A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Transcriptome Profiling for Searching Cold Hardiness-Related Genes in Peach Tree (*Prunus persica*) Shoots

복숭아나무 신초의 내한성 관련 유전자 탐색을 위한 전사체 분석

BY

JUNHYUNG PARK

FEBRUARY, 2019

MAJOR IN HORTICULTURAL SCIENCE AND BIOTECHNOLOGY

DEPARTMENT OF PLANT SCIENCE

THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

Transcriptome Profiling for Searching Cold Hardiness-Related Genes in Peach Tree (*Prunus persica*) Shoots

Junhyung Park

Major in Horticultural Science and Biotechnology
Department of Plant Science
The Graduate School of Seoul National University

ABSTRACT

Cold hardiness of peach (*Prunus persica*) trees varies with cultivar. To search the genes responsible for the cultivar difference, the transcriptomes from two peach cultivars showing different cold hardiness were compared by using next generation sequencing. ‘Soomee’ and ‘Kiraranokiwami’ peach trees are known to be relatively cold-tolerant and -susceptible, respectively. Shoots were collected in October and January from the field-grown 5-year-old trees of the two cultivars. RNAs from the shoots were prepared for the transcriptome analysis. Following the transcriptome sequencing, total bases of 4.8-7.1 Gb were obtained and 94-97% of total reads were mapped to the reference peach genome. Totally, 190 and 176 differentially expressed genes (DEGs) were found

in October and January, respectively, from the two cultivars. Gene set enrichment analysis revealed that most of the DEGs belonged to cell wall macromolecule catabolic process, signal transduction, ADP binding, trehalose biosynthetic process, and integral component of plasma membrane along with several uncharacterized proteins and long noncoding RNAs. The in silico results were validated by performing reverse transcript quantitative polymerase chain reaction against the DEGs showing the significant fold change both in October and January. The present results demonstrate the cultivar difference of peach trees in cold hardiness at transcriptome level.

Key words**:** cold stress, cultivar difference, differentially expressed genes, gene set enrichment analysis, transcriptome analysis

Student number**:** 2017-23627

CONTENTS

ABSTRACT ····· i

CONTENTS ····· iii

LIST OF TABLES ····· iv

LIST OF FIGURES ····· v

INTRODUCTION ····· 1

MATERIALS AND METHODS ····· 4

RESULTS AND DISCUSSION ····· 9

LITERATURE CITED ····· 21

ABSTRACT IN KOREAN ····· 27

LIST OF TABLES

Table 1. Sequencing results of ‘Soomee (SM)’ and ‘Kiraranokiwami (KK)’ peach tree shoots ····· 11

Table 2. Top 10 up-regulated DEGs from ‘Soomee’ over ‘Kiraranokiwami’ peach tree shoots in October and January ····· 12

Table 3. Top 10 down-regulated DEGs from ‘Soomee’ over ‘Kiraranokiwami’ peach tree shoots in October and January ····· 13

Table 4. InterPro families of DEGs from ‘Soomee’ over ‘Kiraranokiwami’ peach tree shoots in October ····· 16

Table 5. InterPro families of DEGs from ‘Soomee’ over ‘Kiraranokiwami’ peach tree shoots in January ····· 17

LIST OF FIGURES

Fig. 1. LT_{50} values for ‘Soomee (SM)’ and ‘Kiraranokiwami (KK)’ peach tree shoots in October and January ····· 10

Fig. 2. Gene set enrinchment analysis results from ‘Soomee’ over ‘Kiraranokiwami’ peach tree shoots in October (A) and January (B) ····· 15

Fig. 3. Quantitative PCR results for validating transcriptome analysis of ‘Soomee (SM)’ and ‘Kiraranokiwami (KK)’ peach tree shoots in October and January ····· 19

INTRODUCTION

Abnormal cold waves during warm winter or spring season can seriously injure fruit tree buds and flowers, lowering fruit production. Peach trees (*Prunus persica*), which bloom in early spring, are also exposed to the danger of chilling and frost injury. Understanding the mechanism of cold acclimation and function of cold hardiness-related genes is necessary to develop cultural practices to cope with cold stress and to breed cold-tolerant peach cultivars. After peach genome was sequenced (Verde et al., 2013), expressions of the genes, including cold response genes, have been investigated. However, most of the studies in peach trees have focused on seasonal changes of their expression and on specific tissues or proteins (Artlip et al., 2013; Bassett et al., 2015; Jiao et al., 2017).

Many perennial plants, including fruit trees, obtain cold tolerance by exposure to low non-freezing temperature (Thomashow, 1999), called cold acclimation. During cold acclimation, numerous genes from various pathways are involved in this process. In *Arabidopsis*, cold acclimation is initiated by C-repeat binding factor (CBF) pathway, affected by temperature, red/far-red light ratio, and circadian clock, which controls expression of cold responsive (COR) genes inducing cold hardiness (Wisniewski, 2014).

A number of genes involved in cold acclimation process have been discovered from various woody plant species. For instance, *CBF* genes, which related to transcriptional control and main part of CBF pathway, have been

discovered from sour cherry (Owens et al., 2002), sweet cherry (Kitashiba et al., 2004), birch (Welling and Palva, 2008), peach (Wisnieswki et al., 2011), and almond (Barros et al., 2012). Moreover, overexpression of *AtCBF1* gene from *Arabidopsis* on *Populus balsamifera* subsp. *trichocarpa* enhanced the cold hardiness of leaves and stems (Benedict et al., 2006), and CBF genes from *Vitis* species also increase abiotic stress tolerance in *Arabidopsis* (Kobayashi et al., 2012; Li et al., 2013; Siddiqua and Nassuth, 2011; Takahura et al., 2011). These results indicate that there are conserved cold stress responsive mechanism between herbaceous and woody plants. In eucalyptus, however, *CBF* genes were regulated differently from those in *Arabidopsis* (El Kayal et al., 2006), suggesting the presence of species-specific mechanism of CBF pathway.

Transcriptome analysis related to cold acclimation and cold hardiness had been performed in several woody plants. Most of the studies focused on seasonal change of transcriptome during cold acclimation process, and results show that differentially expressed gene (DEG) groups are associated with cold signal transduction, cold response, cell membrane stabilization, sugar metabolism, and oxidative stress (Chen et al., 2017; Gaete-Loyola et al., 2017; Guerra et al., 2015; Wang et al., 2013; Xu et al., 2014). However, comparative studies with different cold tolerant cultivars have not been performed frequently in woody plants, whereas in herbaceous plants like rice (Shen et al., 2014) or ryegrass (Abeybayake et al., 2015).

In this study, two peach cultivars showing different cold hardiness were compared at the transcriptome level to identify cold hardiness-related genes that determine the cultivar difference.

MATERIALS AND METHODS

Plant materials

For transcriptome analysis and cold hardiness determination, bud-attached and -detached shoots were collected in October and January, from 5-year-old cold-tolerant 'Soomee (SM)' and cold-susceptible 'Kiraranokiwami (KK)' peach trees grown at the experimental orchard of National Institute of Horticultural and Herbal Science, Wanju, Korea (35° 50' N, 127° 01' E). Three trees of each cultivar were used for biological replications. Bud-attached shoots were collected for cold hardiness determination, while bud-detached shoots were immediately chilled with liquid N_2, powdered by using a TissueLyser II (Qiagen, Venlo, Netherlands) and stored at −75°C before RNA extraction.

RNA extraction

Total RNA was extracted from frozen bud-detached shoots as described by Gambino et al. (2008) with slight modifications. All utensils used in the extraction were autoclaved at 120°C for 30 min. All solutions were made with diethyl pyrocarbonate (DEPC)-treated water and autoclaved at 120°C for 30 min. Extraction buffer (2% hexadecyltrimethylammonium bromide, 2% polyvinylpyrrolidone-40, 2 M NaCl, 0.1 M Tris-HCl pH 8.0, 25 mM EDTA pH 8.0, and 2% of β-mercaptoethanol added just before use) were heated at 65°C in

a 15-mL tube. After heating, 900 μL of the extraction buffer were added to a 2-mL microfuge tube, containing approximately 50-100 mg of sample powder, and incubated at 65ºC for 10 min. An equal volume of chloroform:isoamyl alcohol (24:1, v/v) was added, vortexed for 5 s, and centrifuged at 11,000 g at 4ºC for 10 min. The supernatant of 750 μL was recovered and extraction with chloroform:isoamyl alcohol was performed again. The supernatant of 600 μL was transferred to a new 2-mL tube and equal volume of 6 M LiCl solution was added. The mixture was incubated on ice for 30 min and centrifuged at 21,000 g at 4ºC for 20 min to precipitate RNA. Pellet was resuspended in 500 μL of pre-heated (65ºC) SSTE buffer (0.5% sodium lauryl sulfate, 1 M NaCl, 1 M Tris-HCl pH 8.0, 10 mM EDTA pH 8.0), while gentle shaking. Equal volume of chloroform:isoamyl alcohol was added and then centrifuged at 11,000 g at 4ºC for 10 min. The supernatant of 400 μL was transferred to a new 1.5-mL tube, 280 mL of ice-cold isopropanol was added and the mixture was centrifuged at 21,000 g at 4ºC for 15 min. Pellet was washed with 1 mL of 70% ethanol by centrifuging at 11,000 g at 4ºC for 10 min, dried, and resuspended in 20 μL of DEPC-treated water. Finally, the solution was heated at 65ºC for 5 min to dissolve RNA completely.

Quality and purity of extracted RNA samples were assessed by A_{260}/A_{280}, A_{260}/A_{230} ratio, and RNA integrity number using Nanodrop ND 1000 (Thermo Fisher Scientific, Wilmington, DE, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany).

Transcriptome analysis

RNA sequencing

Total 12 libraries were constructed with a TruSeq RNA sample prep kit v2 (Illumina, San Diego, CA, USA), and sequenced by using HiSeq 2500 system (Illumina). Quality of the produced data was confirmed by FastQC v0.11.5 program, and unwanted artifacts like adaptor sequence and short length reads (< 36 bp) were trimmed from the raw data by using Trimmomatic 0.32 program. The trimmed reads were mapped to the reference peach genome (GCF_000346465.2) using HISAT2 and Bowtie2 program, and pooled into four groups (SM10, SM1, KK10, and KK1). Expression levels of known genes were calculated by FPKM (fragments per kilobase of transcript per million mapped reads) method using StringTie 1.3.3b program.

DEGs identification

To identify DEGs effectively, genes showed zero FPKM in any samples were removed from analysis process. The trimmed data were normalized by quantile normalization method after taking $\log_2$ (FPKM+1), and statistical analyses were performed with fold change value and independent t-test. Functional annotation and gene set enrichment analysis were also performed by DAVID tool, based on gene ontology (GO) term and KEGG database.

Quantitative polymerase chain reaction (qPCR) analysis

To validate DEG identification results, qPCR analyses were performed with the genes showing significant fold change between October and January. First

strand cDNAs were synthesized from the same RNA samples used for RNA sequencing, by using an AccuPower RT premix (Bioneer, Daejeon, Korea). Primer sets were designed by NCBI Primer-BLAST, and peach glyceraldehyde-3-phosphate dehydrogenase gene was used as a reference. Relative expression levels of the genes were determined with a LightCycler 480 system (Roche Diagnostics, Basel, Switzerland) and an AccuPower 2X Greenstar qPCR master mix (Bioneer).

Cold hardiness determination

Cold hardiness of shoots was determined by electrolyte leakage (EL) analysis described by Pagter et al. (2008) and Lee et al. (2012) with slight modifications. Bud-attached shoots were cut into 8 cm pieces and randomly divided into six groups. Four groups were incubated in a programmable bath circulator (RW-2040G, Jeio Tech, Seoul, Korea), cooled at a rate of −2°C/h until target temperature, and maintained at the target temperature for 2 h. Four target temperatures were selected from −5 to −35°C. One group was incubated in a refrigerator at 5°C for 2 h, and the other group was incubated in a deep freezer at −75°C for 24 h. The incubated samples were thawed at 0°C, and all temperatures were recorded every second using a data logger (CR-1000 M, Campbell Scientific, Inc., Logan, UT, USA) with a copper-constantan thermocouple.

Following cold treatment, buds were separated from shoots and internode

part of the shoots were cut into 1 cm pieces. Five pieces of each sample were shaken in a 50-mL tube containing 10 mL distilled water on an orbital shaker (Supertech^{TM} Orbital Shaker, SeouLin Bioscience, Seoul, Korea) at 125 rpm at room temperature for 24 h. Electrical conductivity (EC) was measured using an EC meter (Orion Star A215, Thermo Scientific, Waltham, MA, USA), and measured again after autoclaving at 120^{o}C for 30 min. Percent injury was calculated as described by Arora et al. (1992), and adjusted using the equation in the report of Yu et al. (2017). Median lethal temperature (LT_{50}) was calculated using the Gompertz function. Adjusted injury value of each cultivar and month were resampled 30 times as described by Arora et al. (2004) to obtain efficient LT_{50} estimates without repeating entire experiment.

RESULTS AND DISCUSSION

Cold hardiness of the peach tree shoots was expressed by LT_{50} values determined based on EL from the shoots subjected to various cold temperatures. SM shoots showed lower LT_{50} than KK shoots both in October and January (Fig. 1). The LT_{50} values of both cultivars were lower in January than in October, indicating that their shoots became cold hardier during winter. However, the cold hardiness difference between the two cultivars were not significantly changed.

Following the transcriptome sequencing on SM and KK shoots sampled in October and January, the trimmed reads obtained were ranged from 54,420,355 to 66,451,463 with GC content of 45.8-46.3% and Q30 ratio of 93.8-95.5% (Table 1). The total bases of average 5.5-6.7 Gb were obtained and 96.7-97% of the total reads were mapped to the reference peach genome. Based on the mapping and FPKM calculation, totally 190 and 176 DEGs were found in October and January, respectively, from the two cultivars. In October, 115 up-regulated and 75 down-regulated DEGs were identified between SM and KK shoots, whereas 82 up-regulated and 94 down-regulated DEGs were identified in January. Top 10 up-regulated and down-regulated DEGs from SM over KK shoots in October and in January were listed in Tables 2 and 3, respectively. Most of the known DEGs in the top 10 lists were related to disease

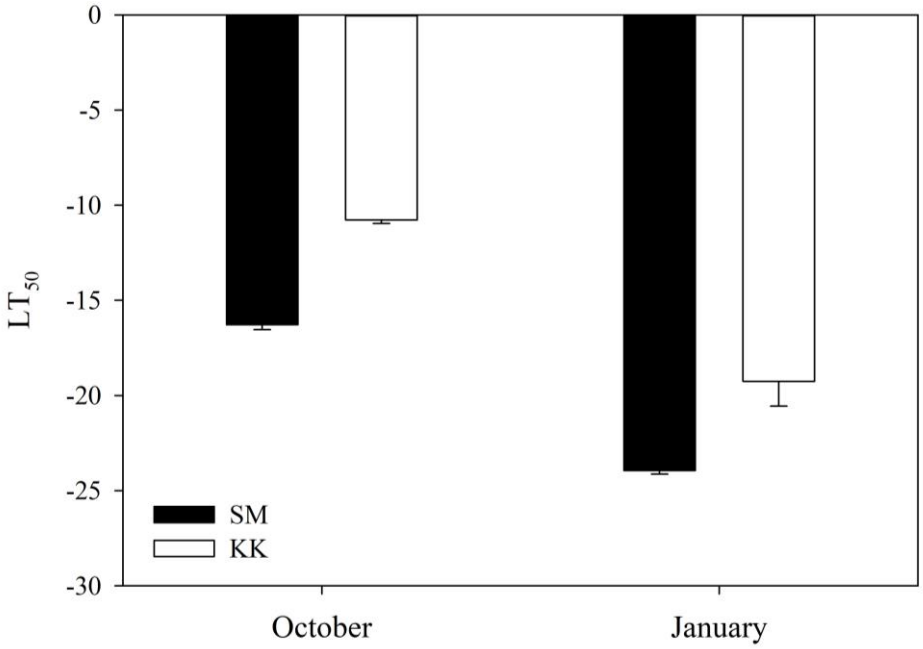

Fig. 1. LT_{50} values for 'Soomee (SM)' and 'Kiraranokiwami (KK)' peach tree shoots in October and January. Vertical bars indicate standard errors of the means (n=30).

Table 1. Sequencing results of 'Soomee (SM)' and 'Kiraranokiwami (KK)' peach tree shoots.

Cultivar	Month	Total reads	Total bases	GC content (%)	Q30 (%)	Mapping ratio (%)
SM	October	66,451,463	6,683,605,100	46.3	95.4	97.0
	January	54,420,355	5,463,673,888	45.8	93.9	96.7
KK	October	61,359,941	6,171,339,345	46.2	95.5	97.0
	January	63,556,044	6,381,156,539	45.8	93.8	96.7

Read length is 101 bp, and each value is the mean of three biological replicates.

Table 2. Top 10 up-regulated DEGs from 'Soomee' over 'Kiraranokiwami' peach tree shoots in October and January.

Month	Product	Fold change
October	Uncharacterized LOC109947120	51.4***
	Uncharacterized LOC18783343	11.8***
	Zinc finger CCCH domain-containing protein 48-like	8.44***
	Small ubiquitin-related modifier 2	7.27**
	TMV resistance protein N	7.15**
	Uncharacterized LOC109950486	5.64***
	Uncharacterized LOC18768878	5.42*
	Uncharacterized LOC18783966	4.78***
	Dynamin-related protein 4C	4.46*
	Gibberellin-regulated protein 14	4.27*
January	Uncharacterized LOC109947120	33.5***
	Uncharacterized LOC18783198	9.96***
	Uncharacterized LOC18783343	9.70**
	Leaf lust 10 disease-resistance locus receptor-like protein kinase-like 2.2	8.96***
	Zinc finger CCCH domain-containing protein 48-like	8.24***
	TMV resistance protein N	7.35***
	Uncharacterized LOC109950486	5.97***
	Squalene monooxygenase	5.84***
	Receptor-like protein 12	5.69**
	Abscisic stress-ripening protein 3	5.53**

*, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively.

Table 3. Top 10 down-regulated DEGs from 'Soomee' over 'Kiraranokiwami' peach tree shoots in October and January.

Month	Product	Fold change
October	Uncharacterized LOC18777218	–19.4***
	Uncharacterized LOC109948783	–11.1***
	TMV resistance protein N	–6.87***
	Disease resistance protein At4g27190	–5.10**
	Chaperone protein dnaJ 11, chloroplastic	–4.66*
	MDIS1-interacting receptor like kinase 2	–3.83**
	F-box/kelch-repeat protein At1g15670	–3.74*
	Uncharacterized LOC109949772	–3.68**
	12-Oxophytodienoate reductase 2	–3.68**
	Uncharacterized protein DDB_G271670	–3.34**
January	Uncharacterized LOC18777218	–15.2***
	MLP-like protein 28	–7.66**
	MLP-like protein 328	–6.40*
	TMV resistance protein N	–6.00***
	MLP-like protein 329	–5.88*
	Uncharacterized LOC109948783	–5.32***
	MLP-like protein 328	–5.12*
	MDIS1-interacting receptor like kinase 2	–4.57***
	Glutathione S-transferase U17	–4.43**
	Cysteine-rich repeat secretory protein 38	–4.36**

*, **, *** Significant at $P < 0.05$, 0.01, or 0.001, respectively.

resistance or biotic stress, and one-third of the total were uncharacterized proteins. Furthermore, most up-regulated protein in October and January was same, uncharacterized LOC109947120. Similarly, most down-regulated protein was uncharacterized LOC18777218 in October and January. According to the UniProt database, LOC18777218 might have one transmembrane domain, implying that this protein is one of the membrane integrated proteins.

Gene set enrichment analysis based on GO and KEGG database revealed that the DEGs related to defense response and ADP binding were abundantly detected both in October and January (Fig. 2). Although the DEGs related to response to biotic stimulus and signal transduction were also detected both in October and January, they were higher in January than in October (Fig. 2A, B). However, the DEGs related to cell wall macromolecule catabolic process, chitin catabolic process, trehalose biosynthetic pathway, chitinase activity, chitin binding, and integral component of plasma membrane were detected only in October (Fig. 2A), whereas those related to flavonoid biosynthetic process, L-phenylalanine catabolic process, coenzyme binding, heme binding, monooxygenase activity, squalene monooxygenase activity, and phenylalanine ammonia-lyase activity were detected only in January (Fig. 2B). However, only 32 (16.8%) and 37 (21.0%) DEGs from October and January were annotated by KEGG database, respectively. Annotation based on the InterPro database also showed that most of the annotated DEGs included defense response related domains both from October and January (Tables 4, 5).

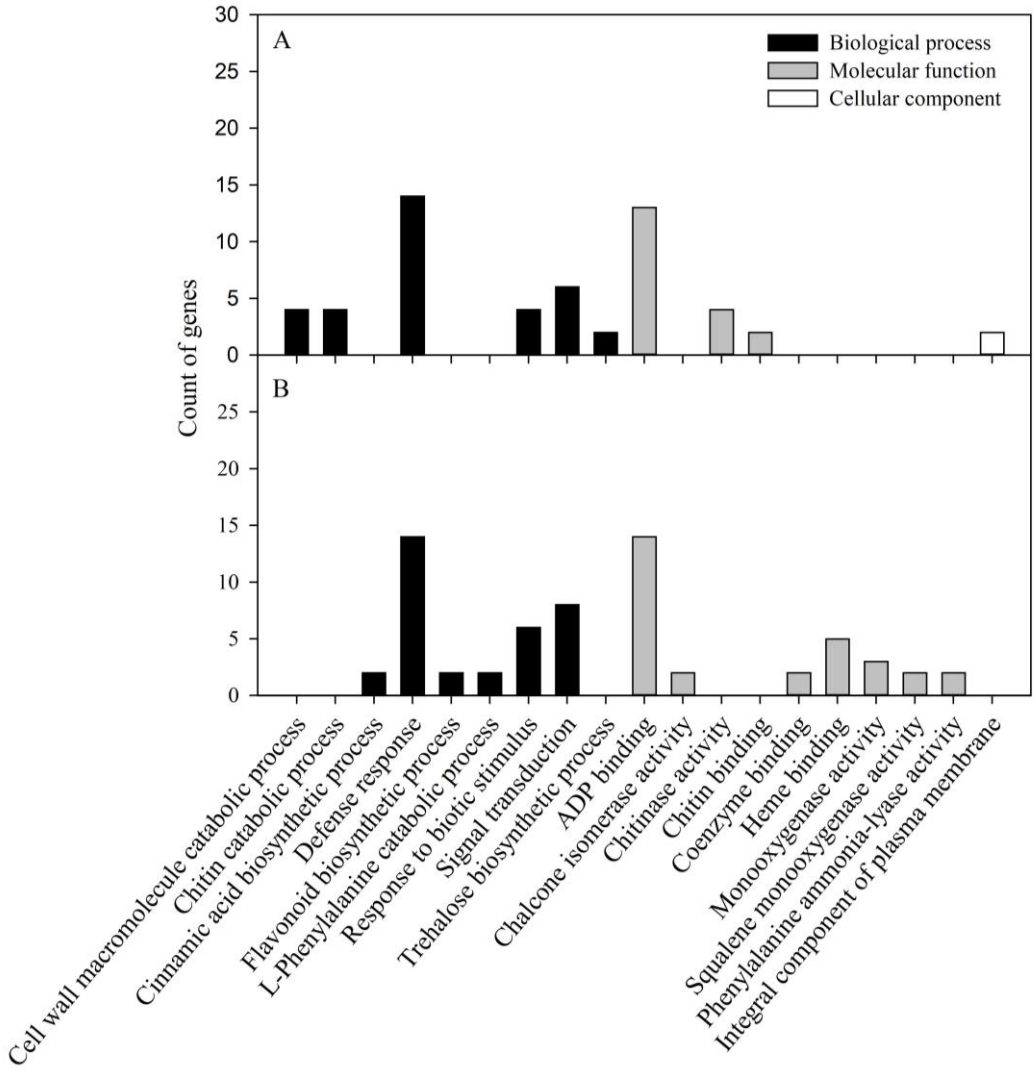

Fig. 2. Gene set enrinchment analysis results from ‘Soomee’ over ‘Kiraranokiwami’ peach tree shoots in October (A) and January (B).

Table 4. InterPro families of DEGs from ‘Soomee’ over ‘Kiraranokiwami’ peach tree shoots in October.

InterPro ID	Description	No. of DEGs
IPR002182	NB-ARC	13
IPR000726	Glycoside hydrolase, family 19, catalytic	4
IPR023346	Lysozyme-like domain	4
IPR024949	Bet v I type allergen	4
IPR000916	Bet v I domain	4
IPR027417	P-Loop containing nucleoside triphosphate hydrolase	18
IPR016283	Glycoside hydrolase, family 19	3
IPR000157	Toll/interleukin-1 receptor homology (TIR) domain	6
IPR011991	Winged helix-turn-helix DNA-binding domain	6
IPR023393	START-like domain	4
IPR013785	Aldolase-type TIM barrel	4
IPR018371	Chitin-binding, type 1, conserved site	2
IPR001002	Chitin-binding, type 1	2
IPR006598	Lipopolysaccharide-modifying protein	2

Table 5. InterPro families of DEGs from ‘Soomee’ over ‘Kiraranokiwami’ peach tree shoots in January.

InterPro ID	Description	No. of DEGs
IPR002182	NB-ARC	15
IPR000157	Toll/interleukin-1 receptor homology (TIR) domain	8
IPR000916	Bet v I domain	6
IPR023393	START-like domain	6
IPR027417	P-Loop containing nucleoside triphosphate hydrolase	15
IPR023144	Phenylalanine ammonia-lyase, shielding domain	2
IPR001106	Aromatic amino acid lyase	2
IPR022313	Phenylalanine/histidine ammonia-lyases, active site	2
IPR005922	Phenylalanine ammonia-lyase	2
IPR000425	Major intrinsic protein	1
IPR023271	Aquaporin-like	1
IPR016088	Chalcone isomerase, 3-layer sandwich	2
IPR017972	Cytochrome P450, conserved site	3
IPR002401	Cytochrome P450, E-class, group I	3
IPR013698	Squalene epoxidase	2
IPR003591	Leucine-rich repeat, typical subtype	5
IPR001509	NAD-dependent epimerase/dehydratase	2
IPR008948	L-Aspartase-like	2
IPR001002	Chitin-binding, type 1	2
IPR016087	Chalcone isomerase	2
IPR024083	Fumarase/histidase, N-terminal	2
IPR018371	Chitin-binding, type 1, conserved site	2

The in silico results were validated by performing qPCR against the DEGs showing the significant fold change both in October and January (Fig. 3). The present results demonstrated the cultivar difference of peach trees in cold hardiness at transcriptome level.

Most of the DEGs found in the present study were associated with defense response, ADP binding, and signal transduction, but almost one-third of the DEGs were uncharacterized. Furthermore, well-known cold hardiness-related genes like *CBF/DREB* genes, sugar metabolism involved genes, and dehydrin were not differentially expressed between the two cultivars. Therefore, the cultivar difference of cold hardiness might also be influenced by many uncharacterized genes. Further studies on the uncharacterized proteins showing significant fold change are required to identify the genes affecting the cold hardiness difference between the peach tree cultivars.

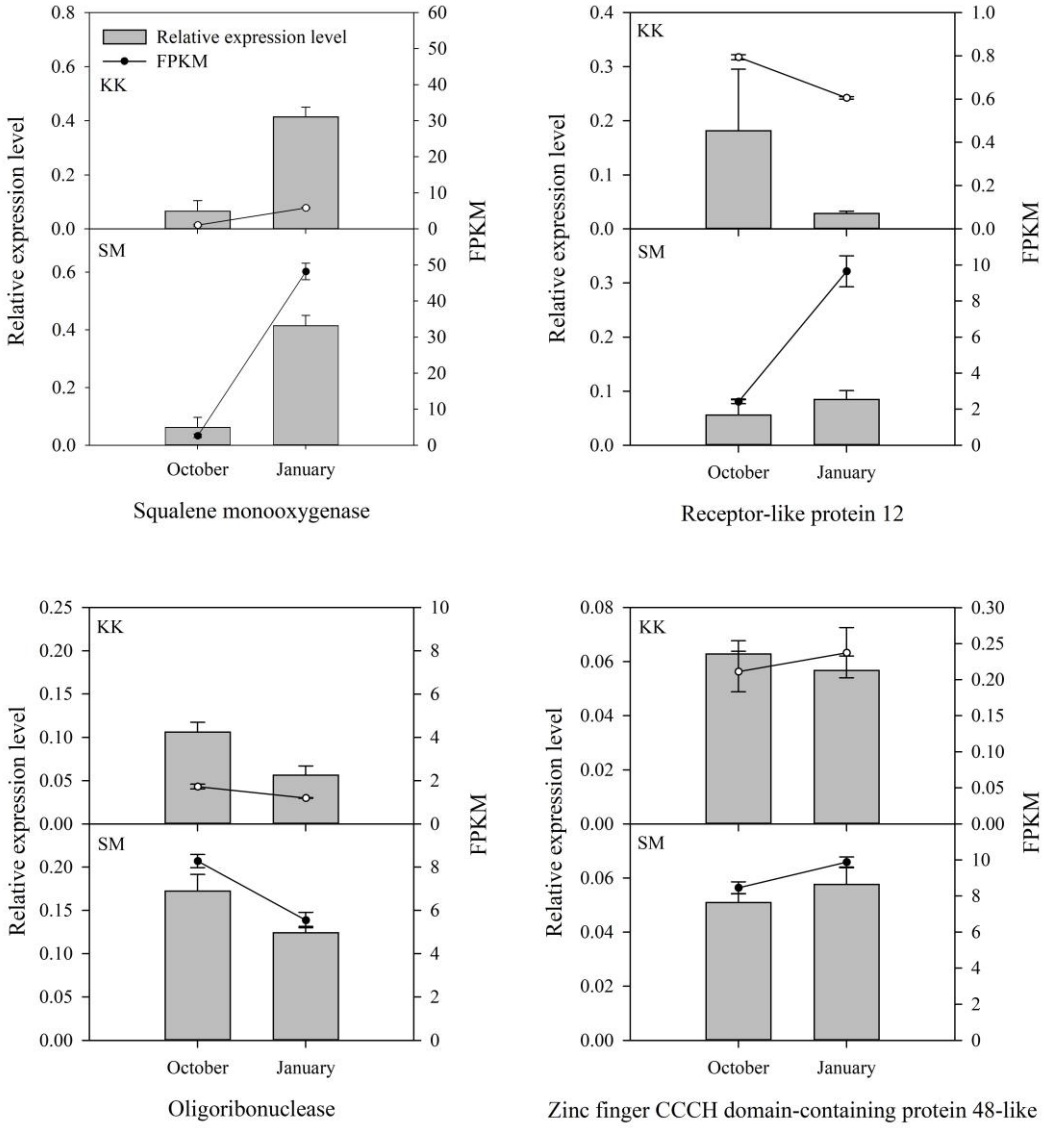

Fig. 3. Quantitative PCR results for validating transcriptome analysis of 'Soomee (SM)' and 'Kiraranokiwami (KK)' peach tree shoots in October and January. Vertical bars indicate standard errors of the means (n=3).

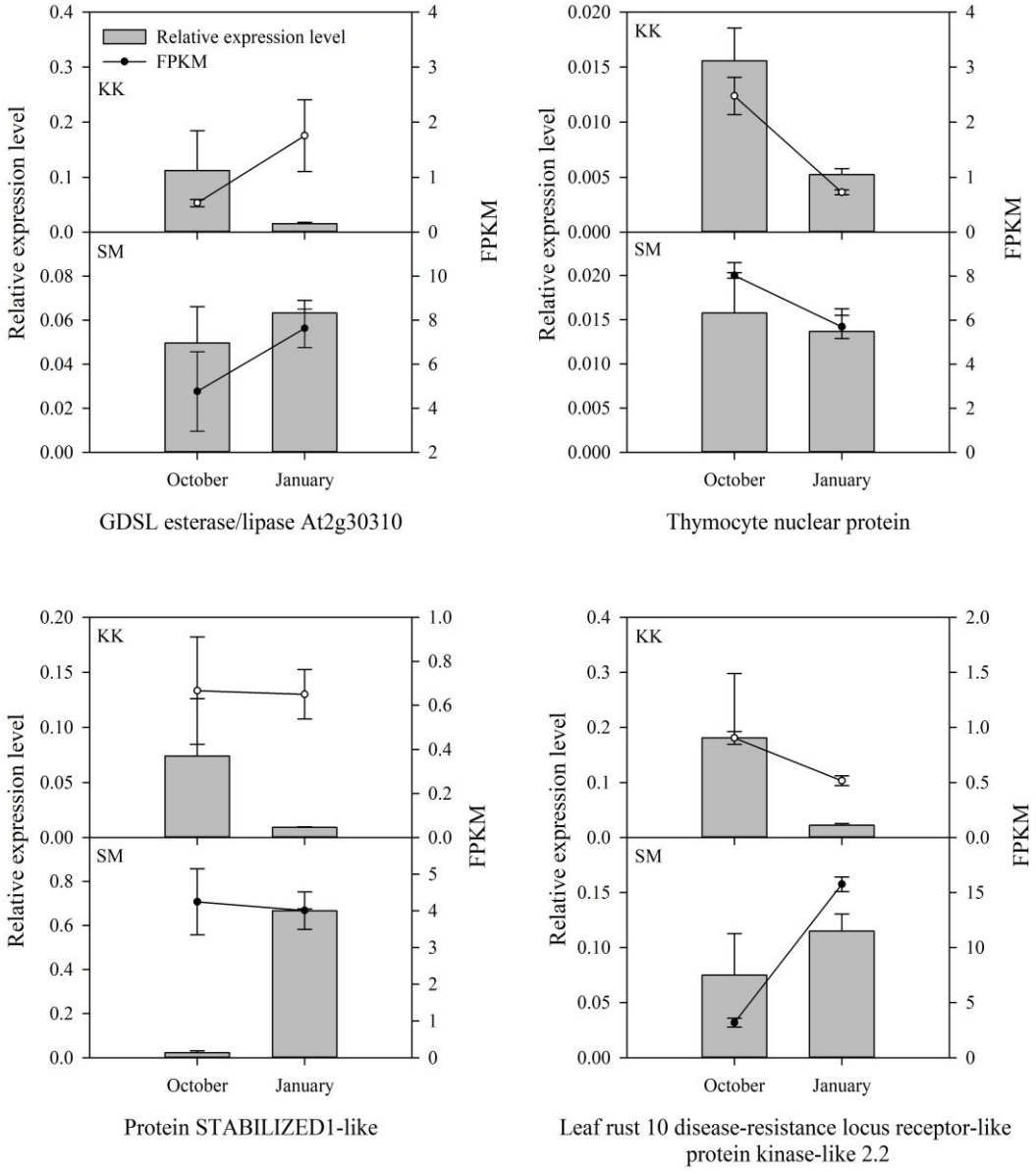

Fig. 3. Continued.

LITERATURE CITED

Abeynayake, S.W., S. Byrne, I. Nagy, K. Jonaviciene, T.P. Etzerodt, B. Boelt, and T. Asp. 2015. Changes in *Lolium prenne* transcriptome during cold acclimation in two genotypes adapted to different climatic conditions. BMC Plant Biol. 15: 250-263.

Arora, R., L.J. Rowland, E.L. Ogden, A.L. Dhanaraj, C.O. Marian, M.K. Ehlenfeldt, and B. Vinyard. 2004. Dehardening kinetics, bud development, and dehydrin metabolism in blueberry cultivars during deacclimation at constant, warm temperatures. J. Am. Soc. Hort. Sci. 129: 667-674.

Arora, R., M.E. Wisniewski, and R. Scorza. 1992. Cold acclimation in genetically related (sibling) deciduous and evergreen peach (*Prunus persica* [L.] Batsch). Plant Physiol. 99: 1562-1568.

Artlip, T.S., M.E. Wisniewski, C.L. Bassett, and J.L. Norelli. 2013. CBF gene expression in peach leaf and bark tissues is gated by a circadian clock. Tree Physiol. 33: 866-877.

Barros, P.M., N. Gonçalves, N.J.M. Saibo, and M.M. Oliveira. 2012. Functional characterization of two almond C-repeat-binding factors involved in cold response. Tree Physiol. 32: 1113-1128.

Bassett, C.L., K.M. Fisher, and R.E. Farrell. 2015. The complete peach dehydrin family: Characterization of three recently recognized genes. Tree Genet. Genom. 11: 126.

Benedict, C., J.S. Skinner, R. Meng, Y. Chang, R. Bhalerao, N.P.A. Huner, C.E. Finn, T.H.H. Chen, and V. Hurry. 2006. The CBF1-dependent low temperature signaling pathway, regulon, and increase in freeze tolerance are conserved in *Populus* spp. Plant Cell Environ. 29: 1259-1272.

Chen, J., X. Yang, X. Huang, S. Duan, C. Long, J. Chen, and J. Rong. 2017. Leaf transcriptome analysis of a subtropical evergreen broadleaf plant, wild oil-tea camellia (*Camelia oleifera*), revealing candidate genes for cold acclimation. BMC Genomics 18: 211-224.

El Kayal, W., G. Keller, C. Debayles, R. Kumar, D. Weier, C. Teulieres, and C. Marque. 2006. Regulation of tocopherol biosynthesis through transcriptional control of tocopherol cyclase during cold hardening in *Eucalyptus gunii*. Physiol. Plant. 126: 221-223.

Gaete-Loyola, J., C. Lagos, M.F. Beltran, S. Valenzuela, V. Emhart, and M. Fernandez. 2017. Transcriptome profiling of *Eucalyptus nutens* reveals deeper insight into the molecular mechanism of cold acclimation and deacclimation process. Tree Genet. Genom. 13: 37-54.

Gambino, G., I. Perrone, and I. Gribaudo. 2008. A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. Phytochem. Anal. 19: 520-525.

Guerra, D., A. Lamontanara, P. Bagnaresi, L. Orru, F. Rizza, S. Zelasco, D. Beghe, T. Ganino, D. Pagani, L. Cattivelli, and E. Mazzucotelli. 2015. Transcriptome changes associated with cold acclimation in leaves of olive

tree (*Olea europaea* L.). Tree Genet. Genom. 11: 113-136.

Jiao, Y., Z. Shen, and J. Yan. 2017. Transcriptome analysis of peach [*Prunus persica* (L.) Batsch] stigma in response to low-temperature stress with digital gene expression profiling. J. Plant Biochem. Biotechnol. 26: 141-148.

Kitashiba, H., T. Ishizaka, K. Isuzugawa, K. Nishimura, and T. Suzuki. 2004. Expression of a sweet cherry *DREB1*/*CBF* ortholog in *Arabidopsis* confers salt and freezing tolerance. J. Plant Physiol. 161: 1171-1176.

Kobayashi, M., H, Horiuchi, K. Fujita, Y. Takahura, and S. Suzuki. 2012. Characterization of grape C-repeat-binding factor 2 and B-box-type zinc finger protein in transgenic *Arabidopsis* plants under stress conditions. Mol. Biol. Rep. 39: 7933-7939.

Lee, J.H., D.J. Yu, S.J. Kim, D. Choi, and H.J. Lee. 2012. Intraspecies differences in cold hardiness, carbohydrate content, and β-amylase gene expression of *Vaccinium corymbosum* during cold acclimation and deacclimation. Tree Physiol. 32: 1533-1540.

Li, J., N. Wang, H. Xin, and S. Li. 2013. Overexpression of *VaCBF4*, a transcription factor from *Vitis amurensis*, improves cold tolerance accompanying increased resistance to drought and salinity in *Arabidopsis*. Plant Mol. Biol. Rep. 31: 1518-1528.

Owens, C.L., M.F. Thomashaw, J.F. Hancock, and A.F. Iezzoni. 2002. CBF orthologs in sour cherry and strawberry and the heterologous expression of

CBF1 in strawberry. J. Am. Soc. Hort. Sci. 127: 489-494.

Pagter, M., C.R. Jensen, K.K. Peterson, F. Liu, and R. Arora. 2008. Changes in carbohydrates, ABA, and bark proteins during seasonal cold acclimation and deacclimation in *Hydrangea* species differing in cold hardiness. Physiol. Plant. 134: 473-485.

Shen, C., D. Li, R. He, Z. Fang, Y. Xia, J. Gao, H. Shen, and M. Cao. 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.

Siddiqua, M. and A. Nassuth. 2011. *Vitis CBF1* and *Vitis CBF4* differ in their effect on *Arabidopsis* abiotic stress tolerance, development, and gene expression. Plant Cell Environ. 34: 1345-1359.

Takhura, Y., M. Kobayashi, and S. Suzuki. 2011. Low-temperature-induced transcription factors in grapevine enhance cold tolerance in transgenic *Arabidopsis* plants. J. Plant Physiol. 168: 967-975.

Thomashow, M.F. 1999. Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. Annu. Rev. Plant Biol. 50: 571-599.

Verde, I., A.G. Abbott, S. Scalabrin, S. Jung, S. Shu, F. Marroni, T. Zhebentyayeva, M.T. Dettori, J. Grimwood, F. Cattonaro, A. Zuccolo, L. Rossini, J. Jenkins, E. Vendramin, L.A. Meisel, V. Decroocq, B. Sosinski, S. Prochnik, T. Mitros, A. Policriti, G. Cipriani, L. Dondini, S. Ficklin, D.M. Goodstein, P. Xuan, C.D. Fabbro, V. Aramini, D. Copetti, S. Gonzalez, D.S. Horner, R. Falchi, S. Lucas, E. Mica, J. Maldonado, B. Lazzari, D.

Bielenberg, R. Pirona, M. Miculan, A. Barakat, R. Testolin, A. Stella, S. Tartarini, P. Tonutti, P. Arus, A. Orellana, C. Wells, D. Main, G. Vizzotto, H. Silva, F. Salamini, J. Schmutz, M. Morgante, and D.S. Rokhsar. 2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication, and genome evolution. Nat. Genet. 45: 487-494.

Wang, X.C., Q.Y. Zhao, C.L. Ma, Z.H. Zhang, H.L. Cao, Y.M. Kong, C. Yue, X.Y. Hao, L. Chen, J.Q. Ma, J.Q. Jin, X. Li, and Y.J. Yang. 2013. Global transcriptome profiles of *Camellia sinensis* during cold acclimation. BMC Genomics 14: 415-418.

Welling, A. and E.T. Palva. 2008. Involvement of CBF transcription factors in winter hardiness in birch. Plant Physiol. 147: 1199-1211.

Wisniewski, M.E., J.L. Norelli, C.L. Bassett, T.S. Artlip, and D. Macarisin. 2011. Ectopic expression of a novel peach (*Prunus persica*) *CBF* transcription factor in apple (*Malus domestica*) results in short-day induced dormancy and increased cold hardiness. Planta 233: 971-983.

Wisniewski, M.E., A. Nassuth, C. Teulieres, C. Marque, L.J. Rowland, P.B. Chao, and A. Brown. 2014. Genomics of cold hardiness in woody plants. Crit. Rev. Plant Sci. 33: 92-124.

Xu, W., R. Li, N. Zhang, F. Ma, Y. Jiao, and Z. Wang. 2014. Transcriptome profiling of *Vitis amurensis*, an extremely cold-tolerant Chinese wild *Vitis* species, reveals candidate genes and events that potentially connected to

cold stress. Plant Mol. Biol. 86: 527-541.

Yu, D.J., J.Y. Hwang, S.W. Chung, H.D. Oh, S.K. Yun, and H.J. Lee. 2017. Changes in cold hardiness and carbohydrate content in peach (*Prunus pesica*) trunk bark and wood tissues during cold acclimation and deacclimation. Sci. Hort. 219: 45-52.

ABSTRACT IN KOREAN

복숭아 나무(*Prunus perisca*)의 내한성은 품종마다 다르다. 이러한 품종 간 차이를 유발하는 유전자들을 찾기 위해 서로 다른 내한성을 갖는 두 복숭아 품종의 전사체를 차세대 염기 서열 분석법을 사용해 비교하였다. 복숭아 나무 중 '수미'는 상대적으로 내한성이 강하고, '키라라노키와미'는 내한성이 약한 것으로 알려져 있다. 노지에서 키운 두 품종의 5년생 나무에서 10월과 1월에 가지를 채취하였으며, 1년생 가지에서 추출한 RNA를 전사체 분석에 사용하였다. 전사체 서열 분석 결과, 4.8-7.1Gb 크기의 정보를 얻었으며, 전체 단편 중 94-97%가 표준 복숭아 유전체에 매핑되었다. 두 품종 간에서 차별적으로 발현하는 유전자들이 10월에서 190개, 1월에서 262개가 발견되었다. Gene set enrichment 분석으로 몇몇 특정되지 않은 단백질들과 long noncoding RNA들을 포함하는 대부분의 차별 발현 유전자들이 세포벽 고분자 분해 과정, 신호 전달, ADP 결합, 트레할로스 생합성 과정, 그리고 세포막 필수 구성 요소에 속한다는 것을 밝혀내었다. 컴퓨터로 생산된 결과들은 역전사 반응 후 10월과 1월에서 유의미한 변화량을 보인 차별 발현 유전자들에 대해 정량적 중합 효소 연쇄 반응을 시행하여 검정하였다. 본 연구에서의 결과는 전사체 수준에서 복숭아 나무의 품종 간 내한성 차이를 나타내고 있다.