as response to low non-freezing temperature [41]. Thirteen *WRKY* genes were reported to be regulated only by cold, drought, and salinity in rice [42]. The hot pepper *CaWRKY1* gene was found among the up-regulated cold stress genes [43]. In *Populus* transcriptome, 61 *PtrWRKY* genes were induced as response to various biotic/abiotic stresses, including cold [44]. Fifteen grapevine *VvWRKY* genes have been reported to show the cold stress-induced expression pattern [45]. Previous studies showed that *WRKY* genes play an important role in cold response pathway. We have identified four *WRKY* genes with clone names such as STMEG07, STMGO55, STMCN79, and STMIY73 at 4-, 8-, and 48-h time periods of chilling-stressed potato. The clone STMEG07 expressed at 4-h time period with 2.69-fold and showed similarity to tobacco *WRKY4* gene with DNA-binding TF activity. The clone STMGO55 expressed at 4- and 8-h time periods with 1.58- and 1.27-folds, respectively,

and demonstrated similarity to *Arabidopsis WRKY12* gene. The clone STMCN79 expressed at 8-h time period with 0.91-fold and showed similarity to *Arabidopsis WRKY33* gene with protein binding and DNA-binding TF activities in various tissues. The clone STMIY73 also expressed at 48-h time period with 1.18-fold and showed similarity to tobacco *WRKY* gene.

Another TF family identified in this study is the CCAAT-box binding (*HAP3*). In *Arabidopsis*, HAP complex consists of three different subunits, namely as HAP2, HAP3, and HAP5. Each individual HAP subunit has been shown to be involved in the regulation of many physiological processes and stress tolerance [46]. HAP2 and HAP3 subunits have been mainly reported to involve in drought or osmotic stress tolerance [47, 48]. The overexpression of *HAP5A* was demonstrated to confer the cold tolerance in *Arabidopsis* plants [49]. We have identified four CCAAT-box binding (*HAP3*) TF clones such as STMHL13, STMIN14, STMGF80, and STMII37 at four time points of chilling-stressed potato. The clone STMHL13 expressed at 8-h time period with 2.43-fold and showed similarity to *Arabidopsis NFYB8* gene with DNA-binding TF and protein heterodimerization activities in various cell types. The clone STMIN14 expressed at 12-h time period with 2.86-fold and demonstrated a similarity to *Arabidopsis AT3G58610* gene with coenzyme binding and ketol-acid reductoisomerase activities. The clone STMGF80 expressed at 48-h time point with 2.17-fold and showed similarity to *Arabidopsis NFYB3* (*HAP3C*) gene. The clone STMII37 expressed at 48-h time point with 1.92-fold and demonstrated similarity to rice *HAP3A* gene.

bZIP is another TF family identified in this study with its four members. This family has been shown to regulate many physiological processes such as seed maturation, floral development, photo-morphogenesis, and stress and hormone signaling [50]. The overexpression of *OsbZIP52* gene was reported to significantly increase the cold and drought sensitivity in rice [51]. Grapevine TF *VvbZIP23* was demonstrated to be induced by a wide spectrum of abiotic stresses, including cold [52]. The overexpressed wheat *TabZIP60* significantly enhanced the drought, salt, and cold tolerance in *Arabidopsis* [53]. Soybean *GmbZIP44*, *62*, and *78* genes conferred the salt and cold tolerance in transgenic *Arabidopsis* [54]. We have identified four *bZIP* TF genes with clone names such as STMEO01, STMGP13, STMES62, and STMJO92 at three time points of chilling-stressed potato. The clone STMEO01 and STMES62 expressed at 4- and 48-h time points with 2.33- and 1.11-folds, respectively, and showed similarity to tobacco *BZI-1* gene with DNA-binding TF activity. The clone STMGP13 expressed at 12-h time point with 1.20-fold and demonstrated similarity to *Arabidopsis ATBZIP60* gene. The clone STMJO92 expressed at 48-h time points with 0.75-fold and showed similarity to soybean BZIP TF *ATB2* gene with DNA-binding TF activity. Overall, it seems that a large number of TF genes in potato are induced as response to cold stress to either develop a cold acclimation or regulate other cold-related physiological processes.

Chilling Stress-Induced Signaling

Plants develop cold response/acclimation as a result of highly complex signaling network, but it necessitates the involvement of a number of intra- and intercellular molecules [55]. In this study, we have identified the receptor kinases, MAP kinases, calcium, light, G-protein, some sugar, and nutrient physiology related molecules, phosphoinositides, and 14-3-3 proteins in signaling pathway (BIN 30) (Fig. 4e). However, main focus will be on the protein kinases and calcium because of their primary functions in cold stress pathway. We identified two families of kinases, including receptor kinases and MAP kinases (Fig. 4e). Receptor-like kinases (RLKs) have important functions in perception and transduction of extracellular stimuli [55].

Moreover, RLKs are key regulators in plant growth and development and in stress responses [56, 57]. In *Arabidopsis*, more than 600 RLK-encoding genes and in rice, over 1000 RLK-encoding genes have been reported [58, 59]. In the present study, we have identified 11 up-regulated receptor kinase genes with clone names such as STMDQ19 (4-h point/3.84-fold; 48-h point/1.56-fold), STMET61 (4-h point/3.45-fold; 8-h point/0.60-fold), STMJE19 (4-h point/1.98-fold; 48-h point/1.49-fold), STMIZ38 (4-h point/1.20-fold; 8-h point/2.04-fold), STMDP55 (4-h point/0.73-fold; 48-h point/1.11-fold), STMIF31 (4-h point/0.65-fold; 48-h point/0.97-fold), STMIB37 (4-h point/3.31-fold), STMJG85 (4-h point/0.84-fold), STMJH20 (12-h point/2.34-fold), STMGC91 (48-h point/0.75-fold), STMJN32 (48-h point/0.79-fold) in chilling-induced potato. The presence of wide spectrum of chilling stress-responsive receptor kinases in potato could indicate their important functions in cold or cold-related pathways. However, three clones such as STMDQ19 (4-h point/3.84-fold), STMET61 (4-h point/3.45-fold), and STMIB37 (4-h point/3.31-fold) have demonstrated highest expression fold over 3 at 4-h time period. The clone STMDQ19 showed similarity to tomato *LhirPtoB* gene with ATP binding and protein serine/threonine kinase activities. The clone STMET61 was similar to *Arabidopsis CLV1* gene with ATP binding, protein kinase, and receptor serine/threonine kinase binding activities. The clone STMIB37 was similar to *Arabidopsis AT1G67720* gene with ATP binding and protein serine/threonine kinase activities.

In addition, mitogen-activated protein kinases (MAPKs) play important roles in downstream signaling by mediating between receptors and other intracellular signaling networks in plants [60]. Moreover, they could be also activated by various stresses simultaneously, thereby; they function as convergent points in stress transduction pathway [61, 62]. *Arabidopsis* genome was reported to encode nearly 60 MAPKKKs, 10 MAPKKs, and 20 MAPKs [60]. Thus, expressed MAP kinase genes in chilling-stressed potato could possibly modulate the cold stress-related transduction cascade to converge the signaling in order for developing a cold response/acclimation. We have identified five up-regulated MAP kinase genes with clone names such as STMIP25 (4-h point/0.93-fold), STMET25 (8-h point/1.93-fold), STMHH86 (12-h point/1.07-fold), STMEA92 (48-h point/0.63 point), and STMEF20 (48-h point/1.80-fold) in chilling-induced potato.

Moreover, Ca^{2+} functions as a second messenger in regulation of cold stress signaling pathway [63]. Upon cold stress, cytosolic Ca^{2+} concentration immediately rises up to a level of designated Ca^{2+} signatures for cold. This designated cytoplasmic Ca^{2+} signature is decoded by Ca^{2+} sensors like Calmodulins (CaM), Calmodulin-like proteins (CMLs), Ca^{2+}-dependent protein kinases (CDPKs), Calcineurin B-like proteins (CBLs), and their interacting kinases (CIPKs) to transduce the signal intracellularly [64, 65]. Therefore, differentially expressed Ca-related genes in chilling-stressed potato could have major functions in intracellular signal transduction, thereby, in development of cold acclimation. We have identified 12 calcium-related genes with clone names such as STMDV44 (4-h point/3.42-fold), STMDG68 (4-h point/2.96-fold), STMHY91 (4-h point/1.81-fold), STMCH25 (4-h point/0.97-fold), STMJH13 (4-h point/0.69-fold), STMEN67 (8-h point/2.09-fold), STMEP25 (8-h point/0.85-fold), STMID19 (8-h point/1.82-fold; 48-h point/1.02-fold), STMJA91 (12-h point/2.17-fold; 48-h point/1.12-fold), STMDM25 (48-h point/2.44-fold), STMGB13 (48-h point/0.81-fold), and STMIT08 (48-h point/0.80-fold). The clone STMDV44 showed the highest expression fold with 3.42 at 4-h time period and was similar to tomato *CLB1* and *Arabidopsis AT3G60950* gene with endonuclease and exonuclease activities.

Chilling Stress-Induced Hormone Regulation

An accumulating evidence shows that synergistic or antagonistic hormone cross-talks play important roles in adaptation/response to abiotic stresses [66]. Plant hormones often rapidly change the gene expression of target genes by modulating the transcriptional factors [67]. In this study, we have identified the differentially expressed phytohormone genes of abscisic acid, auxin, brassinosteroid, cytokinin, ethylene, jasmonate, gibberellin, and salicylic acid in chilling-stressed potato plant.

Endogenous abscisic acid (ABA) levels mainly increases as response to osmotic (drought, salinity) stress but some extent to cold. The exogenous ABA applications were also reported to induce a number of cold-responsive genes, but ABA role in regulation of cold-responsive genes is not clear yet [60]. However, many cold- and drought-inducible genes share common motifs such as DRE/CRT and ABRE in their promoters [68], suggesting the existence of a cross-talk between ABA-independent and ABA-dependent transduction cascades [18]. Therefore, cold stress could either directly induce ABA genes or decreased water availability resulting from cold stress indirectly induces the ABA genes. We have identified two ABA up-regulated genes with clone names such as STMEV91 (4-h point/3.97-fold) and STMHK55 (48-h point/1.72-fold). The clone STMEV91 showed 3.97 highest expression fold at 4-h time period and was similar to *Arabidopsis FIP1* gene with putative vesicle transport activity. Auxin regulates many physiological processes including plant growth and development and abiotic stress response [69]. Shibasaki et al. [70] reported that cold stress affects the auxin transport pathway in *Arabidopsis* by inhibiting the various intracellular proteins including auxin efflux carriers. In a different study of *Arabidopsis*, the expression profile of *ARF* and *Aux/IAA* gene family members were demonstrated to be changed during cold acclimation [71]. Transcriptome analysis of rice revealed that at least 154 genes were auxin-induced and 50 auxin-repressed under one or more stress conditions including desiccation, salt, and cold [69]. We have identified 12 auxin-induced genes with clone names such as STMCM67 (4-h point/3.60-fold), STMCB07 (4-h point/2.02-fold; 8-h point/1.83-fold), STMEY49 (4-h point/0.85-fold; 8-h point/1.05-fold), STMJB61 (8-h point/0.99-fold), STMCF91 (8-h point/1.53-fold), STMCI37 (8-h point/0.90-fold), STMHY44 (12-h point/1.79-fold), STMEN19 (12-h point/1.15-fold), STMGE49 (12-h point/1.64-fold), STMHG73 (12-h point/0.97-fold), STMGT44 (48-h point/0.79-fold), and STMJB61 (48-h point/1.12-fold). The clone STMCM67 expressed with 3.60 highest fold at 4-h point and showed similarity to *Arabidopsis ILL4* gene with IAA-Ala conjugate hydrolase and metallopeptidase activities in root, stem, and flower. Induction of a number of auxin-related genes in potato could indicate the modulation of auxin transport by cold stress. Ethylene (ET) modulates various physiological processes including germination, fruit ripening, organ abscission, pathogen, senescence, and biotic/abiotic stresses [72]. Ethylene signaling was reported to negatively regulate the freezing tolerance; increased ethylene level resulted in decreased freezing tolerance while inhibiting ethylene biosynthesis/signaling conferred the enhanced freezing tolerance in *Arabidopsis* [73]. A similar negative correlation was also demonstrated between ET level and freezing tolerance in *Medicago truncatula* [74]. However, an ET biosynthesis inhibitor 1-methylcyclopropene (1-MCP) decreased the cold tolerance in tomato, showing a positive effect on cold tolerance [75]. This indicates that ET's role on cold tolerance could be species-dependent. However, further studies are needed to confirm whether positive or negative regulatory role of ET on cold tolerance in potato plant. We have identified five ethylene up-regulated genes with clone names such as STMHA07 (4-h point/3.17-fold), STMHQ43 (4-h point/0.92-fold), STMEA01 (8-h point/1.12-fold), STMDZ32 (48-h point/0.70-fold), and STMJH61 (48-h point/1.84-fold). The clone STMHA07 showed 3.17 highest fold at 4-h time point and was similar to tomato *ACS5* gene with 1-

aminocyclopropane-1-carboxylate synthase and pyridoxal phosphate binding activities. In addition, we have also identified some other phytohormone-related genes of cytokinin, brassinosteroid, jasmonate, gibberellin, and salicylic acid (Fig. 4d). In the view of above-mentioned findings, phytohormones seem to collaboratively coordinate with each other to improve the plant adaptability to cold stress.

Chilling Stress-Induced Lipid Metabolism Regulation

Plant cold acclimation involves a wide spectrum of transcriptional regulators that modulate cold-regulated (*COR*) genes, some of which encode lipid metabolism-related proteins and enzymes to protect the plant cell from freezing injury [76]. The primary site of freezing damage is the cellular membranes. Cold stress-related changes in membrane composition and fluidity, thereby in lipid metabolism have crucial importance in cold acclimation process [14, 15]. The content of di-unsaturated fatty acids (FAs) and phospholipids in plasma membrane increases to reduce the cold-induced membrane damage, and this leads to membrane rigidification [77]. Membrane rigidification acts as a physical signal to induce plasma membrane proteins, which then activate other downstream signaling networks to maintain membrane stability and integrity [78]. In addition, biosynthesis of desaturases was reported to be up-regulated to enhance the cold tolerance [79]. By contrast, some lipid metabolism-related molecules involved in FA synthesis and elongation, phospholipid and steroids/squalene synthesis, and lipase and lysophospholipase degradation were also reported to be down-regulated either as response to cold-related reduced growth or to preserve the cold-induced altered lipid composition [71, 77]. In this study, we have identified six up-regulated FA synthesis and elongation genes, 10 up-regulated lipid degradation genes, two up-regulated FA desaturation genes, one up-regulated phospholipid synthesis gene, six up-regulated exotics (steroids, squalene, etc.) genes, and one up-regulated lipid transfer protein gene (Fig. 4b). In lipid metabolism, FA synthesis and elongation, lipid degradation, and exotics (steroids, squalene, etc.) genes were mainly expressed. The up-regulated FA synthesis and elongation genes included with clone names such as STMGC43 (4-h point/3.25-fold), STMDB67 (4-h point/1.96-fold; 8-h point/0.70-fold), STMDQ79 (12-h point/0.77-fold), STMFA38 (48-h point/0.70-fold), STMHN19 (48-h point/0.97-fold), and STMJJ74 (48-h point/0.67-fold). The clone STMGC43 demonstrated 3.25 highest fold at 4-h time point and showed similarity to *Elaeis oleifera* palmitoyl-acyl carrier protein thioesterase gene with thiolester hydrolase activity. The up-regulated lipid degradation genes included the clones such as STMIU19 (4-h point/2.84-fold; 12-h point/2.01-fold), STMJP61 (4-h point/1.94-fold), STMDV25 (4-h point/0.89-fold), STMJN86 (8-h point/1.15-fold), STMHR01 (12-h point/1.59-fold), STMJG61 (12-h point/1.40-fold), STMIG79 (12-h point/1.81-fold), STMIT79 (12-h point/1.90-fold), STMGW07 (48-h point/2.22-fold), and STMIH07 (48-h point/3.09-fold). The clone STMIH07 demonstrated the highest expression fold with 3.09 at 48-h time period and showed similarity to *Arabidopsis PLA2-ALPHA* gene with calcium ion binding and phospholipase A2 activities. The up-regulated exotics (steroids, squalene, etc.) genes included the clones such as STMJA61 (8-h point/1.17-fold), STMHL68 (8-h point/2.51-fold), STMDB31 (12-h point/0.89-fold), STMDH37 (12-h point/2.36-fold), STMGX37 (48-h point/1.75-fold), and STMIF80 (48-h point/1.03-fold). Therefore, it seems that genes involved in chilling-induced lipid metabolism and their expression patterns could lead the potato to alter its membrane composition, thereby, to transduce the downstream signaling network to develop a cold response/acclimation.

Chilling Stress-Induced Photosynthetic Regulation

Photosynthesis has crucial importance as being a universal stress sensor in green plants. Photosystem I (PS I) and II (PS II) and Rubisco are thought to be primary stress sensors in the chloroplast. The stress-related changes in these sensors cause to generate stress signals like production of reactive oxygen species (ROSs), change of sugar levels, change of redox reactions from photosynthetic electron transport system, and energy imbalance, which accordingly lead to the metabolic/molecular reprograming of stress adaptation [80, 81]. The photosynthetic genes were previously reported to be down-regulated as response to cold stress [82]. A number of genes in light signaling have been also demonstrated to be down-regulated in medium to long-term cold exposure; however, short-term exposure mainly up-regulated the genes, probably resulting from a temporary "shock state" before cold adaptation [11]. We have identified nine up-regulated and 15 down-regulated light reaction genes in chilling-stressed potato (Fig. 4a). The up-regulated light reaction genes included the clones such as STMEA37 (4-h point/3.27-fold), STMEY61 (8-h point/1.19-fold), STMJB01 (8-h point/1.48-fold), STMCH07 (48-h point/2.69-fold), STMCO92 (48-h point/0.62-fold), STMCS92 (48-h point/0.74-fold), STMCX85 (48-h point/1.12-fold), STMEU31 (48-h point/1.96-fold), and STMGQ55 (48-h point/0.85-fold). It seems that as low temperatures caused to oxidative damage, plants may have minimized this damage by down-regulating the light reactions genes, which otherwise could increase the oxidative damage. Abiotic stress factors can also change the rates of primary photochemical reactions and reduce the enzyme activities of Calvin cycle [80]. Low temperatures were also reported to inhibit electron transport by altering thylakoid lipids and to significantly decrease the enzymatic reaction rates involved in C, N, and S reduction [83]. We identified that five Calvin cycle genes were up-regulated and seven down-regulated, while one photorespiration gene were up-regulated and three down-regulated in chilling-stressed potato (Fig. 4a). This implicates that cold stress may have reductive effects on enzyme activities in Calvin cycle and photorespiration, resulting in down-regulated gene activities. The up-regulated Calvin cycle genes included clones such as STMES37 (4-h point/2.36-fold; 12-h point/3.21-fold), STMCP14 (4-h point/1.14-fold), STMCB56 (8-h point/0.67-fold), STMCP20 (8-h point/1.91-fold), and STMIY49 (12-h point/1.00). The clone STMES37 showed 3.21 highest expression fold at 12-h time point and was similar to tomato ribulose bisphosphate carboxylase/oxygenase activase gene with ATP binding activity. In addition, one photorespiration gene with clone name STMCE67 was up-regulated at 4-h time point with 1.19-fold.

In conclusion, we have identified the overall expression profile of genes at four time points (4, 8, 12, and 48 h) of chilling-exposed (+4 °C) potato plant, with a particular emphasis on DEGs. A number of genes with diverse biological functions have been found to be differentially expressed in chilling-stressed potato; however, main focus was on the genes related with transcription factors, phytohormones, lipid metabolism, signaling, and photosynthesis. We believe that findings of this study will be a valuable theoretical knowledge in terms of understanding the chilling-induced tolerance mechanisms in cultivated potato species as well as expanding their cultivation to different climatologic areas. In addition, identified genes could be used as potential candidate genes in engineering and for developing the cold stress-resistant food crops.

References

1. Evers, D., Bonnechère, S., Hoffmann, L., & Hausman, J. F. (2007). Physiological aspects of abiotic stress response in potato. *Belgian Journal of Botany*, *141–150*.
2. Jefferies, R. A., & MacKerron, D. K. L. (1993). Responses of potato genotypes to drought. II. Leaf area index, growth and yield. *Annals of Applied Biology*, *122*(1), 105–112.
3. Chinnusamy, V., Zhu, J. K., & Sunkar, R. (2010). Gene regulation during cold stress acclimation in plants. In R. Sunkar (Ed.), *Plant stress tolerance*. Methods in Molecular Biology, 639 (pp. 39–55). Totowa, NJ: Humana Press.
4. Floris, M., Hany, M., Elodie, L., Christophe, R., & Benoit, M. (2009). Post-transcriptional regulation of gene expression in plants during abiotic stress. *International Journal of Molecular Sciences*, *10*, 3168–3185.
5. Le, M. Q., Pagter, M., & Hincha, D. K. (2015). Global changes in gene expression, assayed by microarray hybridization and quantitative RT-PCR, during acclimation of three Arabidopsis thaliana accessions to sub-zero temperatures after cold acclimation. *Plant Molecular Biology*, *87*(1-2), 1–15.
6. Oufir, M., Legay, S., Nicot, N., Van, M. K., Hoffmann, L., Renaut, J., Hausman, J. F., & Evers, D. (2008). Gene expression in potato during cold exposure: changes in carbohydrate and polyamine metabolisms. *Plant Science*, *175*, 839–852.
7. Chawade, A., Lindlöf, A., Olsson, B., & Olsson, O. (2013). Global expression profiling of low temperature induced genes in the chilling tolerant japonica rice Jumli Marshi. *PLoS One*, *8*(12), e81729.
8. Laudencia-Chingcuanco, D., Ganeshan, S., You, F., Fowler, B., Chibbar, R., & Anderson, O. (2011). Genome-wide gene expression analysis supports a developmental model of low temperature tolerance gene regulation in wheat (*Triticum aestivum* L.). *BMC Genomics*, *12*, 299.
9. Svensson, J. T., Crosatti, C., Campoli, C., Bassi, R., Stanca, A. M., Close, T. J., & Cattivelli, L. (2006). Transcriptome analysis of cold acclimation in barley Albina and Xantha mutants. *Plant Physiology*, *141*, 257–270.
10. Chawade, A., Linden, P., Brautigam, M., Jonsson, R., Jonsson, A., Moritz, T., & Olsson, O. (2012). Development of a model system to identify differences in spring and winter oat. *PLoS One*, *7*, e29792.
11. Fowler, S., & Thomashow, M. F. (2002). Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *Plant Cell*, *14*, 1675–1690.
12. Evers, D., Legay, S., Lamoureux, D., Hausman, J. F., Hoffmann, L., & Renaut, J. (2012). Towards a synthetic view of potato cold and salt stress response by transcriptomic and proteomic analyses. *Plant Molecular Biology*, *78*(4-5), 503–514.
13. Solanke, A. U., & Sharma, A. K. (2008). Signal transduction during cold stress in plants. *Physiology and Molecular Biology of Plants*, *14*(1-2), 69–79.
14. Chinnusamy, V., Zhu, J., & Zhu, J. K. (2007). Cold stress regulation of gene expression in plants. *Trends in Plant Science*, *12*, 444–451.
15. Renaut, J., Planchon, S., Oufir, M., Hausman, J. F., Hoffmann, L., & Evers, D. (2009). , Identification of proteins from potato leaves submitted to chilling temperature. In L. V. Gusta, M. Wisniewski, & K. K. Tanino (Eds.), *Plant cold hardiness: from the laboratory to the field* (pp. 279–292). Wallingford: CAB Internationnal.
16. Guy, C., Kaplan, F., Kopka, J., Selbig, J., & Hincha, D. K. (2008). Metabolomics of temperature stress. *Physiologia Plantarum*, *132*, 220–235.
17. Verslues, P. E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J., & Zhu, J. K. (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant Journal*, *45*, 523–539.
18. Shinozaki, K., Yamaguchi-Shinozaki, K., & Seki, M. (2003). Regulatory network of gene expression in the drought and cold stress responses. *Current Opinion in Plant Biology*, *6*, 410–417.
19. Nakashima, K., & Yamaguchi-Shinozaki, K. (2006). Regulons involved in osmotic stress-responsive and cold stress-responsive gene expression in plants. *Physiologia Plantarum*, *126*, 62–71.
20. Kreps, J. A., Wu, Y., Chang, H. S., Zhu, T., Wang, X., & Harper, J. F. (2002). Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. *Plant Physiology*, *130*, 2129–2141.
21. Winfield, M. O., Lu, C., Wilson, I. D., Coghill, J. A., & Edwards, K. J. (2010). Plant responses to cold: transcriptome analysis of wheat. *Plant Biotechnology Journal*, *8*, 749–771.
22. Zhang, T., Zhao, X., Wang, W., Pan, Y., Huang, L., Liu, X., & Fu, B. (2012). Comparative transcriptome profiling of chilling stress responsiveness in two contrasting rice genotypes. *PLoS One*, *7*(8), e43274.
23. Tommasini, L., Svensson, J. T., Rodriguez, E. M., Wahid, A., Malatrasi, M., Kato, K., & Close, T. J. (2008). Dehydrin gene expression provides an indicator of low temperature and drought stress: transcriptome-based analysis of barley (*Hordeum vulgare* L.). *Functional & Integrative Genomics*, *8*(4), 387–405.

24. William, R. (2006). The association among gene expression responses to nine abiotic stress. *Treatments in Arabidopsis Thaliana Genetics*, *174*, 1811–1824.
25. Thimm, O., Bläsing, O., Gibon, Y., Nagel, A., Meyer, S., et al. (2004). Mapman: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *The Plant Journal*, *37*, 914–939.
26. Rotter, A., Usadel, B., Baebler, S., Stitt, M., & Gruden, K. (2007). Adaptation of the MapMan ontology to biotic stress responses: application in Solanaceous species. *Plant Methods*, *3*, 10.
27. Song, Y., Chen, Q., Ci, D., & Zhang, D. (2013). Transcriptome profiling reveals differential transcript abundance in response to chilling stress in *Populus simonii*. *Plant Cell Reports*, *32*(9), 1407–1425.
28. Tian, D. Q., Pan, X. Y., Yu, Y. M., Wang, W. Y., Zhang, F., Ge, Y. Y., & Liu, X. J. (2013). De novo characterization of the Anthurium transcriptome and analysis of its digital gene expression under cold stress. *BMC Genomics*, *14*(1), 827.
29. Wang, X. C., Zhao, Q. Y., Ma, C. L., Zhang, Z. H., Cao, H. L., Kong, Y. M., & Yang, Y. J. (2013). Global transcriptome profiles of Camellia sinensis during cold acclimation. *BMC Genomics*, *14*(1), 415.
30. Xu, W., Zhang, N., Jiao, Y., Li, R., Xiao, D., & Wang, Z. (2014). The grapevine basic helix-loop-helix (bHLH) transcription factor positively modulates CBF-pathway and confers tolerance to cold-stress in Arabidopsis. *Molecular Biology Reports*, *41*(8), 5329–5342.
31. Kim, S. A., Ahn, S. Y., Kim, S. H., Han, J. H., & Yun, H. K. (2014). Expression of basic helix-loop-helix transcripts during low temperature treatments in grapevines. *The Korean Society of Breeding Science*, *2*(2), 110–116.
32. Huang, X. S., Wang, W., Zhang, Q., & Liu, J. H. (2013). A basic helix-loop-helix transcription factor, PtrbHLH, of Poncirus trifoliata confers cold tolerance and modulates peroxidase-mediated scavenging of hydrogen peroxide. *Plant Physiology*, *162*(2), 1178–1194.
33. Feng, X. M., Zhao, Q., Zhao, L. L., Qiao, Y., Xie, X. B., Li, H. F., & Hao, Y. J. (2012). The cold-induced basic helix-loop-helix transcription factor gene MdCIbHLH1 encodes an ICE-like protein in apple. *BMC Plant Biology*, *12*(1), 22.
34. Wang, Y. J., Zhang, Z. G., He X. J , Zhou, H. L., Wen, Y. X., Dai, J. X., Zhang, J. S., & Chen, S. Y. (2003). A rice transcription factor OsbHLH1 is involved in cold stress response. Theoretical and Applied Genetics, 107, 1402–1409.
35. Seo, J. S., Joo, J., Kim, M. J., Kim, Y. K., Nahm, B. H., Song, S. I., & Choi, Y. D. (2011). OsbHLH148, a basic helix-loop-helix protein, interacts with OsJAZ proteins in a jasmonate signaling pathway leading to drought tolerance in rice. *The Plant Journal*, *65*(6), 907–921.
36. Fursova, O. V., Pogorelko, G. V., & Tarasov, V. A. (2009). Identification of ICE2, a gene involved in cold acclimation which determines freezing tolerance in Arabidopsis thaliana. *Gene*, *429*(1), 98–103.
37. Peng, H. H., Shan, W., Kuang, J. F., Lu, W. J., & Chen, J. Y. (2013). Molecular characterization of cold-responsive basic helix-loop-helix transcription factors MabHLHs that interact with MaICE1 in banana fruit. *Planta*, *238*(5), 937–953.
38. Chen, L., Song, Y., Li, S., Zhang, L., Zou, C., & Yu, D. (2012). The role of WRKY transcription factors in plant abiotic stresses. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1819*(2), 120–128.
39. Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., & Shinozaki, K. (2002). Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *The Plant Journal*, *31*(3), 279–292.
40. Zhou, Q. Y., Tian, A. G., Zou, H. F., Xie, Z. M., Lei, G., Huang, J., & Chen, S. Y. (2008). Soybean WRKY-type transcription factor genes, GmWRKY13, GmWRKY21, and GmWRKY54, confer differential tolerance to abiotic stresses in transgenic Arabidopsis plants. *Plant Biotechnology Journal*, *6*(5), 486–503.
41. Mare, C., Mazzucotelli, E., Crosatti, C., Francia, E., & Cattivelli, L. (2004). Hv-WRKY38: a new transcription factor involved in cold-and drought-response in barley. *Plant Molecular Biology*, *55*(3), 399–416.
42. Ramamoorthy, R., Jiang, S. Y., Kumar, N., Venkatesh, P. N., & Ramachandran, S. (2008). A comprehensive transcriptional profiling of the WRKY gene family in rice under various abiotic and phytohormone treatments. *Plant and Cell Physiology*, *49*(6), 865–879.
43. Hwang, E. W., Kim, K. A., Park, S. C., Jeong, M. J., Byun, M. O., & Kwon, H. B. (2005). Expression profiles of hot pepper (Capsicum annuum) genes under cold stress conditions. *Journal of Biosciences*, *30*(5), 657–667.
44. Jiang, Y., Duan, Y., Yin, J., Ye, S., Zhu, J., Zhang, F., & Luo, K. (2014). Genome-wide identification and characterization of the Populus WRKY transcription factor family and analysis of their expression in response to biotic and abiotic stresses. *Journal of Experimental Botany, 65*, 6629–6644.
45. Wang, L., Zhu, W., Fang, L., Sun, X., Su, L., Liang, Z., & Xin, H. (2014). Genome-wide identification of WRKY family genes and their response to cold stress in Vitis vinifera. *BMC Plant Biology*, *14*(1), 103.

46. Li, L., Yu, Y., Wei, J., Huang, G., Zhang, D., Liu, Y., & Zhang, L. (2013). Homologous HAP5 subunit from Picea wilsonii improved tolerance to salt and decreased sensitivity to ABA in transformed Arabidopsis. *Planta*, *238*(2), 345–356.
47. Chen, N. Z., Zhang, X. Q., Wei, P. C., Chen, Q. J., Ren, F., Chen, J., & Wang, X. C. (2007). AtHAP3b plays a crucial role in the regulation of flowering time in Arabidopsis during osmotic stress. *Journal of Biochemistry and Molecular Biology*, *40*, 1083–1089.
48. Nelson, D. E., Repetti, P. P., Adams, T. R., Creelman, R. A., Wu, J., Warner, D. C., Anstrom, D. C., Bensen, R. J., Castiglioni, P. P., & Donnarummo, M. G. (2007). Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proceedings of the National academy of Sciences of the United States of America*, *104*, 16450–16455.
49. Shi, H., Ye, T., Zhong, B., Liu, X., Jin, R., & Chan, Z. (2014). AtHAP5A modulates freezing stress resistance in Arabidopsis through binding to CCAAT motif of AtXTH21. *New Phytologist*, *203*(2), 554–567.
50. Nijhawan, A., Jain, M., Tyagi, A. K., & Khurana, J. P. (2008). Genomic survey and gene expression analysis of the basic leucine zipper transcription factor family in rice. *Plant Physiology*, *146*(2), 333–350.
51. Liu, C., Wu, Y., & Wang, X. (2012). bZIP transcription factor OsbZIP52/RISBZ5: a potential negative regulator of cold and drought stress response in rice. *Planta*, *235*(6), 1157–1169.
52. Tak, H., & Mhatre, M. (2013). Cloning and molecular characterization of a putative bZIP transcription factor VvbZIP23 from Vitis vinifera. *Protoplasma*, *250*(1), 333–345.
53. Zhang, L., Zhang, L., Xia, C., Zhao, G., Liu, J., Jia, J., & Kong, X. (2014). A novel wheat bZIP transcription factor, TabZIP60, confers multiple abiotic stress tolerances in transgenic Arabidopsis. *Physiologia Plantarum*, *153*(4), 538–554.
54. Liao, Y., Zou, H. F., Wei, W., Hao, Y. J., Tian, A. G., Huang, J., & Chen, S. Y. (2008). Soybean GmbZIP44, GmbZIP62 and GmbZIP78 genes function as negative regulator of ABA signaling and confer salt and freezing tolerance in transgenic Arabidopsis. *Planta*, *228*(2), 225–240.
55. Osakabe, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., & Tran, L. S. P. (2013). Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress. *Journal of Experimental Botany*, *64*(2), 445–458.
56. Hwang, S. G., Kim, D. S., & Jang, C. S. (2011). Comparative analysis of evolutionary dynamics of genes encoding leucine-rich repeat receptor-like kinase between rice and Arabidopsis. *Genetica*, *139*, 1023–1032.
57. Marshall, A., Aalen, R. B., Audenaert, D., Beeckman, T., Broadley, M. R., Butenko, M. A., & De Smet, I. (2012). Tackling drought stress: receptor-like kinases present new approaches. *The Plant Cell Online*, *24*(6), 2262–2278.
58. Dardick, C., Chen, J., Richter, T., Ouyang, S., & Ronald, P. (2007). The rice kinase database. A phylogenomic database for the rice kinome. *Plant Physiology*, *143*, 579–586.
59. Shiu, S. H., & Bleecker, A. B. (2003). Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis. *Plant Physiology*, *132*, 530–543s.
60. Huang, G. T., Ma, S. L., Bai, L. P., Zhang, L., Ma, H., Jia, P., & Guo, Z. F. (2012). Signal transduction during cold, salt, and drought stresses in plants. *Molecular Biology Reports*, *39*(2), 969–987.
61. Chinnusamy, V., Schumaker, K., & Zhu, J. K. (2003). Molecular genetic perspectives on cross-talk and specificity in abiotic stress signaling in plants. *Journal of Experimental Botany*, *55*(395), 225–236.
62. Morrison, D. K. (2012). MAP kinase pathways. *Cold Spring Harbor Perspectives in Biology*, *4*(11), a011254.
63. Reddy, A. S., Ali, G. S., Celesnik, H., & Day, I. S. (2011). Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. *Plant Cell*, *23*, 2010–2032.
64. DeFalco, T. A., Bender, K. W., & Snedden, W. A. (2010). Breaking the code: Ca2+ sensors in plant 371 signaling. *Biochemistry Journal*, *425*, 27–40.
65. Batistic, O., & Kudla, J. (2012). Analysis of calcium signaling pathways in plants. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1820*(8), 1283–1293.
66. Santner, A., & Estelle, M. (2009). Recent advances and emerging trends in plant hormone signaling. *Nature*, *459*(7250), 1071–1078.
67. Peleg, Z., & Blumwald, E. (2011). Hormone balance and abiotic stress tolerance in crop plants. *Current Opinion in Plant Biology*, *14*(3), 290–295.
68. Narusaka, Y., Nakashima, K., Shinwari, Z. K., Sakuma, Y., Furihata, T., Abe, H., Narusaka, M., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2003). Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of Arabidopsis rd29A gene in response to dehydration and high-salinity stresses. *Plant Journal*, *34*, 137–148.
69. Jain, M., & Khurana, J. P. (2009). Transcript profiling reveals diverse roles of auxin-responsive genes during reproductive development and abiotic stress in rice. *FEBS Journal*, *276*(11), 3148–3162.
70. Shibasaki, K., Uemura, M., Tsurumi, S., & Rahman, A. (2009). Auxin response in Arabidopsis under cold stress: underlying molecular mechanisms. *The Plant Cell Online*, *21*(12), 3823–3838.

71. Hannah, M. A., Heyer, A. G., & Hincha, D. K. (2005). A global survey of gene regulation during cold acclimation in Arabidopsis thaliana. *PLoS Genetics*, *1*, 179–196.
72. Chen, Y. F., Etheridge, N., & Schaller, G. E. (2005). Ethylene signal transduction. *Annals of Botany*, *95*(6), 901–915.
73. Shi, Y., Tian, S., Hou, L., Huang, X., Zhang, X., Guo, H., & Yang, S. (2012). Ethylene signaling negatively regulates freezing tolerance by repressing expression of CBF and type-A ARR genes in Arabidopsis. *The Plant Cell Online*, *24*(6), 2578–2595.
74. Zhao, M., Liu, W., Xia, X., Wang, T., & Zhang, W. H. (2014). Cold acclimation-induced freezing tolerance of Medicago truncatula seedlings is negatively regulated by ethylene. *Physiologia Plantarum*, *152*(1), 115–129.
75. Zhao, D., Shen, L., Fan, B., Yu, M., Zheng, Y., Lv, S., & Sheng, J. (2009). Ethylene and cold participate in the regulation of LeCBF1 gene expression in postharvest tomato fruits. *FEBS Letters*, *583*(20), 3329–3334.
76. Guo, L., Yang, H., Zhang, X., & Yang, S. (2013). Lipid transfer protein 3 as a target of MYB96 mediates freezing and drought stress in Arabidopsis. *Journal of Experimental Botany*, *64*(6), 1755–1767.
77. Uemura, M., Joseph, R. A., & Steponkus, P. L. (1995). Cold acclimation of Arabidopsis thaliana (effect on plasma membrane lipid composition and freeze-induced lesions). *Plant Physiology*, *109*, 15–30.
78. Yadav, S. K. (2010). Cold stress tolerance mechanisms in plants. A review. *Agronomy for Sustainable Development*, *30*, 515–527.
79. Amiri, R. M., Yur'eva, N. O., Shimshilashvili, K. R., Goldenkova-Pavlova, I. V., Pchelkin, V. P., Kuznitsova, E. I., & Nosov, A. M. (2010). Expression of acyl-lipid Δ12-desaturase gene in prokaryotic and eukaryotic cells and its effect on cold stress tolerance of potato. *Journal of Integrative Plant Biology*, *52*(3), 289–297.
80. Biswal, B., Joshi, P. N., Raval, M. K., & Biswal, U. C. (2011). Photosynthesis, a global sensor of environmental stress in green plants: stress signalling and adaptation. *Current Science (Bangalore)*, *101*(1), 47–56.
81. Koc, I., Filiz, E., & Tombuloglu, H. (2015). Assessment of miRNA expression profile anddifferential expression pattern of target genes in cold-tolerant and cold-sensitive tomato cultivars. *Biotechnology & Biotechnological Equipment*, doi:10.1080/13102818.2015.1061447
82. Savitch, L. V., Barker-Astrom, J., Ivanov, A. G., Hurry, V., Oquist, G., et al. (2001). Cold acclimation of Arabidopsis thaliana results in incomplete recovery of photosynthetic capacity, associated with an increased reduction of the chloroplast stroma. *Planta*, *214*, 295–303.
83. Huner, N. P. A., Oquist, G., & Sarhan, F. (1998). Energy balance and acclimation to light and cold. *Trends in Plant Science*, *3*, 224–230.