Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2015) 39: 1021-1032

doi:10.3906/bot-1503-25

Application of data analysis in cold stress: a case study of *Nicotiana benthamiana*

İbrahim KOÇ[1,2], Zihni Onur ÇALIŞKANER[1], Ertuğrul FİLİZ[3,*]

[1]Department of Molecular Biology and Genetics, Faculty of Science, Gebze Technical University, Gebze, Kocaeli, Turkey
[2]Department of Crop Sciences, University of Illinois at Urbana-Champaign, Champaign, IL, USA
[3]Department of Crop and Animal Production, Çilimli Vocational School, Düzce University, Düzce, Turkey

Received: 16.03.2015 • **Accepted/Published Online:** 28.09.2015 • **Printed:** 21.12.2015

Abstract: Cold stress is a major environmental factor in plant life cycles. *Nicotiana benthamiana*, which belongs to the family Solanaceae, is one of the most commonly used model species in plant–microbe interaction studies. In total, 5205 differentially expressed genes were identified under cold stress in *N. benthamiana*. Of these, 5029 were upregulated and 176 were downregulated within four time periods (4 h, 12 h, 24 h, and 48 h). The common up- and downregulated genes were identified as 692 and 6, respectively. The functional annotations of these genes were studied and these common genes involved in protein, RNA, miscellaneous enzyme families, signaling, stress, lipid, and carbohydrate metabolisms were enriched by using MapMan ontology. In addition, a total of 22 cold-inducible transcription factors were enriched, including subsets of the zinc finger family, *bHLH*, *E2F/DP*, *bZIP*, *SET* domain, *GRAS*, *MYB*, *ARF*, *CO*-like, Homeobox, and *DOF* zinc finger family members. Our findings will pave the way for understanding the expression of cold-inducible genes as a response to cold stress in *Nicotiana* species. This study will also be a valuable resource for crop improvement studies under abiotic stress conditions for *Nicotiana* plants.

Key words: Solanaceae, abiotic stress, gene expression, differentially expressed genes, chilling stress

1. Introduction

Cold stresses including chilling (<20 °C) and freezing (<0 °C) temperatures negatively affect plant growth and development and seed production. Plants struggle with cold stress by improving stress tolerance (Bray et al., 2000; Chinnusamy et al., 2007). Chilling decreases the membrane fluidity by causing the impairment of unsaturated membrane lipids and freezing temperatures lead to membrane damage by severe cellular dehydration, associated with ice formation (Wang et al., 2006; Solanke and Sharma, 2008). In the cold stress pathway, cytosolic Ca^{2+} is considered as an important second messenger in low-temperature signal transduction (Figure 1). Calmodulin (CaM), CaM domain-containing protein kinases (CDPKs), calcineurin B-like proteins (CBLs), and CBL-interacting protein kinases (CIPKs) are among the major Ca^{2+} sensors in plants (Solanke and Sharma, 2008). Thanks to microarray technologies, a large number of cold stress-responsive genes have been identified in various plant species. These genes include three main groups: 1) signaling components (protein kinases and transcription factors), 2) functional components (enzymes in metabolic pathways, aquaporins, etc.), and 3) small noncoding RNAs, namely micro-RNAs (miRNAs) (Shen et al., 2014; Koc et al., 2015a). Moreover, many transcription factor genes, including the *WRKY* family, DRE-binding protein (*DREB*) family, zinc-finger family, ethylene-responsive element binding factor (*ERF*) family, *MYB* family, basic helix–loop–helix (*bHLH*) family, basic-domain leucine zipper (*bZIP*) family, *NAC* family, and homeodomain transcription factor families and retrotransposons are also activated with harsh stress conditions (Shinozaki et al., 2003; Koc et al. 2015b). A class of *DREB/CBF* transcription factors, which bind to *DRE/CRT cis*-elements in the promoter regions of target genes, is commonly known for pathways in cold-inducible genes (Maruyama et al., 2009). Recent studies of *Arabidopsis thaliana* have also demonstrated the importance of *DREB/CBF* transcription factors in cold stress. In addition, ICE1, MYB15, and CAMTA3 proteins have been identified as regulators of *DREB1/CBF* gene expression (Chinnusamy et al., 2007; Doherty et al., 2009). Thus, biotic/abiotic stress conditions in plants cause significant changes in global gene expression. In *A. thaliana*, it has been reported that nearly 30% of the transcriptome is regulated by abiotic stress, and 2409 genes have been determined to have considerable

* Correspondence: ertugrulfiliz@gmail.com

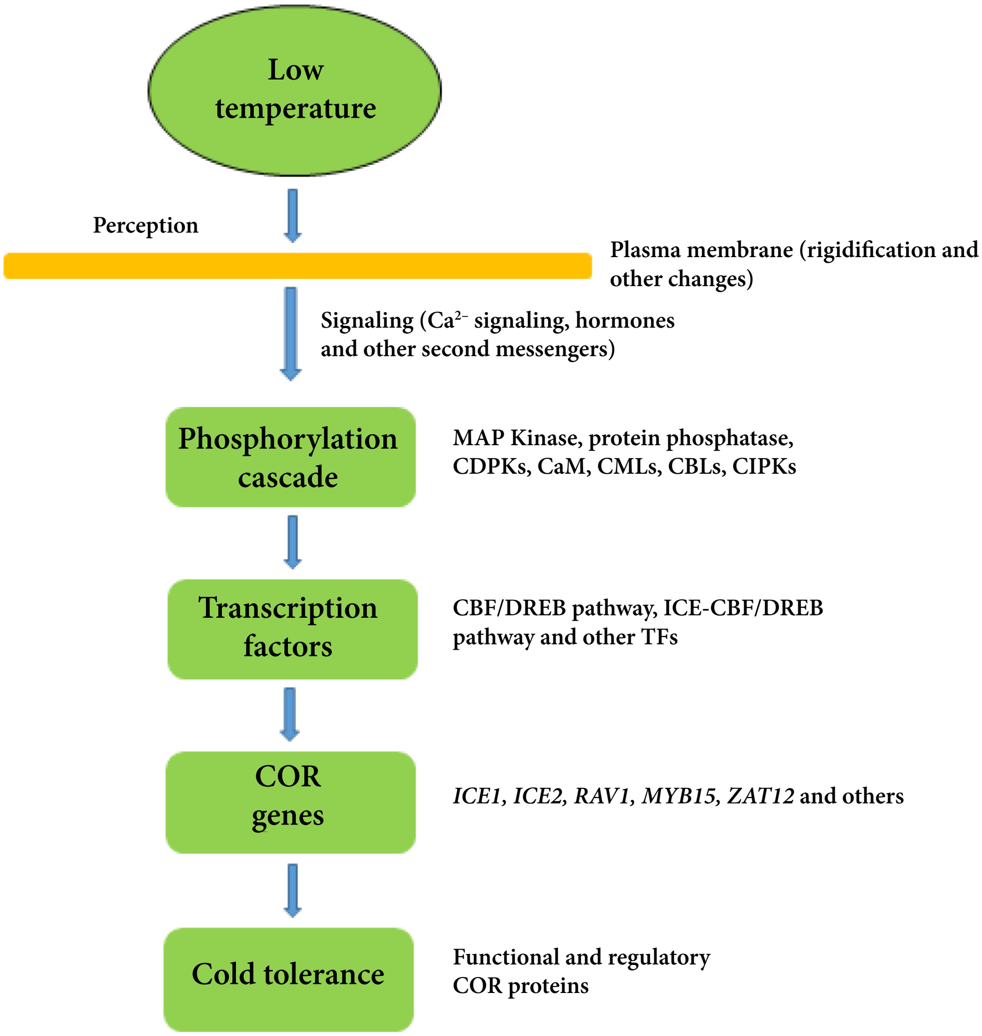

Figure 1. Cold signaling pathway in *Arabidopsis* (modified from Rahman, 2013).

importance for cold, salt, and drought tolerance (Kreps et al., 2002). Microarray expression analyses offer important opportunities for the systematic evaluation of biological responses. Huge numbers of transcript data were evaluated and visualized easily with various tools (Urbanczyk-Wochniak et al., 2006).

N. benthamiana is one of the most commonly used model species in studies of plant–microbe interactions and other research topics. Its genome contains 19 chromosomes (~3 Gb). It belongs to the family Solanaceae, and thus it is a close relative of tomato (*Solanum lycopersicum*) and potato (*S. tuberosum*) (Goodin et al., 2008; Bombarely et al., 2012). In this study, we aimed to identify and characterize the differentially expressed genes (DEGs) at four time points (4 h, 12 h, 24 h, and 48 h) of cold-stressed *N. benthamiana* plants. We have specifically focused on the common up- and downregulated genes for all the time periods. We think that the findings of this study will significantly contribute to the Solanaceae genomics in order to understand the cold acclimation mechanisms exclusively in *Nicotiana* species.

2. Materials and methods

The gene expression data of *N. benthamiana* plants showing differential expression under cold stress were supplied by the GEO database (http://www.ncbi.nlm.nih.gov/geo/) of the NCBI. The expression data of cold stress were obtained from GSE8203 by using the MATLAB program. Researchers subjected to *N. benthamiana* cold stress at 4 °C. In the experiment, TIGR10 K potato microarrays containing 15.264 cDNAs (http://www.jcvi.org/potato/sol_ma_microarrays.shtml) were used. We retrieved the gene expression values in log2 form for four time points (4 h, 12 h, 24 h, and 48 h). The fold change between stress and control conditions was expressed by stress/control formula. The log ratios below –2 and above +2 were selected for down- and upregulated genes, respectively. Biological replicates were not performed in the experiment. Since there was no replication, we determined common up- and downregulated genes at all time points to increase the reliability of the study. We should note that expression values of up- and downregulated genes were nearly same for all time points (Table S1; on the journal's website). Annotation and functional characterization was assigned by using MapMan (Stu_TIGR.m02 August07). MapMan implementation in the current study was helpful since it facilitates biological interpretation and provides a global overview of the results (Rotter et al., 2007).

3. Results

3.1. Identification of genes involved in cold acclimation

Absolute values of log2 ratio (≥2 and ≤–2) were used as thresholds to confirm the significance of DEGs. Cold-induced genes were identified based on the fold change of each gene (Figure 2; Table S1). A total of 5205 DEGs were identified. Of these, 5029 were upregulated and 176 were downregulated. The common up- and downregulated genes were found as 692 and 6, respectively.

3.2. Functional annotation of common DEGs

Although a large number of cold-responsive DEGs have been identified in cold-stressed *N. benthamiana*, only common up- and downregulated genes were annotated (Figure 3; Table S1). MapMan BINs were applied to understand the biological significance of DEGs. This approach is based on the generation of a dictionary of terms that use canonical syntax for knowledge representation (Kim and Caetano-Anollés, 2010). For upregulated genes, 387 DEGs (56%) were annotated in 28 major BINs, while 305 genes (44%) could not be assigned to any ontology. The top ten upregulated genes in cold stress were distributed among protein metabolism (BIN 29, 13%), RNA metabolism (BIN 27, 8.1%), miscellaneous enzyme families (BIN26, 4.6%), signaling pathway genes (BIN 30, 4.1%), stress genes (BIN 20, 3.6%), cell (BIN 31, 2.7%), lipid metabolism (BIN 11, 2.4%), transport (BIN 34, 2.3%), cell wall and amino acid metabolism (BIN 10 and 13, 1.9%), and secondary metabolism, hormones, and development (BIN 16, 17, and 33, 1.7%). Downregulated genes were distributed among amino acid metabolism (BIN 13, 1), nucleotide metabolism (BIN 23, 1), and protein metabolism (BIN 29). However, four downregulated genes could not be assigned to any ontology.

The major BINs contained high relative gene numbers and were found in a broad range of subgroups. Genes in the protein pathway (BIN 29) were identified as mostly involved in protein degradation; posttranslational modification (PTM) and protein synthesis were also identified. In the RNA pathway (BIN 27), a large number of upregulated putative transcription factors were identified to belong to the auxin response factor (*ARF*) family, basic helix–loop–helix family (*bHLH*), *bZIP* transcription factor family, *C2H2* zinc finger family, *C3H* zinc finger family, *E2F/DP* transcription factor family, *G2*-like transcription factor family (*GARP*), *GRAS* transcription factor family, homeobox transcription factor family, *MYB* domain transcription factor family, *SET*-domain transcriptional regulator family, squamosa promoter binding protein family, and nucleosome/chromatin assembly factor group (Table 1; Figure 4).

Miscellaneous enzyme families (BIN 26) contained various metabolic upregulated genes such as cytochrome P450, glutathione S transferases, UDP glucosyl and glucoronyl transferases, lipid transfer protein (LTP) family protein, and alcohol dehydrogenases. The stress pathway (BIN 20) contained various subgroups related with cold, drought/salt, heat, and PR10 proteins. In secondary

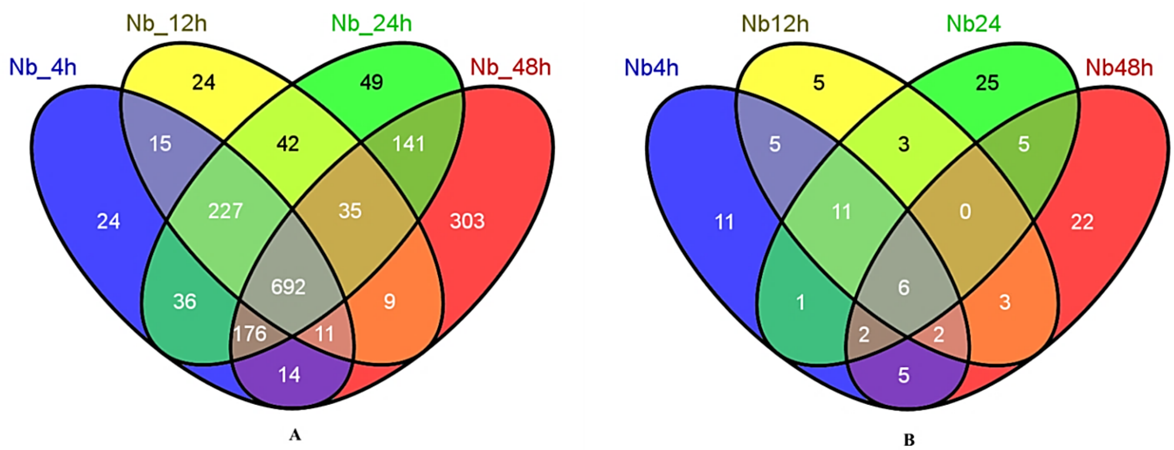

Figure 2. Number of differentially expressed genes in *N. benthamiana* at four time points (4, 12, 24, 48 h). A and B show up- and downregulated genes, respectively.

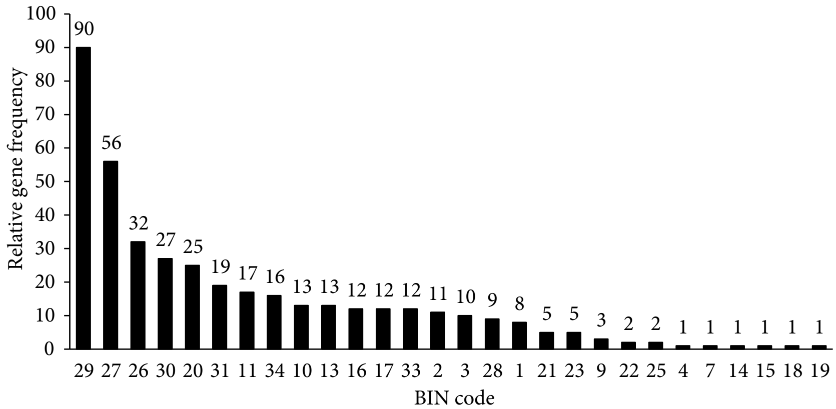

Figure 3. Distribution of *N. benthamiana* upregulated genes among MapMan BINs. These genes were classified into MapMan BINs and the number of items was counted for each BIN. BIN 1, Photosynthesis; BIN 2, Major carbohydrates; BIN 3, Minor carbohydrates; BIN 4, Glycolysis; BIN 5, Fermentation; BIN 6, 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.

metabolism (BIN 16), anthocyanins, dihydroflavonols, carotenoids, phosphomevalonate kinase, terpenoids, betaine, phenylpropanoids, and wax genes were detected. The signaling pathway (BIN 30) contained a wide range of subgroups such as receptor kinases, calcium signaling, G-proteins, sugar and nutrient physiology, light, and phosphinositides (Figure 5A). In hormone metabolism (BIN 17), abscisic acid synthesis-degradation, auxin signal transduction, cytokinin signal transduction, ethylene signal transduction, ethylene synthesis-degradation, and gibberellin induced-regulated-responsive-activated genes were upregulated (Figure 5B). In photosynthesis metabolism (BIN 1), some photosystem II, Calvin cycle, and photorespiration genes were upregulated (Figure 5C). In lipid metabolism (BIN 11), fatty acid (FA) synthesis and elongation (acetyl CoA carboxylation, ACP desaturase, ACP protein, ACP thioesterase, acyl CoA ligase, enoyl ACP reductase, long chain fatty acid CoA ligase, enoyl CoA hydratase, and phospholipid synthesis) and steroid-squalene synthases were identified (Figure 5D). Expression levels of cold-induced upregulated genes were visualized with their putative functions in metabolism by using the "Metabolism overview" pathway (Figure 6). Particularly, light reactions, lipid reactions, and major-minor carbohydrate metabolisms were upregulated based on enrichment analysis.

Based on downregulated genes, one gene of amino acid, protein, and nucleotide metabolisms was identified as downregulated, while three downregulated genes were not assigned.

4. Discussion

4.1. Analysis of differentially expressed genes

Cold stress induces many physiological and biochemical mechanisms in cells in order to alleviate or overcome stress factors. Besides, lower temperatures could affect many metabolic events such as water and nutrient uptake, membrane fluidity, and protein and nucleic acid conformation (Winfield et al., 2010). In the current study, transcriptomic data of cold-stressed *N. benthamiana* plants were evaluated at different time points (4, 12, 24, and 48 h) in order to identify up- and downregulated genes.

Microarray analysis of chilling-tolerant rice cultivar JM (Jumli Marshi) under cold stress showed that 4636 (1490 upregulated and 3146 downregulated) genes were significantly differentially expressed. The number of DEGs in four common cold-induced rice cultivars was reported as 182 (Chawade et al., 2013). In *Populus simonii*, 5267 genes were reported to be upregulated while 6359 were downregulated under cold stress (Song et al., 2013). In tropical flower *Anthurium andraeanum*, a total of 4363 genes were identified to be significantly changed under cold stress and nearly 30% of genes were found to be cold-inducible (Tian et al., 2013). In the three wheat varieties of Harnesk, Paragon, and Solstice, over 2% of the whole transcriptome exhibited an expression level of greater than two-fold change in response to cold stress. In these varieties, 1711 genes were upregulated while 1402 were downregulated, with 394 common genes (Winfield et al., 2010). In tea plant (*Camellia sinensis*), 1770 differentially expressed genes were reported; of these, 1168 were

Table 1. Differentially expressed genes involved in TFs in response to cold stress.

Clone name	BIN code	Annotation	TF family	log2 ratio			
				4 h vs. control	12 h vs. control	24 h vs. control	48 h vs. control
STMFB31	27.3.11	C2H2 zinc finger family	ZFM (zinc finger family)	3.30	3.37	3.41	3.27
STMGB27	27.3.11	C2H2 zinc finger family	ZFM (zinc finger family)	3.00	3.68	3.86	3.87
STMGT65	27.3.11	C2H2 zinc finger family	ZFM (zinc finger family)	3.08	2.37	3.45	3.33
STMIF84	27.3.11	C2H2 zinc finger family	ZFM (zinc finger family)	3.79	3.47	3.87	4.57
STMCN84	27.3.6	Basic helix–loop–helix family	bHLH	2.93	2.82	2.81	3.18
STMET64	27.3.6	Basic helix–loop–helix family	bHLH	2.56	2.64	2.80	2.95
STMGI14	27.3.6	Basic helix–loop–helix family	bHLH	3.20	3.19	3.42	3.26
STMDJ55	27.3.69	SET-domain transcriptional regulator family	SET	3.79	3.25	4.07	3.90
STMHA02	27.3.69	SET-domain transcriptional regulator family	SET	3.08	3.35	3.33	3.27
STMCJ45	27.3.39	AtSR transcription factor family	AtSR	2.42	2.52	2.62	2.49
STMCN34	27.3.62	Nucleosome/chromatin assembly factor group		3.35	3.16	3.99	3.47
STMCX47	27.3.52	Global transcription factor group		2.47	2.44	2.44	2.07
STMDB34	27.3.7	Constans-like zinc finger family	C2C2(Zn) CO-like	2.42	3.30	3.68	2.50
STMDC51	27.3.67	Putative DNA-binding protein		2.82	2.70	3.08	2.56
STMDC60	27.3.67	Putative DNA-binding protein		2.87	2.31	3.02	3.62
STMDM59	27.3.67	Putative DNA-binding protein		3.16	2.94	3.33	2.26
STMEH47	27.3.67	Putative DNA-binding protein		3.01	3.22	3.28	3.89
STMEL21	27.3.67	Putative DNA-binding protein		2.27	2.59	2.96	3.16
STMEV67	27.3.67	Putative DNA-binding protein		3.08	3.13	3.32	3.13
STMHK14	27.3.67	Putative DNA-binding protein		3.47	3.89	3.86	3.14
STMEG82	27.3.18	E2F/DP transcription factor family	E2F/DP	2.74	2.67	2.97	3.06
STMEH69	27.3.4	Auxin response factor family	ARF	2.89	2.09	3.42	3.38
STMEK50	27.3.20	G2-like transcription factor family	GARP	2.33	2.50	2.57	3.09
STMEO01	27.3.35	bZIP transcription factor family	bZIP	2.43	2.54	2.85	3.1
STMEV20	27.3.22	Homeobox transcription factor family	HB	3.05	3.39	3.66	3.84
STMEV22	27.3.21	GRAS transcription factor family	GRAS	2.69	2.84	3.29	2.59
STMGA07	27.3.26	MYB-related transcription factor family		4.03	3.65	4.24	4.11
STMGC50	27.3.25	MYB domain transcription factor family	MYB	3.32	3.71	3.87	3.61
STMGJ85	27.3.28	Squamosa promoter binding protein family	SBP	4.05	4.44	3.89	3.59
STMGN12	27.3.73	Zn-finger(CCHC)	ZFM (zinc finger family)	3.77	3.97	3.95	4.50
STMGW14	27.3.12	C3H zinc finger family	ZFM (zinc finger family)	3.75	3.98	3.69	3.32
STMGX55	27.3.64	PHOR1		3.48	3.09	4.19	3.82
STMIF10	27.3.8	C2C2(Zn)	DOF zinc finger family	2.84	2.66	3.55	3.58
STMII17	27.3.5	ARR	ARR	2.99	2.14	2.48	4.43
STMIP82	27.3.44	Chromatin remodeling factors		2.41	2.01	2.86	2.81

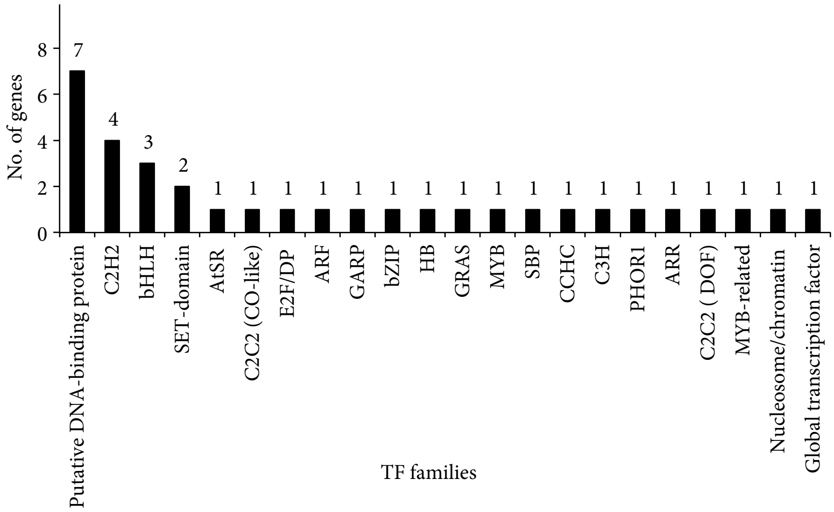

Figure 4. Differentially expressed transcription factor encoding genes. Numbers of cold-induced TF genes were identified from MapMan annotations. Unclassified TFs are not shown.

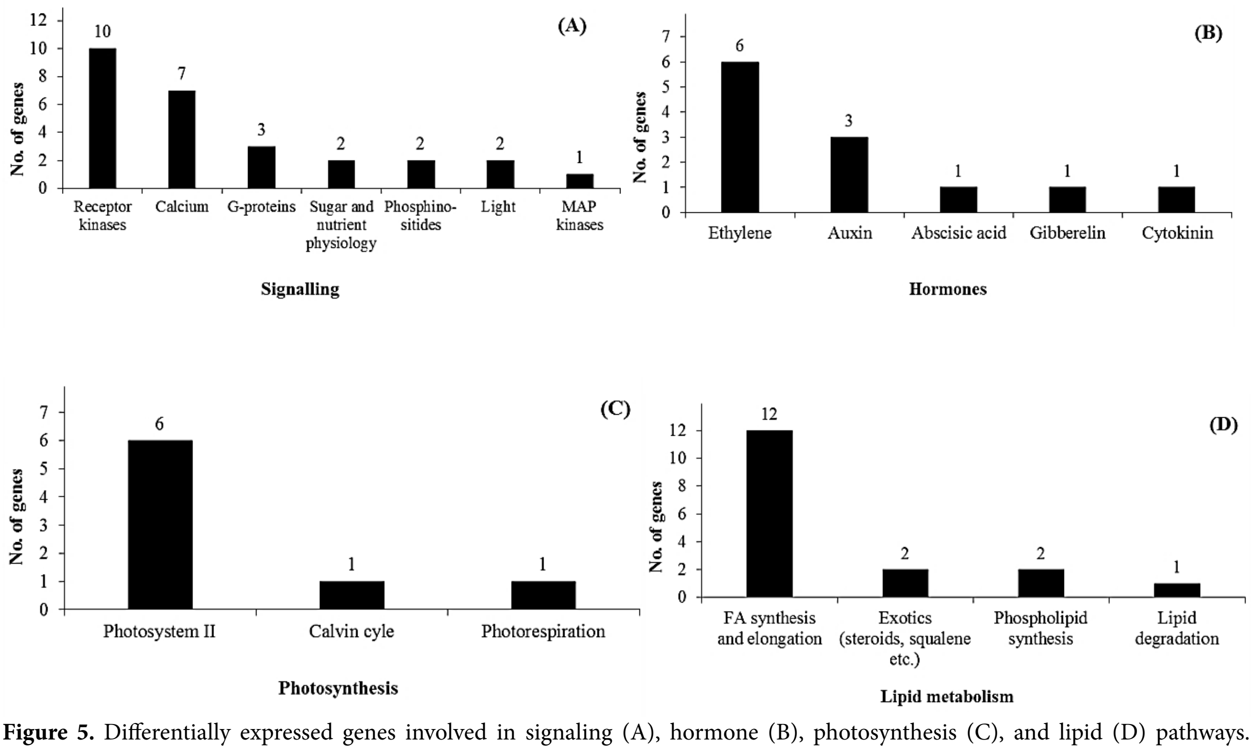

Figure 5. Differentially expressed genes involved in signaling (A), hormone (B), photosynthesis (C), and lipid (D) pathways. Numbers of cold-induced genes were identified from MapMan annotations.

upregulated and 602 were downregulated under cold stress (Wang et al., 2013). In this study, we have identified a total of 5205 DEGs. Of these, 5029 were upregulated and 176 were downregulated genes, and 698 genes were found to be common. This indicates that plant response to cold stress varies depending on plant genomic background against cold stress. According to the "Metabolism overview" pathway analysis (Figure 6), major and minor carbohydrate metabolisms, light reactions, and lipid metabolism were observed to have upregulated strikingly, suggesting that these genes may play important roles in response to cold stress in *N. benthamiana*.

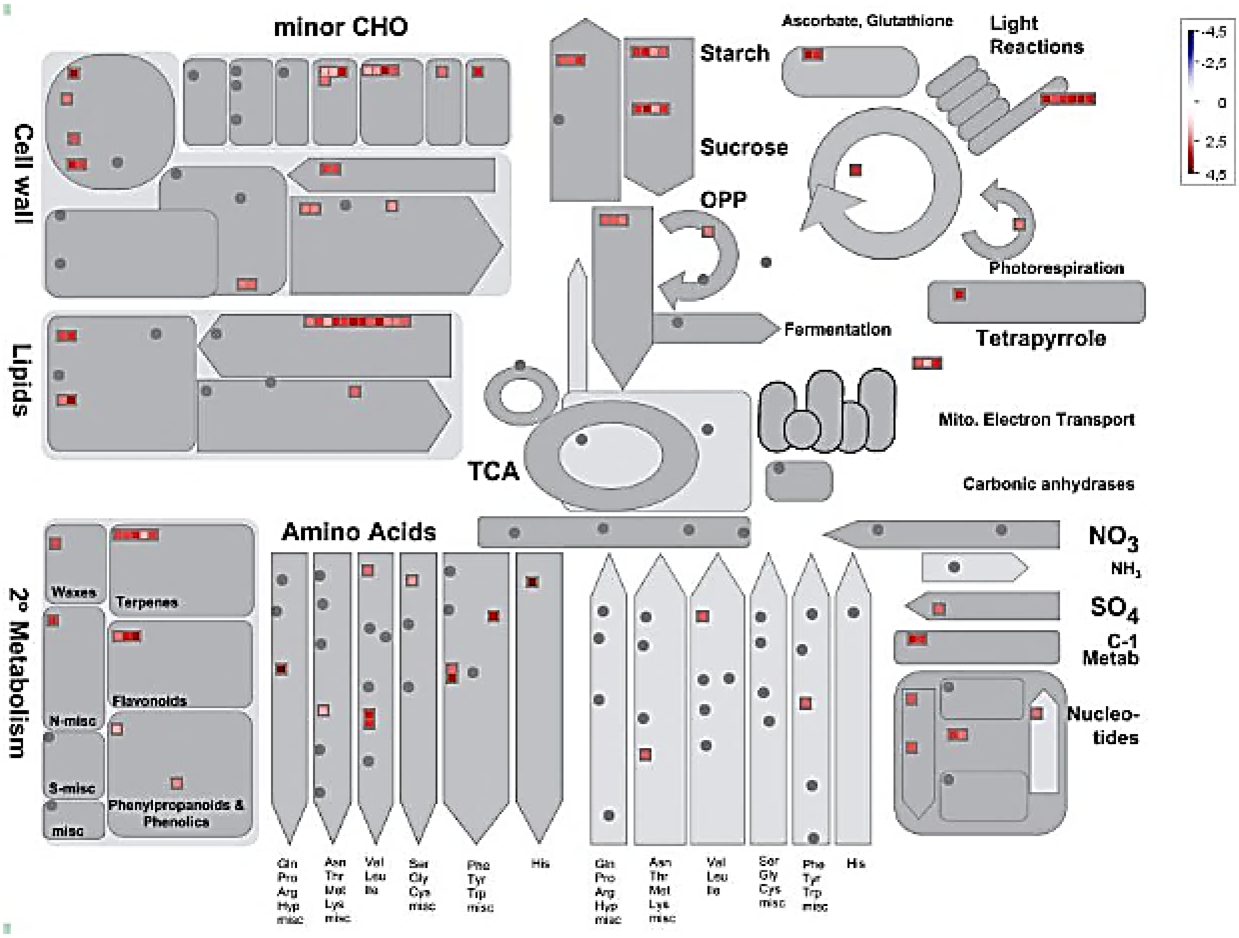

Figure 6. The "Metabolism overview" MapMan pathway was used for visualization of transcriptional changes in common upregulated genes (at least a two-fold change in expression) at 4 h with putative functions in metabolism. Red represents higher expression level in cold-stressed samples.

4.2. Transcription factors responsive to cold stress

Transcription factors have essential functions in plant development and stress tolerance (Chinnusamy et al., 2007). In model plants, TFs regulate many target genes by direct binding to *cis*-elements in promoter regions (Zhang et al., 2009). Forty-two upregulated genes encoding putative TFs were found in *N. benthamiana*, but among all the TFs, 22 TFs were enriched (Table 1; Figure 4). The most abundant TF was found as putative DNA binding factor with seven members, followed by the zinc-finger family with six members, basic helix–loop–helix family (*bHLH*) with three members, and *SET* domain TFs with two members. Zinc finger proteins (*ZFP*s) are important TFs with cysteines and/or histidines coordinating zinc atom(s). Cys2/His2 (*C2H2*)-type ZFPs containing the *EAR* transcriptional repressor domain play important roles in plants under biotic/abiotic stress conditions (Singh et al., 2010).

The *C2H2*-type zinc finger of *A. thaliana* 6 (At*ZAT6*) was transcriptionally stimulated by salt, dehydration, cold stress treatments, and pathogen infection (Shi et al., 2014). In *Anthurium* plants, zinc-figure proteins were abundantly accumulated within the initial first hour under cold stress condition (Tian et al., 2013). In *Eucalyptus grandis*, transcription of Egr*ZFP1-6* rapidly increased 2 h after cold treatment. Expressions of the Egr*ZFP1-7* gene were also detected in cold and salt resistance (Wang et al., 2014). In rice, seven Zn-finger TFs, both homeodomain and *C2H2*-type, were identified as binding to the promoter of Os*DREB1B* (Figueiredo et al., 2012). It can be suggested that *ZFP* TFs could play key roles in cold stress pathways in *N. benthamiana* and could crosstalk among stress signaling pathways. In cold stress, a well-documented cold signaling pathway is the C-repeat binding factor/DRE binding factor (*CBF*/*DREB*) transcriptional regulatory cascade (Thomashow, 1999). Interestingly, *CBF* TFs were not commonly upregulated in *N. benthamiana*. However, tomato, a close relative of *N. benthamiana*, has a complete *CBF* cold response pathway, but its *CBF* regulon differs from that of *Arabidopsis* (Zhang et al., 2004). For this case, *CBF* cold-responsive pathway genes in *N. benthamiana* need further validation. Basic helix–loop–helix (*bHLH*)-type transcription factors play important roles in the stress-adaptive regulation network (Xu et al., 2014). These TFs contained a bHLH motif with conserved amino acids, including two functionally distinct regions (N-terminal basic region and helix–loop–helix region) (Li et al., 2006). *Arabidopsis bHLH* gene *ICE1* was upregulated by cold and salt but not by dehydration (Chinnusamy et al., 2003). In tea plant (*Camellia sinensis*), several *bHLH* genes were upregulated by cold (Wang et al., 2012). In trifoliate orange (*Poncirus trifoliata*), Ptr*bHLH* transcript was constantly induced by cold (Huang et al., 2013). In apple (*Malus domestica*), *bHLH* gene Md*CIbHLH1* (cold-induced bHLH1) that encodes an ICE-like protein was significantly induced in response to cold stress (Feng et al., 2012). *MYB* TFs play key roles in the ABA-dependent pathway of stress signaling for upregulation of abiotic stress-responsive genes. Plant MYB proteins are classified into three main groups: R2R3-MYB, R1R2R3-MYB, and MYB-related proteins (Stracke et al., 2001). The *MYB* transcription family was mostly stimulated late under cold stress conditions in *Arabidopsis* (Fowler and Thomashow, 2002). MYB and bHLH proteins frequently interact with each other to regulate transcription (Ramsay and Glover, 2005). In this study, upregulation of *MYB* and *bHLH* TFs may indicate the crucial role of these proteins in coping with cold stress conditions.

The *A. thaliana* genome has more than 30 genes encoding SET-domain proteins and it is considered that they play essential roles in epigenetic regulation of gene expression and chromatin structure. These proteins can be classified into two groups: the polycomb group (PcG) and the trithorax group (trxG), which are important regulators in development (Thorstensen et al., 2008). In this study, the upregulated SET-domain may contribute to the transcriptional regulation to withstand cold stress in *N. benthamiana*. Overall, about 25 types of TF families were upregulated (Table 1). This indicates that cold stress could induce many TFs in response to adverse environmental conditions.

4.3. Signaling network response to cold stress

Plants perceive signals and stimuli by receptors and generate adaptive responses to the conditions. Plant protein kinases such as CDPKs and MAPKs are considered to play important roles in cellular signaling (Osakabe et al., 2013). Receptor-like kinase (RLK) proteins have important functions in signal transduction pathways (Shiu and Bleecker, 2001a). RLK protein kinases were identified as one of the largest gene families in the *Arabidopsis* genome with about 610 members, which are encoded by a multigene family (Shiu and Bleecker, 2001b), and about 1131 members in the rice genome (Shiu et al., 2004). RLKs contain a signal sequence, an amino-terminal domain with a transmembrane region, and a carboxyl-terminal kinase domain (Torii, 2000). These RLKs also play key roles in homeostatic mechanisms underlying the abiotic stress response and integrating environmental and plant hormone signaling (Shiu and Bleecker, 2001a; Dievart and Clark, 2004). In the present study, 10 receptor kinase genes have been shown to be significantly differentially expressed under cold stress (Figure 4). This indicates that protein kinases play important roles in detection of cold stress in *N. benthamiana*. It is well established that Ca^{2+} acts as a key messenger in regulation of growth and developmental processes and plays vital functions in stress signaling, i.e. cold stress (Reddy et al., 2011). Cytosolic free calcium concentration rises immediately in cold stress, indicating that calcium influx plays essential roles in response to environmental stresses (Knight et al., 1996). Calcium/calmodulin-mediated related genes can be classified into three main groups: 1) Ca^{2+}-dependent protein kinase (*CPK*), 2) calcineurin B-like protein (*CBL*), and 3) calmodulin (*CaM*) (DeFalco et al., 2010). In accordance with that, seven signaling genes (notably *CaM* (2) and *CPK* (2) genes) were upregulated (Figure 4), proposing that calcium/calmodulin-mediated related genes may play vital roles in cold acclimation process in *N. benthamiana*.

4.4. Hormone-related genes in cold stress conditions

Phytohormones play important roles in plant responses to cold stress. Ethylene is one of the most important regulatory hormones in environmental responses to stress conditions as well as having various physiological roles, including germination, fruit ripening, organ abscission, pathogen, response, and senescence (Chen et al., 2005). Ethylene response factors (*ERF*), which are a large multigene family, play important roles in responses to the ethylene signal and in regulation of gene expression in response to biotic/abiotic stresses (Zhang et al., 2008). ERF proteins contain the AP2/ERF domain structure, in which nearly 60 amino acids are involved in DNA binding. However, most ERF members recognize *cis*-element GCC-box (AGCCGCC) (Ohme-Takagi and Shinshi, 1995). In this study, seven ethylene genes were found to be highly expressed under cold stress conditions (Figure 4). It was reported that, in tomato, the Sl-ERF.B.3 (*Solanum lycopersicum* ethylene response factor B.3) gene, which belongs to the *ERF* family, was induced by cold, heat, and flooding stresses (Klay et al., 2014). Considering our results, it can be suggested that *ERF*s may regulate the responses and/or cold acclimation with constant transcriptional patterns in Solanaceae.

Auxin (indole-3-acetic acid, IAA) is the first discovered plant hormone and plays important roles in various metabolic processes, including flower organ development, plant morphogenesis, root patterning, and vascular tissue differentiation (Davies, 1995; Zhao, 2010). Cold stress basically targets intracellular auxin transport in *Arabidopsis* root. In addition, cold stress inhibits the intracellular trafficking of various proteins including auxin efflux carriers. Auxin signaling mutants *axr1* and *tir1* respond to cold treatment as the wild-type, proposing that cold stress alters auxin transport in preference to auxin signaling (Shibasaki et al., 2009). In rice, analysis of transcript profiling showed that many auxin-responsive genes play roles in response to cold stress (Jain and Khurana, 2009). Similarly, auxin genes were upregulated in *N. benthamiana*, suggesting that auxin transport may be affected by cold stress; therefore, auxin pathways were reregulated in response to cold stress. However, auxin signaling regulation in response to cold stress still remains to be investigated.

4.5. Genes related to photosynthesis

Photosynthesis is unquestionably a dominant sensor of stress in plants. Chloroplast-specific stress-sensing mechanisms detect stress-induced changes, including energy imbalance, changes of cellular sugar level, and redox homeostasis in components of thylakoids. These changes initiate signaling cascades, which consequently cause the genetic reprograming for stress adaptation (Biswal et al., 2011). Among cell organelles, chloroplasts, and especially chlorophyll biosynthesis, are rapidly affected under cold stress. Alterations in Chl antenna complexes cause an imbalance in photosystem II (PS II) (Ensminger et al., 2006). PS II is a protein complex with some polypeptides including subunits and chemical moieties that play important roles in electrochemical reactions (Renger, 2010). Results of previous studies showed that low-temperature stress inhibits the repair of PS II but does not affect photodamage to PS II (Murata et al., 2007). In this study, eight photosynthesis genes were upregulated with six photosystem II genes; one is the Calvin cycle gene and the other is the photorespiration gene (Figure 5C), indicating that particularly the photosystem II pathway was upregulated in order to cope with cold stress conditions for preventing photosystem damages in N. *benthamiana*.

4.6. Lipid metabolism-associated genes in cold stress conditions

Cold stress decreases the fluidic nature of cellular membranes and increases their rigidity. The content of fatty acid unsaturation and phospholipids result in cold acclimation and causes membrane rigidification (Los and Murata, 2004). Plant membrane lipids show a tendency to change from gel to liquid-crystalline phase in response to cold stress (Badea and Basu, 2009). Membrane rigidification was perceived by membrane proteins of plant cells, and these signals are transduced and many signaling pathways are activated to protect its membrane stability and integrity (Orvar et al., 2000; Yadav, 2010). In *N. benthamiana*, 17 genes involved in lipid metabolism were upregulated, and fatty acid (FA) synthesis and elongation genes (12 of 17) were found to be highly expressed (Figure 5D). Expression of the stearoyl-ACP desaturase (*w-9*) gene involved in fatty acid (FA) synthesis and elongation raises the cold tolerance out of increased desaturation of the fatty acids for control of membrane damage in potato. In potato, content of plasma membrane unsaturated fatty acids showed 5% to 10% changes under cold stress (De Palma et al., 2008). In this study, these expression patterns indicate that lipid metabolism may be reregulated for cold acclimation under cold stress.

4.7. Transport-related genes in cold stress conditions

Porins are members of β-barrel proteins with diverse functions in prokaryotes and eukaryotes. They are localized in outer membranes of mitochondria and in plastids in eukaryotes (Benz, 1994; Fischer et al., 1994). One important family of these proteins is the voltage-dependent anion channel (VDAC) family in eukaryotes (Wandrey et al., 2004). In *Arabidopsis* five *VDAC* isoforms (Clausen et al., 2004) and in rice three isoforms were identified (Roosens et al., 2000). *VDAC*s are considered to play important roles in regulation of metabolite transport between mitochondria and cytoplasm (Homblé et al., 2012). Transporting of anions, cations, ATP, Ca^{2+}, and metabolites is mediated by VDACs with connections between mitochondria and other parts of the cell (Shoshan-Barmatz et al., 2006). Expression of *VDAC*s in plants can be regulated by different abiotic/biotic stresses such as salinity, cold, drought, and pathogen defense. *VDAC* genes that were determined as salinity-inducibles gene in pearl millet were upregulated by drought, cold, and salicylic acid, but not by abscisic acid (Desai et al., 2006). Accordingly, porin and metabolite transporters genes were upregulated in *N. benthamiana* (Table S1). These transcriptional changes may be expected in response to cold stress for transporting metabolites in connections between mitochondria and cytoplasm.

In conclusion, we focused on transcriptional changes in *N. benthamiana* for common up- and downregulated cold stress genes. A number of genes involved in diverse biological or molecular pathways have been identified, but increased transcripts related to transcription factors, lipid metabolism, signaling, and photosynthesis pathways may play essential functions in the protection of *Nicotiana* under adverse conditions of cold stress. Results of this study will provide insights into the molecular mechanisms of *N. benthamiana* during the cold acclimation process. In addition, it could be a valuable resource to find new cold-related genes for improving the resistant plants for low-temperature conditions, especially members of the family Solanaceae.

Acknowledgment

This study was supported by a grant from TÜBİTAK (the Scientific and Technological Research Council of Turkey, no. 1059B141400573).

References

Badea C, Basu SK (2009). The effect of low temperature on metabolism of membrane lipids in plants and associated gene expression. Plant Omics 2: 78–84.

Benz R (1994). Permeation of hydrophilic solutes through mitochondrial outer membranes: review on mitochondrial porins. Biochim Biophys Acta 1197: 167–196.

Biswal B, Joshi PN, Raval MK, Biswal UC (2011). Photosynthesis, a global sensor of environmental stress in green plants: stress signaling and adaptation. Curr Sci 101: 47–56.

Bombarely A, Rosli HG, Vrebalov J, Moffett P, Mueller LA, Martin GB (2012). A draft genome sequence of *Nicotiana benthamiana* to enhance molecular plant-microbe biology research. Mol Plant Microbe In 25: 1523–1530.

Bray E, Bailey-Serres J, Weretilnyk E (2000). Responses to abiotic stresses. In: Buchanan BB, Gruissem W, Jones RL, editors. Biochemistry and Molecular Biology of Plants. Rockville, MD, USA: American Society of Plant Biologists, pp. 1158–1203.

Chawade A, Lindlof 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: e81729.

Chen Y, Etheridge N, Schaller GE (2005). Ethylene signal transduction. Ann Bot 95: 901–915.

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

Clausen C, Ilkavets I, Thompson R, Philippar K, Vojta A, Möhlmann T, Neuhaus E, Fulgosi H, Soll J (2004). Intracellular localization of VDAC proteins in plants. Planta 220: 30–37.

Davies PJ (1995). Plant Hormones. Dordrecht, the Netherlands: Kluwer Academic Publishers.

DeFalco TA, Bender KW, Snedden WA (2010). Breaking the code: Ca^{2+} sensors in plant signaling. Biochem J 425: 27–40.

De Palma M, Grillo S, Massarelli I, Costa A, Balogh G, Vigh L, Leone A (2008). Regulation of desaturase gene expression, changes in membrane lipid composition and freezing tolerance in potato plants. Mol Breeding 21: 15–26.

Desai MK, Mishra RN, Verma D, Nair S, Sopory SK, Reddy MK (2006). Structural and functional analysis of a salt stress inducible gene encoding voltage dependent anion channel (VDAC) from pearl millet (*Pennisetum glaucum*). Plant Physiol Biochem 44: 483–493.

Dievart A, Clark SE (2004). LRR-containing receptors regulating plant development and defense. Development 131: 251–261.

Doherty CJ, Van Buskirk HA, Myers SJ, Thomashow MF (2009). Roles for *Arabidopsis CAMTA* transcription factors in cold-regulated gene expression and freezing tolerance. Plant Cell 21: 972–984.

Ensminger I, Bosch F, Huner NPA (2006). Photo stasis and cold acclimation: sensing low temperature through photosynthesis. Physiol Plant 126: 28–44.

Feng XM, Zhao Q, Zhao LL, Qiao Y, Xie XB, Li HF, Yao YX, You CX, Hao YJ (2012). The cold-induced basic helix-loop-helix transcription factor gene MdCI*bHLH1* encodes an ICE-like protein in apple. BMC Plant Biol 12: 22.

Figueiredo DD, Barros PM, Cordeiro AM, Serra TS, Lourenco T, Chander S, Margarida Oliveira M, Saibo NJM (2012). Seven zinc-finger transcription factors are novel regulators of the stress responsive gene Os*DREB1B*. J Exp Bot 63: 3643–3656.

Fischer K, Weber A, Brink S, Arbinger B, Schunemann D, Borchert S, Heldt HW, Popp B, Benz R, Link TA et al. (1994). Porins from plants: molecular cloning and functional characterization of two new members of the porin family. J Biol Chem 269: 25754–25760.

Fowler S, Thomashow MF (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.

Goodin MM, Zaitlin D, Naidu RA, Lommel SA (2008). *Nicotiana benthamiana*: Its history and future as a model for plant-pathogen interactions. Mol Plant-Microbe Interact 21: 1015–1026.

Homblé F, Krammer EM, Prévost M (2012). Plant VDAC: Facts and speculations. Biochim Biophys Acta 1818: 1486–1501.

Huang XS, Wang W, Zhang Q, Liu JH (2013). A basic helix - loop - helix transcription factor, Ptr*bHLH*, of *Poncirus trifoliata* confers cold tolerance and modulates peroxidase-mediated scavenging of hydrogen peroxide. Plant Physiol 162: 1178–1194.

Jain M, Khurana JP (2009). Transcript profiling reveals diverse roles of auxin-responsive genes during reproductive development and abiotic stress in rice. FEBS J 276: 3148–3162.

Kim KM, Caetano-Anollés G (2010). Emergence and evolution of modern molecular functions inferred from phylogenomic analysis of ontological data. Mol Biol Evol 27: 1710–1733.

Klay I, Pirrello J, Riahi L, Bernadac A, Cherif A, Bouzayen M, Bouzid S (2014). Ethylene response factor *Sl-ERF.B.3* is responsive to abiotic stresses and mediates salt and cold stress response regulation in tomato. Scientific World Journal 2014: 167681.

Knight H, Trewavas AJ, Knight MR (1996). Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation. Plant Cell 8: 489–503.

Koc I, Filiz E, Tombuloglu H (2015a). Assessment of miRNA expression profile and differential expression pattern of target genes in cold-tolerant and cold-sensitive tomato cultivars. Biotechnol Biotechnol Equip 29: 851–860.

Koc I, Vatansever R, Ozyigit II, Filiz E (2015b). Identification of differentially expressed genes in chilling-induced potato (*Solanum tuberosum* L.); a data analysis study. Appl Biochem Biotech 177: 792–811.

Kreps JA, Wu Y, Chang HS, Zhu T, Wang X, Harper JF (2002). Transcriptome changes for *Arabidopsis* in response to salt, osmotic, and cold stress. Plant Physiol 130: 2129–2141.

Li XX, Duan XP, Jiang HX, Sun YJ, Tang YP, Yuan Z, Guo JK, Liang WQ, Chen L, Yin JY et al. (2006). Genome-wide analysis of basic/helix-loophelix transcription factor family in rice and *Arabidopsis*. Plant Physiol 141: 1167–1184.

Los DA, Murata N (2004). Membrane fluidity and its roles in the perception of environmental signals. Biochim Biophys Acta 1666: 142–157.

Maruyama K, Takeda M, Kidokoro S, Yamada K, Sakuma Y, Urano K, Fujita M, Yoshiwara K, Matsukura S, Morishita Y et al. (2009). Metabolic pathways involved in cold acclimation identified by integrated analysis of metabolites and transcripts regulated by *DREB1A* and *DREB2A*. Plant Physiol 150: 1972–1980.

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007). Photoinhibition of photosystem II under environmental stress. Biochim Biophys Acta 1767: 414–421.

Ohme-Takagi M, Shinshi H (1995). Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 7: 173–182.

Orvar BL, Sangwan V, Omann F, Dhindsa RS (2000). Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity. Plant J 23: 785–794.

Osakabe Y, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2013). Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress. J Exp Bot 64: 445–458.

Rahman A (2013). Auxin: a regulator of cold stress response. Physiol Plantarum 147: 28–35.

Ramsay NA, Glover BJ (2005). MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. Trends Plant Sci 10: 63–70.

Reddy AS, Ali GS, Celesnik H, Day IS (2011). Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. Plant Cell 23: 2010–2032.

Renger G (2010). The light reaction of photosynthesis. Curr Sci 98: 1305–1319.

Roosens N, Al Bitar F, Jacobs M, Homble F (2000). Characterization of a cDNA encoding a rice mitochondrial voltage-dependent anion channel and its gene expression studied upon plant development and osmotic stress. BBA-Biomembranes 1463: 470–476.

Rotter A, Usadel B, Baebler S, Stitt M, Gruden K (2007). Adaptation of the MapMan ontology to biotic stress responses: application in solanaceous species. Plants Meth 3: 10.

Shen C, Li D, He R, Fang Z, Xia Y, Gao J, Shen H, Cao M (2014). Comparative transcriptome analysis of RNA-Seq data for cold-tolerant and cold-sensitive rice genotypes under cold stress. J Plant Biol 57: 337–348.

Shi H, Wang X, Ye T, Chen F, Deng J, Yang P, Zhang Y, Chan Z (2014). The cysteine2/ histidine 2-type transcription factor zinc finger of *Arabidopsis thaliana* 6 modulates biotic and abiotic stress responses by activating salicylic acid-related genes and C-repeat- binding factor genes in *Arabidopsis*. Plant Physiol 165: 1367–1379.

Shibasaki K, Uemura M, Tsurumi S, Rahman A (2009). Auxin response in *Arabidopsis* under cold stress: underlying molecular mechanisms. Plant Cell 21: 3823–3838.

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003). Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6: 410–417.

Shiu SH, Bleecker AB (2001a). Plant receptor-like kinase gene family: diversity, function, and signaling. Sci STKE 113: re22.

Shiu SH, Bleecker AB (2001b). Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. P Natl Acad Sci USA 98: 10763–10768.

Shiu SH, Karlowski WM, Pan R, Tzeng YH, Mayer KF, Li WH (2004). Comparative analysis of the receptor-like kinase family in *Arabidopsis* and rice. Plant Cell 16: 1220–1234.

Shoshan-Barmatz V, Israelson A, Brdiczka D, Sheu SS (2006). The voltage-dependent anion channel (VDAC): function in intracellular signalling, cell life and cell death. Curr Pharm Design 12: 2249–2270.

Singh AK, Sopory SK, Wu R, Singla-Pareek SL (2010). Transgenic approaches. In: Pareek A, Sopory SK, Bohnert HJ, Govindjee, editors. In: Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation. Berlin, Germany: Springer, pp. 417–450.

Solanke AU, Sharma AK (2008). Signal transduction during cold stress in plants. Physiol Mol Biol Plants 14: 69–79.

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 Rep 32: 1407–1425.

Stracke R, Werber M, Weisshaar B (2001). The *R2R3-MYB* gene family in *Arabidopsis thaliana*. Curr Opin Plant Biol 4: 447–456.

Thomashow MF (1999). Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol 50: 571–599.

Thorstensen T, Grini PE, Mercy IS, Alm V, Erdal S, Aasland R, Aalen RB (2008). The *Arabidopsis* SET-domain protein ASHR3 is involved in stamen development and interacts with the *bHLH* transcription factor aborted microspores (AMS). Plant Mol Biol 66: 47–59.

Tian DQ, Pan XY, Yu YM, Wang WY, Zhang F, Ge YY, Shen XL, Shen FQ, Liu XJ (2013). *De novo* characterization of the *Anthurium* transcriptome and analysis of its digital gene expression under cold stress. BMC Genomics 14: 827.

Torii KU (2000). Receptor kinase activation and signal transduction in plants: an emerging picture. Curr Opin Plant Biol 3: 361–367.

Urbanczyk-Wochniak E, Usadel B, Thimm O, Nunes-Nesi A, Carrari F, Davy M, Blasing O, Kowalczyk M, Weicht D, Polinceusz A et al. (2006). Conversion of MapMan to allow the analysis of transcript data from solanaceous species: effects of genetic and environmental alterations in energy metabolism in the leaf. Plant Mol Biol 60: 773–792.

Wandrey M, Trevaskis B, Brewin N, Udvardi MK (2004). Molecular and cell biology of a family of voltage dependent anion channel porins in *Lotus japonicas*. Plant Physiol 134: 182–193.

Wang S, Wei XL, Cheng LJ, Tong ZK (2014). Identification of a C2H2-type zinc finger gene family from *Eucalyptus grandis* and its response to various abiotic stresses. Biol Plantarum 58: 385–390.

Wang X, Li W, Li M, Welti R (2006). Profiling lipid changes in plant response to low temperatures. Physiol Plant 126: 90–96.

Wang XC, Zhao QY, Ma CL, Zhang ZH, Cao HL, Kong YM, Yue C, Hao XY, Chen L, Ma JQ et al. (2013). Global transcriptome profiles of *Camellia sinensis* during cold acclimation. BMC Genomics 14: 415.

Wang Y, Jiang CJ, Li YY, Wei CL, Deng WW (2012). Cs*ICE1* and Cs*CBF1*: two transcription factors involved in cold responses in *Camellia sinensis*. Plant Cell Rep 31: 27–34.

Winfield MO, Lu C, Wilson ID, Coghill JA, Edwards KJ (2010). Plant responses to cold: transcriptome analysis of wheat. Plant Biotechnol J 8: 749–771.

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*. Mol Biol Rep 41: 5329–5342.

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

Zhang CZ, Fei SZ, Warnke S, Li LJ, Hannapel D (2009). Identification of genes associated with cold acclimation in perennial ryegrass. J Plant Physiol 166: 1436–1445.

Zhang G, Chen M, Chen X, Xu Z, Guan S, Li LC, Li A, Guo J, Mao L, Ma Y (2008). Phylogeny, gene structures, and expression patterns of the *ERF* gene family in soybean (*Glycine max* L.). J Exp Bot 59: 4095–4107.

Zhang X, Fowler SG, Cheng H, Lou Y, Rhee SY, Stockinger EJ, Thomashow MF (2004). Freezing sensitive tomato has a functional *CBF* cold response pathway, but a *CBF* regulon that differs from that of freezing-tolerant *Arabidopsis*. Plant J 39: 905–919.

Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J (2001). A role for Flavin monooxygenase-like enzymes in auxin biosynthesis. Science 291: 306–309.

Table S1. Expression values of up- and downregulated genes for all time points.

Clone name	4 h FC	12 h FC	24 h FC	48 h FC
STMCA65	2.83	3.106	2.943	2.406
STMCA69	2.59	2.838	2.962	3.444
STMCA92	2.43	2.233	2.799	2.832
STMCB12	3.463	3.316	3.671	3.639
STMCB28	2.87	2.692	2.729	2.87
STMCB38	4.003	3.516	3.774	3.66
STMCB52	3.206	2.893	3.198	3.279
STMCC07	3.309	2.87	3.302	3.239
STMCC37	4.158	4.023	4.159	4.222
STMCC86	2.356	2.623	2.866	3.421
STMCC93	3.855	2.217	3.819	3.546
STMCD06	2.186	2.302	2.541	2.526
STMCD19	2.667	3.113	3.055	3.861
STMCD22	2.82	3.02	3.664	3.187
STMCD24	3.099	3.529	3.506	3.279
STMCD76	2.716	2.414	3.488	3.497
STMCE11	2.108	2.205	2.227	2.275
STMCE55	2.551	2.37	2.223	3.729
STMCF13	2.39	2.379	2.281	2.541
STMCF32	3.552	3.365	3.202	3.329
STMCF47	3.163	2.046	3.323	3.615
STMCF53	4.379	3.689	4.093	4.326
STMCF84	3.414	3.587	3.521	3.429
STMCG11	3.892	3.237	4.139	2.526
STMCG54	3.24	2.824	2.858	3.141
STMCG59	2.967	3.093	2.336	2.124
STMCH02	2.698	2.789	2.744	2.718
STMCH18	3.299	2.905	3.376	3.694
STMCH49	3.256	3.642	3.279	3.61
STMCH58	2.134	3.014	3.422	3.597
STMCI73	3.454	3.527	3.276	3.558
STMCI91	3.037	4.68	3.954	3.32
STMCJ42	2.759	2.566	3.644	3.395
STMCJ45	2.42	2.524	2.621	2.493
STMCJ71	3.057	2.384	3.089	2.963
STMCK28	3.104	2.87	3.631	2.689
STMCK71	3.573	3.395	4.03	3.51
STMCK81	2.66	2.609	3.096	2.628
STMCL10	2.651	2.945	3.768	3.387
STMCL12	2.39	2.348	3.38	2.864
STMCL28	2.616	2.501	3.124	3.44
STMCL35	2.134	2.257	2.799	2.227
STMCL39	2.653	2.658	3.269	3.658
STMCL56	2.763	3.096	3.138	3.472
STMCL60	3.589	3.667	4.011	3.036
STMCL87	3.116	3.566	3.731	3.801
STMCM45	2.705	2.519	2.973	2.007
STMCN28	3.463	3.183	3.777	3.18
STMCN31	3.271	3.428	3.057	3.141
STMCN34	3.35	3.165	3.995	3.479
STMCN41	3.046	3.106	3.271	2.973
STMCN65	3.154	2.98	3.432	2.984
STMCN70	3.796	2.92	3.966	3.899

STMCN81	3.668	3.379	3.861	3.475
STMCN84	2.939	2.828	2.816	3.18
STMCO13	2.816	2.805	2.7	3.453
STMCO14	3.736	3.756	3.93	3.888
STMCO31	3.22	3.062	3.136	3.492
STMCO49	4.48	3.916	3.961	3.57
STMCO70	2.59	2.131	2.864	2.536
STMCP52	2.223	2.18	2.662	3.227
STMCP68	2.761	2.928	3.027	3.653
STMCQ22	2.782	2.963	3.384	2.709
STMCQ29	3.369	3.637	4.063	3.972
STMCQ50	3.198	3.385	3.402	2.874
STMCQ89	3.227	3.454	4.097	3.837
STMCR01	3.986	3.959	4.069	3.535
STMCR02	3.693	3.795	3.592	3.455
STMCR39	2.646	2.568	3.036	2.496
STMCR43	3.069	3.679	4.17	4.458
STMCS22	3.399	3.434	3.908	3.336
STMCS30	2.018	2.084	2.087	3.118
STMCS78	3.076	2.705	2.345	4.159
STMCS91	3.801	3.485	3.933	3.811
STMCU01	2.705	2.703	2.353	3.113
STMCU08	3.362	3.524	3.184	3.195
STMCU65	3.157	3.134	3.651	3.004
STMCU75	2.761	3.29	3.578	3.329
STMCV26	2.621	3.016	3.402	2.884
STMCV32	3.197	2.307	2.922	2.614
STMCV47	2.16	2.541	2.748	2.907
STMCX02	2.521	2.556	2.573	2.483
STMCX17	2.725	2.293	2.356	2.66
STMCX46	3.265	3.248	3.346	3.184
STMCX47	2.472	2.441	2.441	2.07
STMCY23	2.733	2.628	2.733	3.163
STMCZ23	2.711	3.531	3.801	2.094
STMCZ33	2.508	2.801	3.332	2.639
STMCZ58	2.561	2.144	2.251	2.373
STMCZ61	3.084	3.424	3.567	5.049
STMDB25	2.573	2.722	2.731	4.801
STMDB32	3.844	3.524	4.076	3.487
STMDB34	2.428	3.303	3.685	2.501
STMDB59	3.087	2.694	3.812	3.525
STMDB81	3.593	2.531	4.013	3.732
STMDC51	2.822	2.707	3.084	2.566
STMDC60	2.878	2.313	3.027	3.625
STMDC77	2.011	2.766	3.341	2.392
STMDD06	4.25	3.919	4.131	4.434
STMDD39	2.651	2.568	2.628	2.331
STMDD94	3.091	3.055	3.623	2.118
STMDE16	2.384	2.359	2.84	2.592
STMDE56	3.076	2.526	2.787	3.572
STMDF07	3.32	3.252	3.113	2.251
STMDF55	2.826	2.248	2.604	3.611
STMDG04	2.411	2.392	3.575	3.016
STMDG23	3.027	2.685	3.68	2.111
STMDG33	3.659	4.263	4.328	3.722
STMDH19	2.716	2.805	3.044	3.609

STMDH25	2.406	2.662	3.023	3.091
STMDH57	2.913	2.503	3.272	3.581
STMDH63	3.163	2.729	3.459	3.141
STMDI22	2.913	2.438	3.151	2.907
STMDI38	2.832	2.483	2.844	3.36
STMDI40	2.834	2.183	3.392	3.128
STMDI45	2.077	2.26	2.011	2.018
STMDI55	3.222	3.268	3.326	3.555
STMDJ55	3.768	3.251	4.078	3.904
STMDJ66	3.18	3.025	2.95	2.95
STMDJ73	3.692	3.409	3.756	3.78
STMDJ78	3.921	4.179	3.975	4.088
STMDJ81	2.384	3.072	2.611	2.834
STMDJ83	3.53	3.119	3.975	4.544
STMDJ90	3.236	2.599	3.285	3.268
STMDJ93	3.581	2.876	3.939	3.391
STMDM04	3.205	3.438	3.233	2.757
STMDM22	2.742	2.824	2.844	2.553
STMDM59	3.16	2.941	3.336	2.263
STMDM77	2.703	2.816	3.597	2.22
STMDO06	3.191	2.828	3.448	3.937
STMDO43	3.272	3.365	3.133	3.142
STMDO58	2.768	2.68	3.251	3.155
STMDP30	3.067	3.18	3.51	3.587
STMDP50	3.548	3.455	3.409	3.776
STMDP54	3.963	3.233	3.85	4.118
STMDP55	2.986	2.583	2.7	3.564
STMDP59	3.077	3.005	3.832	2.93
STMDP61	2.971	2.662	3.021	3.037
STMDP90	2.084	2.05	2.763	3.036
STMDP93	3.725	3.885	4.104	3.869
STMDQ25	3.569	3.625	3.157	3.653
STMDQ31	3.895	3.198	3.75	3.61
STMDQ33	3.705	3.744	3.809	2.811
STMDQ52	2.759	2.452	2.993	2.217
STMDQ63	3.206	3.206	3.718	3.387
STMDQ65	3.039	3.254	3.176	3.297
STMDR21	2.275	2.844	2.785	3.655
STMDR31	3.524	3.73	3.489	3.198
STMDS16	2.658	3.129	3.526	2.488
STMDS32	3.271	3.718	3.725	3.633
STMDS78	2.395	2.86	3.162	2.305
STMDS87	2.406	2	2.755	3.06
STMDT09	3.133	3.124	3.569	2.986
STMDT37	3.869	3.556	3.717	4.214
STMDT44	2.976	3.214	2.687	2.941
STMDT53	3.844	3.436	4.195	3.675
STMDT76	2.822	2.365	3.257	3.198
STMDT77	3.236	3.016	3.414	3.023
STMDT78	2.556	2.198	2.689	2.826
STMDT95	3.35	2.531	3.782	3.155
STMDU06	3.766	3.18	3.236	2.809
STMDU09	3.492	3.782	3.763	2.785
STMDU73	3.729	3.438	3.916	3.638
STMDU92	3.429	4.029	3.807	3.676
STMDV31	3.513	3.511	3.276	3.683

STMDV33	3.263	3.698	4.146	3.876
STMDV55	3.35	3.963	3.611	3.595
STMDV62	2.88	3.081	3.377	3.826
STMDV74	3.011	3.262	3.668	3.727
STMDW13	2.852	2.95	3.039	3.861
STMDW17	2.976	2.901	3.968	4.307
STMDW40	3.425	4.073	3.908	3.506
STMDW41	2.623	2.257	3.61	3.779
STMDW54	3.714	4.159	4.381	4.733
STMDW72	3.654	3.707	3.313	4.153
STMDZ20	4.123	3.959	4.088	3.352
STMDZ24	3.165	3.055	3.519	3.83
STMDZ26	3.42	3.729	3.53	3.31
STMDZ38	3.403	3.172	3.262	2.82
STMDZ46	2.778	2.893	3.053	2.687
STMDZ48	2.444	2.676	2.846	3.23
STMDZ59	3.274	3.536	3.781	3.152
STMDZ61	3.783	3.583	3.738	3.527
STMDZ66	3.007	2.091	2.711	3.004
STMDZ82	2.858	2.824	3.142	2.856
STMEA09	3.461	3.349	3.548	2.759
STMEA61	4.162	3.631	3.995	3.502
STMEA69	3.436	3.742	3.398	3.109
STMEA86	2.763	3.176	3.057	3.025
STMEB59	3.03	2.842	3.719	3.372
STMEC01	3.639	3.966	4.068	4.439
STMEC41	3.483	3.343	4.176	4.367
STMEC50	2.766	2.742	3.06	2.987
STMEC89	3.025	3.2	3.771	4.223
STMED14	2.832	3.444	3.055	3.206
STMED22	3.203	3.297	3.581	2.774
STMED32	3.041	3.476	3.133	3.276
STMED74	3.307	3.876	3.577	3.459
STMED96	2.491	2.287	2.553	2.965
STMEF19	3.039	2.733	3.309	3.546
STMEF54	4.215	3.84	3.162	4.679
STMEF69	3.205	3.32	3.851	3.358
STMEG37	3.966	4.341	4.379	4.499
STMEG61	3.626	3.108	3.335	2.844
STMEG82	2.742	2.674	2.976	3.069
STMEG87	3.136	2.937	3.176	2.751
STMEH43	3.782	3.542	3.861	3.52
STMEH45	3.725	2.467	4.008	3.638
STMEH47	3.011	3.222	3.285	3.898
STMEH69	2.895	2.091	3.426	3.388
STMEH79	2.934	3.219	3.619	3.715
STMEH93	2.501	2.465	2.766	3.266
STMEI02	2.257	2.257	2.316	2.173
STMEI04	2.513	2.278	2.561	2.22
STMEI05	2.025	2.683	3.222	2.799
STMEI16	3.183	3.349	3.608	3.284
STMEI26	2.7	2.414	2.392	3.013
STMEI27	2.934	2.111	2.856	3.091
STMEI62	2.59	2.475	2.811	2.96
STMEI81	2.95	2.768	3.606	3.285
STMEJ69	2.956	2.791	3.183	3.305

STMEK14	3.067	2.491	3.372	3.307
STMEK32	2.618	2.618	3.053	3.61
STMEK34	2.738	2.676	3.034	2.828
STMEK50	2.336	2.506	2.575	3.099
STMEK75	2.646	2.655	2.151	2.498
STMEL21	2.275	2.595	2.963	3.168
STMEL45	3.285	2.696	2.884	2.84
STMEL91	3.192	2.644	3.329	3.605
STMEM13	2.92	2.958	2.797	2.513
STMEM79	3.747	4.009	4.113	3.952
STMEM85	3.95	2.444	3.891	3.562
STMEN25	2.722	2.687	2.826	3.039
STMEO01	2.436	2.548	2.85	3.104
STMEO55	2.797	2.878	2.524	3.216
STMEO64	3.638	3.39	4.184	3.639
STMEP17	3.06	2.428	3.31	2.438
STMEP41	3.597	3.07	3.702	2.967
STMEP51	3.667	2.676	3.973	3.696
STMEP79	2.578	3.072	3.134	3.136
STMEP81	3.288	2.227	3.731	3.515
STMEQ01	2.597	2.546	2.928	2.86
STMEQ14	2.909	3.39	3.093	3.332
STMEQ20	3.503	3.526	3.467	3.52
STMEQ21	3.126	3.462	3.841	3.617
STMEQ68	3.726	4.082	4.382	3.822
STMEQ84	2.722	2.39	3.084	3.469
STMEQ92	3.316	3.703	3.242	3.013
STMER56	2.365	2.233	2.731	2.254
STMER57	2.862	3.151	3.733	2.947
STMER86	2.628	2.772	2.336	3.162
STMES03	3.065	2.77	2.42	2.868
STMES11	3.213	3.108	3.176	2.882
STMES30	2.398	2.926	2.878	3.43
STMES42	2.632	2.39	2.778	2.452
STMES50	2.214	2.138	2.339	2.932
STMES60	3.065	2.59	3.216	3.562
STMES69	2.488	2.709	2.889	2.26
STMES79	3.086	3.154	3.585	4.277
STMES92	3.268	2.886	3.029	4.076
STMET05	2.711	2.676	2.248	2.021
STMET48	2.799	3.062	2.967	2.353
STMET64	2.563	2.646	2.805	2.958
STMET68	3.136	2.905	2.858	2.975
STMEU23	2.876	3.438	3.773	2.748
STMEU29	2.905	2.639	3.555	2.789
STMEU35	3.051	3.489	3.615	2.553
STMEV09	2.322	2.16	2.759	2.491
STMEV20	3.057	3.399	3.662	3.843
STMEV22	2.692	2.844	3.29	2.595
STMEV44	2.521	2.381	3.173	3.58
STMEV63	2.438	2.217	3.272	3.02
STMEV67	3.081	3.136	3.322	3.134
STMEV85	2.676	2.924	2.671	2.969
STMEW28	3.195	3.084	3.242	3.103
STMEW45	2.676	2.854	2.733	3.141
STMEW77	2.387	2.021	2.651	2.818

STMEW79	2.154	2.319	2.43	3.24
STMEW93	2.854	2.95	3.192	3.367
STMEY13	3.585	4.388	3.887	3.94
STMEY18	3.601	3.605	3.752	3.897
STMEY80	3.106	3.399	3.124	3.183
STMEZ10	2.63	2.609	3.106	3.752
STMEZ31	3.846	4.076	4.037	2.872
STMEZ52	3.17	3.099	3.868	3.842
STMEZ63	2.696	2.454	2.801	3.217
STMEZ78	2.834	2.687	3.531	4.104
STMFA28	2.689	2.785	3.449	2.529
STMFB29	2.543	2.154	2.611	2.678
STMFB31	3.303	3.374	3.411	3.271
STMFB37	2.667	2.676	3.284	3.962
STMFB53	3.181	3.278	3.444	2.986
STMGA07	4.038	3.652	4.243	4.117
STMGA40	2.284	2.057	2.776	2.384
STMGA46	3.58	3.977	4.062	4.091
STMGA65	3.429	3.548	3.511	3.648
STMGA67	3.894	3.592	4.054	4.189
STMGA72	3.307	2.519	2.744	3.39
STMGA95	2.655	2.409	2.95	2.922
STMGB11	2.95	2.506	3.009	2.628
STMGB27	3.009	3.688	3.867	3.876
STMGC14	2.223	2.755	3.032	3.645
STMGC29	3.7	3.234	3.944	4.479
STMGC38	3.526	3.932	4.114	3.879
STMGC43	3.282	2.398	2.937	3.956
STMGC49	2.154	2.319	2.93	3.66
STMGC50	3.325	3.711	3.871	3.619
STMGC68	3.363	3.522	3.454	4.118
STMGD28	3.039	2.531	3.128	2.709
STMGD37	3.522	3.29	2.793	3.792
STMGD43	3.709	3.441	3.95	3.492
STMGD80	2.202	2.22	2.727	2.59
STMGE26	3.234	3.797	3.621	3.825
STMGE42	3.643	2.891	3.969	4.528
STMGF55	3.511	2.281	3.065	4.218
STMGF86	3.363	3.485	3.187	3.367
STMGG16	3.051	3.077	3.932	3.05
STMGG51	3.272	2.084	3.8	3.612
STMGH02	2.852	2.718	2.602	2.733
STMGH51	3.64	3.307	3.358	3.099
STMGH91	3.129	3.366	2.862	3.421
STMGI14	3.203	3.197	3.424	3.265
STMGI15	3.231	3.341	3.915	3.442
STMGI35	2.824	2.444	2.475	3.18
STMGI47	2.832	3.087	3.149	2.611
STMGI65	2.795	2.913	2.797	2.551
STMGI69	2.913	3.265	3.319	3.282
STMGJ02	2.446	2.227	2.618	3.231
STMGJ09	3.318	3.768	3.837	3.184
STMGJ11	3.577	2.452	3.927	3.655
STMGJ13	3.979	3.02	3.432	2.674
STMGJ32	2.903	2.744	2.359	2.459
STMGJ62	2.345	2.05	2.254	2.236

STMGJ67	2.828	3.69	3.191	2.852
STMGJ85	4.053	4.448	3.893	3.597
STMGJ96	3.239	3.681	3.717	3.785
STMGL23	2.766	2.742	2.342	2.183
STMGL35	2.949	2.57	3.488	2.982
STMGL86	2.947	2.111	2.88	2.782
STMGM06	2.299	2.339	2.519	2.459
STMGM12	2.926	2.795	3.388	2.891
STMGM14	3.508	3.702	3.459	3.737
STMGM21	2.067	2.186	2.884	3.209
STMGM22	2.414	2.483	2.438	2.278
STMGM76	3.03	2.909	3.918	3.187
STMGN12	3.772	3.97	3.95	4.506
STMGN48	3.707	3.789	3.798	3.685
STMGN55	2.606	3.064	2.899	4.437
STMGN70	3.091	2.943	4.041	3.325
STMGO38	3.686	3.396	3.626	3.228
STMGO52	2.669	2.793	3.058	3.013
STMGO92	3.595	3.725	3.767	3.372
STMGP03	2.962	2.937	3.118	2.789
STMGP37	4.098	4.056	3.983	4.013
STMGP49	3.629	3.329	3.638	3.916
STMGP59	3.163	3.313	3.479	3.272
STMGP60	3.353	2.296	3.534	3.805
STMGP93	3.811	2.556	3.993	3.454
STMGQ18	3.02	2.696	3.256	3.662
STMGQ49	2.632	2.637	2.766	2.553
STMGQ75	2.59	2.848	3.184	3.223
STMGQ85	2.379	2.278	2.202	2.48
STMGQ90	2.599	2.653	2.66	3.319
STMGQ92	3.271	3.736	3.605	3.585
STMGQ93	2.414	2.913	3.385	3.02
STMGS52	3.392	3.591	4.143	3.517
STMGS93	3.111	3.367	3.29	3.262
STMGT40	3.403	3.373	4.037	3.149
STMGT45	3.108	3.61	3.134	3.231
STMGT49	3.552	3.995	4.126	3.774
STMGT51	3.745	3.687	3.748	3.593
STMGT65	3.089	2.379	3.452	3.338
STMGU06	3.561	3.77	3.625	2.114
STMGU07	2.884	2.667	2.889	2.313
STMGU12	3.178	3.243	3.307	3.467
STMGU41	2.42	2.995	3.715	2.526
STMGU74	2.498	2.774	2.98	3.194
STMGV51	3.333	2.949	3.353	3.693
STMGV52	2.516	2.483	3.527	2.599
STMGV63	3.128	3.376	3.963	2.208
STMGV89	3.233	3.144	4.036	3.966
STMGW13	3.821	4.243	4.014	3.659
STMGW14	3.759	3.986	3.693	3.32
STMGW21	2.307	2.29	2.585	2.06
STMGW71	2.962	2.516	3.394	2.625
STMGX49	4.02	3.835	4.027	3.315
STMGX55	3.48	3.098	4.195	3.822
STMGY58	3.034	2.918	3.734	3.733
STMGY68	3.053	3.402	3.592	3.424

STMGY78	2.793	2.818	3.591	2.57
STMGY94	2.795	2.95	3.402	3.668
STMGZ11	3.128	2.223	4.163	4.768
STMGZ19	3.398	3.498	3.489	3.669
STMGZ22	2.48	2.924	3.449	2.74
STMGZ25	2.131	2.417	2.316	3.653
STMGZ65	2.144	2.06	2.381	2.223
STMGZ89	2.66	3.07	3.862	3.696
STMHA02	3.082	3.355	3.332	3.276
STMHA28	3.118	2.998	3.485	3.046
STMHA31	2.755	3.39	2.852	2.903
STMHA35	3	2.642	3.111	2.824
STMHA41	2.846	2.803	3.129	3.009
STMHA65	3.281	2.506	3.595	3.062
STMHA70	4.183	2.722	4.141	3.952
STMHE04	3.186	2.457	3.65	3.661
STMHE13	3.129	3.044	3.275	3.587
STMHE21	3.426	3.556	3.367	2.539
STMHE31	3.099	2.731	3.248	3.362
STMHE49	4.037	3.581	4.024	3.403
STMHE70	2.395	2.018	2.519	2.59
STMHF27	2.506	2.558	3.058	3.782
STMHF46	2.587	3.057	3.485	3.442
STMHF64	2.422	2.457	2.543	3.29
STMHF72	2.595	2	3.349	3.541
STMHF96	3.466	3.617	3.874	2.47
STMHG33	2.305	2.16	2.58	3.216
STMHG71	2.876	2.575	3.24	4.573
STMHH06	3.016	3.079	3.842	3.751
STMHH39	2.202	3.787	3.157	3.572
STMHH43	3.551	3.257	3.535	3.705
STMHI22	3.385	3.233	3.771	3.302
STMHI76	2.313	2.217	2.797	2.176
STMHI78	3.45	3.062	2.669	4.248
STMHJ02	2.202	2.876	2.305	2.759
STMHJ38	3.863	3.349	3.288	3.722
STMHJ61	2.962	3.087	2.491	3.602
STMHK09	3.414	3.434	3.505	3.067
STMHK14	3.478	3.896	3.862	3.149
STMHK17	3.732	3.319	3.43	3.974
STMHK36	4.046	4.099	4.184	4.963
STMHK38	3.284	3.604	3.441	3.345
STMHK65	3.418	3.444	3.86	4.172
STMHL48	3.149	2.918	3.465	3.577
STMHL74	2.793	2.907	3.011	3.142
STMHL84	3.109	3.187	3.462	3.709
STMHN37	2.018	2.018	2.387	3.697
STMHN73	2.254	2.696	3.03	3.392
STMHO84	2.995	2.87	4.085	3.396
STMHP08	2.7	2.186	2.655	2.018
STMHP29	3.752	3.436	4.084	2.928
STMHP38	3.005	3.693	3.272	3.449
STMHP91	2.909	2.965	3.614	4.018
STMHQ03	2.543	2.367	2.989	2.77
STMHQ16	3.214	3.288	3.661	2.824
STMHQ60	2.797	2.449	3.187	3.609

STMHQ69	3.025	3.219	3.678	3.348
STMHQ76	3.096	2.778	3.239	2.692
STMHQ77	2.287	2.333	2.761	2.157
STMHR39	2.452	2.772	2.546	2.217
STMHR72	2.131	2.848	2.236	2.915
STMHR84	2.995	2.074	2.543	3.175
STMHS11	2.795	3.522	3.912	2.084
STMHS69	2.29	2.759	2.433	2.395
STMHS94	2.074	2.098	2.236	2.452
STMHT70	2.74	3.294	3.37	2.828
STMHT73	3.994	3.522	3.566	3.902
STMHT90	3.751	3.181	3.626	3.472
STMHT95	3.623	3.36	3.815	2.236
STMHU04	2.623	2.23	3.454	3.265
STMHU07	2.17	2.438	2.744	4.222
STMHU23	3.178	3.246	3.803	2.313
STMHU56	3.316	3.448	4.24	4.268
STMHU93	2.911	3.131	3.414	3.502
STMHV44	2.709	3.23	3.178	2.816
STMHV51	2.428	3.297	4.058	2.348
STMHV63	2.7	2.671	3.434	2.982
STMHW20	2.818	2.543	2.31	2.444
STMHW77	2.313	2.192	2.299	2.587
STMHW92	3.091	2.795	3.053	3.374
STMHX04	2.935	3.046	3.225	2.021
STMHX15	3.434	2.648	3.969	3.697
STMHX32	2.639	2.503	2.345	2.746
STMHX43	3.074	3.109	3.053	3.266
STMHX58	2.094	3.007	3.2	2.144
STMHX83	3.046	3.002	2.597	3.417
STMHX89	3.448	3.353	3.584	3.331
STMHX91	2.655	2.488	2.852	3.719
STMHY14	3.349	3.633	3.376	3.365
STMHY15	3.256	3.461	3.653	3.733
STMHY22	2.793	3.119	3.307	3.472
STMHY31	3.585	3.758	3.705	3.803
STMHY55	2.406	2.208	2.428	2.403
STMHY64	2.26	3.214	3.783	3.679
STMHY86	2.687	2.88	3.362	3.803
STMHZ36	3.877	3.987	4.044	4.568
STMHZ43	3.342	3.227	3.234	4.244
STMHZ51	2.563	2.387	3.121	2.121
STMIA04	3.216	3.216	3.721	3.251
STMIA05	2.866	2.475	2.395	2.604
STMIA09	3.329	3.463	3.728	3.133
STMIA44	3.831	3.469	3.694	3.445
STMIA69	2.949	2.496	3.027	3.011
STMIA73	2.778	3.198	2.746	2.785
STMIA91	3.761	3.326	3.579	4.021
STMIB24	3.849	3.671	3.983	4.355
STMIB92	4.037	3.944	3.898	3.76
STMIC21	2.414	2.362	3.136	2.818
STMIC31	3.336	3.129	3.027	2.987
STMIC66	2.926	2.787	2.976	2.761
STMIC72	3.328	3.616	3.574	2.452
STMID03	2.84	2.915	3.668	2.359

STMID05	3.118	3.394	3.998	3.973
STMID19	2.414	2.257	2.438	3.936
STMID53	3.025	3.328	3.942	3.952
STMID62	2.975	3.128	3.398	3.271
STMID69	2.26	2.217	2.958	2.604
STMID78	2.727	2.587	3.329	3.835
STMIF10	2.844	2.667	3.557	3.584
STMIF19	2.091	2.459	2.566	3.891
STMIF26	3.831	3.804	3.511	3.532
STMIF38	2.257	3.081	2.954	2.305
STMIF49	2.962	2.444	2.648	2.609
STMIF50	2.664	2.534	2.77	2.511
STMIF61	4.021	4.051	4.024	3.808
STMIF66	3.094	2.667	3.642	3.415
STMIF84	3.795	3.479	3.877	4.573
STMIF91	2.947	2.454	2.736	3.604
STMIF95	2.674	2.566	2.85	2.787
STMIG09	3.381	3.437	3.729	2.738
STMIG53	2.602	2.893	2.982	2.922
STMIG67	3.494	3.039	3.256	3.639
STMIG86	3.731	3.892	3.88	3.572
STMIH28	2.805	3.084	3.609	3.887
STMIH61	3.801	4.316	4.355	4.14
STMIH62	2.901	2.755	3.254	3.624
STMIH82	3.111	2.664	3.084	2.801
STMII05	3.644	3.886	3.852	4.135
STMII13	2.962	2.587	3.002	4.249
STMII17	2.998	2.141	2.488	4.431
STMII31	3.666	3.296	3.425	3.74
STMII36	3.353	4.166	2.587	3.458
STMII40	3.426	3.044	3.988	4.01
STMII96	4.069	3.142	3.961	4.174
STMIJ23	2.854	2.705	3.469	2.989
STMIJ25	3.766	3.407	3.742	3.799
STMIJ32	2.669	2.563	2.342	2.628
STMIJ89	3.605	3.65	3.697	3.434
STMIK01	3.693	3.438	3.511	3.296
STMIK19	3.625	3.621	3.636	3.542
STMIK84	3.731	3.638	3.857	4.126
STMIL13	2.88	2.183	2.553	2.644
STMIL33	3.903	3.359	4.084	4.522
STMIL44	2.348	2.348	2.478	3.954
STMIL51	2.886	2.053	3.136	2.379
STMIL68	3.847	3.638	3.843	3.717
STMIM01	2.759	3.858	3.417	5.005
STMIM29	2.23	2.131	2.114	2.087
STMIM43	2.891	2.874	3.041	3.411
STMIM51	3.476	3.009	3.899	3.426
STMIM55	3.005	2.74	3.26	3.508
STMIM63	2.893	2.778	3.027	2.173
STMIM79	4.517	3.969	4.242	3.956
STMIM83	3.491	2.987	3.406	2.703
STMIM89	3.817	3.817	4.279	4.583
STMIN26	2.787	2.718	2.809	2.87
STMIN65	2.866	3.186	3.744	2.42
STMIN80	3.322	3.687	3.507	3.205

STMIN81	2.606	3.467	3.84	3.366
STMIO49	4.077	3.687	4.063	3.652
STMIO55	2.785	3.265	3.388	3.126
STMIO57	2.485	2.074	3.407	3.336
STMIP72	2.411	2.202	2.091	2.046
STMIP82	2.411	2.018	2.862	2.816
STMIQ05	3.822	3.578	4.299	2.759
STMIQ09	3.103	3.086	3.558	2.176
STMIQ43	3.021	2.991	3.082	3.206
STMIQ72	3.689	3.71	3.294	2.491
STMIQ79	3.219	3.165	3.114	3.256
STMIQ91	3.305	2.428	3.138	4.109
STMIQ93	3.552	2.406	3.851	3.149
STMIR02	3.323	3.707	3.425	3.801
STMIR10	3.284	3.299	3.462	3.039
STMIR13	2.818	3.396	2.828	2.725
STMIR15	3.313	3.757	3.747	3.629
STMIR20	3.121	3.7	3.706	3.638
STMIR68	2.958	3.531	3.037	3.002
STMIR73	2.807	2.685	3.396	2.868
STMIS25	3.121	2.755	2.998	3.074
STMIS57	3.16	3.294	3.683	3.407
STMIS66	3.353	3.312	3.167	3.491
STMIT14	3.411	2.926	3.37	3.216
STMIT50	2.625	2.928	2.293	2.462
STMIT66	3.07	2.39	2.928	2.716
STMIT80	3.945	3.83	3.844	3.732
STMIT84	3.256	2.986	3.402	3.944
STMIU49	3.104	3.26	2.755	2.742
STMIU77	3.18	2.583	2.733	2.696
STMIU79	3.677	2.95	3.557	3.699
STMIV04	2.963	3.082	4.182	3.476
STMIV38	2.299	2.189	2.362	2.563
STMIV40	2.151	2.032	2.111	2.157
STMIV50	2.438	2.111	2.548	3.029
STMIV62	3.055	3.108	2.417	3.413
STMIW49	2.208	3.335	2.63	3.157
STMIW57	2.88	2.671	3.167	3.113
STMIW60	3.758	3.775	4.162	4.407
STMIW78	2.805	2.818	3.142	3.703
STMIX01	2.441	2.782	2.926	2.011
STMIX04	2.281	2.342	3.124	2.856
STMIX06	2.449	2.854	2.599	3.211
STMIX57	2.566	2.774	3.048	3.189
STMIX64	3.543	3.696	4.052	3.892
STMIX65	3.973	3.398	3.826	3.297
STMIX88	3.285	2.868	3.282	2.864
STMIX92	3.643	3.329	3.425	3.178
STMIY27	3.167	3.66	3.556	2.834
STMIY51	3.522	2.457	4.037	3.696
STMIY79	2.753	2.832	2.807	3.63
STMIZ07	3.861	4.269	4.26	4.354
STMIZ61	2.561	2.296	2.157	2.882
STMIZ65	2.84	2.916	3.614	2.646
STMIZ73	2.982	2.748	3.912	3.167
STMIZ84	3.352	3.139	3.485	2.307

STMJA04	2.644	2.257	3.878	2.548
STMJA15	2.342	2.029	2.602	2.791
STMJB20	3.508	3.629	3.284	3.246
STMJB28	3.069	2.74	3.055	2.96
STMJB40	2.916	2.963	3.365	3.213
STMJB45	2.742	2.658	2.711	3.176
STMJB55	3.424	3.632	3.079	3.118
STMJC88	3.084	3.777	3.887	3.411
STMJD09	2.842	3.263	3.697	3.956
STMJD31	2.328	2.611	2.313	2.551
STMJD53	2.578	2.753	3.507	2.989
STMJD56	3.741	3.92	4.247	3.845
STMJD63	2.858	2.978	3.205	3.051
STMJD65	2.325	2.202	2.658	2.101
STMJE02	3.342	3.888	3.659	3.484
STMJE04	2.546	2.233	2.899	2.428
STMJE59	2.751	2.614	3.227	3.245
STMJF05	3.373	3.502	3.399	3.222
STMJF11	3.332	2.995	3.2	2.832
STMJF14	3.484	3.879	3.753	3.67
STMJF48	3.203	2.353	3.296	3.899
STMJF64	3.331	3.248	3.648	3.268
STMJF65	3.5	3.246	3.485	2.971
STMJF69	2.488	2.373	2.74	2.074
STMJF89	2.722	2.328	2.625	2.854
STMJG06	3.685	3.219	4.256	5.151
STMJG13	4.081	3.618	3.757	3.625
STMJG18	3.617	3.118	4.038	3.426
STMJG47	3.141	2.709	3.55	3.487
STMJG53	2.909	2.57	3.758	3.734
STMJG63	2.832	3.197	3.697	3.016
STMJG80	3.07	3.279	3.214	3.106
STMJH11	3.131	3.401	3.863	3.155
STMJH65	2.834	2.319	3.046	2.866
STMJH71	2.984	3.476	3.373	2.508
STMJI08	3.121	3.246	3.474	3.228
STMJI10	2.766	2.441	3.136	3.233
STMJI29	3.22	3.057	4.159	4.233
STMJI32	2.534	2.414	3.116	4.085
STMJI38	3.64	3.847	4.173	3.828
STMJI51	3.379	2.969	3.366	3.466
STMJI55	2.822	2.774	3.319	3.2
STMJJ19	3.254	3.279	3.22	3.032
STMJJ22	3.011	2.889	3.681	3.178
STMJJ31	2.939	3.039	3.108	3.037
STMJJ43	2.387	2.021	2.761	2.947
STMJJ46	2.06	2.043	2.462	2.48
STMJJ74	3.168	3.35	3.335	3.036
STMJJ80	3.242	3.694	3.592	3.44
STMJJ85	4.486	4.932	4.438	3.831
STMJK07	3.512	3.867	4.071	3.825
STMJK46	3.383	2.491	3.857	4.144
STMJK56	2.671	3.126	3.096	2.976
STMJK65	3.469	3.284	3.656	3.552
STMJK67	3.617	3.835	4.01	3.873
STMJL12	2.623	2.516	2.838	2.986

STMJL21	3.099	3.428	3.318	2.998
STMJL46	2.578	2.398	3.343	3.755
STMJL48	2.772	2.411	2.281	2.722
STMJL61	3.757	3.379	3.736	2.976
STMJL86	3.777	3.52	3.639	4.371
STMJM64	2.606	2.648	2.954	2.592
STMJM92	3.442	3.36	3.312	3.359
STMJM94	3.128	2.417	3.048	2.826
STMJN24	2.876	2.698	3.287	2.945
STMJN54	2.742	2.478	3.252	3.745
STMJN76	2.644	2.387	2.467	2.296
STMJN90	2.991	2.722	3.545	3.426
STMJO12	3.655	3.744	3.624	4.349
STMJO23	3.65	3.479	4.009	3.526
STMJO24	3.948	3.647	3.961	4.242
STMJO62	2.984	3.16	3.333	2.272
STMJO96	3.303	3.299	3.418	4.129
STMJP09	4.085	4.164	3.506	3.88
STMJP44	2.563	2.751	2.889	2.744
STMJP50	2.975	3.114	2.801	4.034
STMJP66	3.163	3.285	3.556	3.792
STMJP68	3.841	3.414	3.606	3.612
STMJP85	4.529	3.939	3.71	3.458
STMGQ01	-4.058	-4.643	-5.643	-4.643
STMHL01	-3.836	-4.321	-3.643	-4.643
STMCH95	-3.183	-3.473	-4.643	-5.643
STMJN89	-3.058	-3.643	-2.555	-2.395
STMDG37	-3.836	-4.643	-3.321	-4.643
STMEQ83	-3.836	-4.643	-3.643	-2.942