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Research Article

Comparative transcriptome analysis of heat stress responsiveness between two contrasting ginseng cultivars

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ABSTRACT

Background: *Panax ginseng* has been used in traditional medicine to strengthen the body and mental well-being of humans for thousands of years. Many elite ginseng cultivars have been developed, and ginseng cultivation has become well established during the last century. However, heat stress poses an important threat to the growth and sustainable production of ginseng. Efforts have been made to study the effects of high temperature on ginseng physiology, but knowledge of the molecular responses to heat stress is still limited.

Methods: We sequenced the transcriptomes (RNA-Seq) of two ginseng cultivars, Chunpoong (CP) and Yunpoong (YP), which are sensitive and resistant to heat stress, respectively, after 1- and 3-week heat treatments. Differential gene expression and gene ontology enrichment along with profiled chlorophyll contents were performed.

Results: CP is more sensitive to heat stress than YP and exhibited a lower chlorophyll content than YP. Moreover, heat stress reduced the chlorophyll content more rapidly in CP than in YP. A total of 329 heat-responsive genes were identified. Intriguingly, genes encoding chlorophyll a/b–binding proteins, WRKY transcription factors, and fatty acid desaturase were predominantly responsive during heat stress and appeared to regulate photosynthesis. In addition, a genome-wide scan of photosynthetic and sugar metabolic genes revealed reduced transcription levels for *ribulose 1,5-bisphosphate carboxylase/oxygenase* under heat stress, especially in CP, possibly attributable to elevated levels of soluble sugars.

Conclusion: Our comprehensive genomic analysis reveals candidate loci/gene targets for breeding and functional studies related to developing high temperature–tolerant ginseng varieties.

1. Introduction

Plants experience various abiotic and biotic stresses that adversely affect their growth and productivity worldwide [1–4]. High temperature and heat stress are of a particular concern as temperatures are projected to rise by 6.9°C by the end of this century [5] due to global warming [6–8], and temperature increase of 3–4°C is expected to reduce crop productivity by 15–35% [9]. In addition, it is expected that heat stress along with drought stress reduce agricultural production even more than heat or drought stress alone [10].

The major effects of heat include increased membrane fluidity, protein denaturation, and stimulated production of reactive oxygen species, which cause permanent damage to plant growth and development [4], notably during their reproductive stage [4]. These effects and the plant's response to them can be reflected by alterations to the transcriptome, proteome, and metabolome, and, ultimately, severe cellular injury [8]. Heat stress upregulates about 5% of the plant transcriptome, including genes encoding molecular chaperones and genes involved in signaling, translation, and metabolism, to mediate heat stress responses in plants [4]. Thus, it is essential to identify quantitative trait loci (QTLs)/genes involved in heat stress responses to develop crops with enhanced heat tolerance and ensure global food security.

Korean ginseng (*Panax ginseng* Meyer, hereafter referred to as *P. ginseng* or simply ginseng) is one of the most famous traditional

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medicinal herbs. Ginseng, which has been used for more than 2000 years in Asia, produces triterpene saponins as principle bioactive secondary metabolites with various pharmacological and physiological benefits to humans [11–13]. *P. ginseng* is a shade-loving perennial plant that is susceptible to photoinhibition at light intensities exceeding 500 μmol/m^2 s. In addition, leaves of this plant are burned after exposure to 30°C for more than 5 days, and its growth will completely stop. Owing to this sensitivity to heat, *P. ginseng* plants can be severely damaged in summer and require careful management. Moreover, the increasing temperatures associated with global warming represent an important threat to *P. ginseng* growth and production. A few physiological and morphological studies [14–16] have been conducted to understand the heat response in *P. ginseng*, but a comprehensive study that combines genome, transcriptome, and physiological data to understand the heat response in *P. ginseng* is still needed.

In Korea, the *P. ginseng* cultivar (cv.) Yunpoong (YP) is known to have slightly higher heat tolerance (light saturation point of 400 μmol/m^2 s) than the heat-sensitive cv. Chunpoong (CP) (light saturation point of 200 μmol/m^2 s) [14–16]. In this study, we analyzed and compared transcriptomes of CP and YP under different heat treatments to understand ginseng heat response mechanisms and identify major genes and biological processes affected by heat stress. This study reports the first transcriptome profile in response to heat stress and provides novel candidate target genes for alleviating adverse effects of heat stress in *P. ginseng*.

2. Materials and methods

2.1. Plant materials, growth conditions, and heat treatments

Dormant roots with healthy rhizomes of 1-year-old cv. CP and YP plants were obtained from the Korea Ginseng Corporation (Daejeon, Korea). After storage for 1 month at 4°C to break dormancy, the roots were planted in soil and grown for 4 weeks to

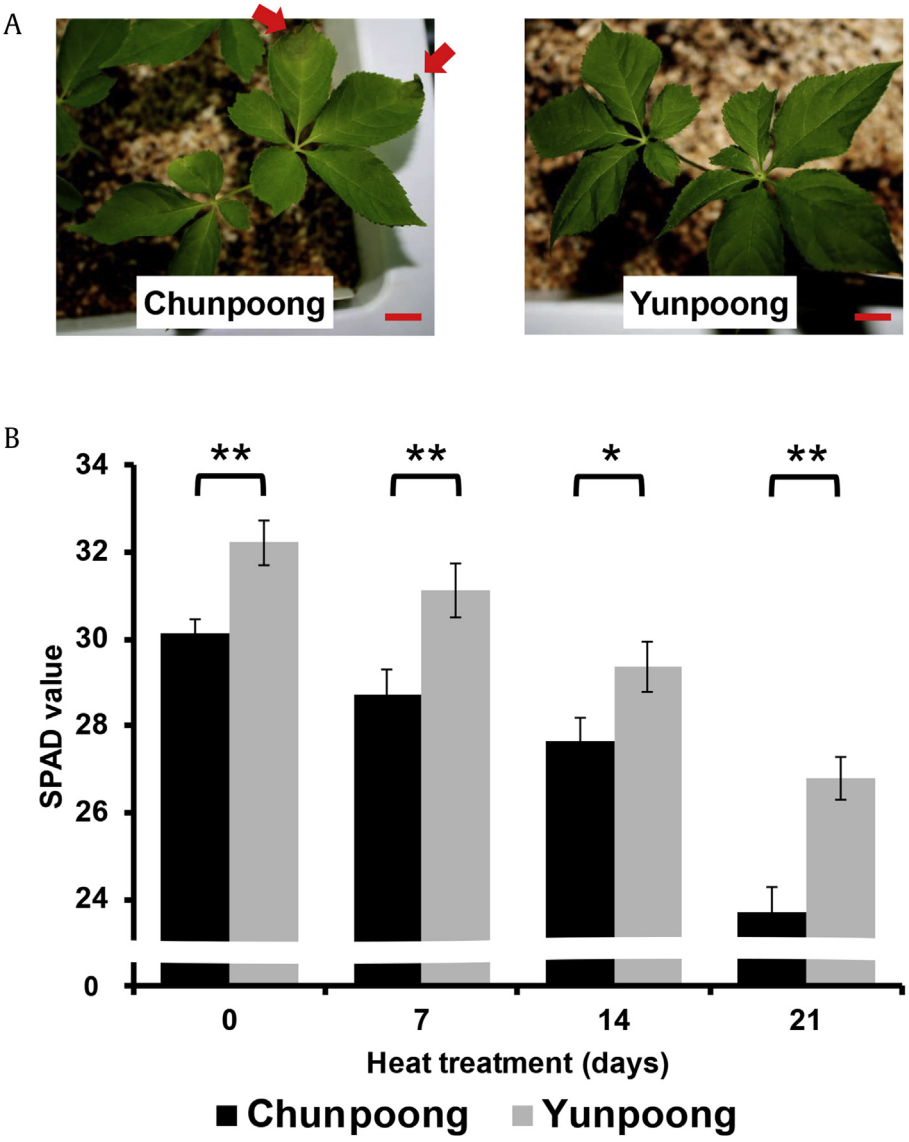

Fig. 1. Leaf burning and chlorophyll content of *P. ginseng* plants under heat stress. (A) Different leaf burning occurrence between CP and YP. After heat treatment for 3 weeks, leaves of CP showed damaged regions (leaf burning, red arrows), whereas those of YP did not show any visibly damaged regions. Scale bars correspond to 1 cm. (B) Changes of chlorophyll content (SPAD values) during heat treatment. Error bars indicate mean ± standard deviation (SD) obtained from more than 20 plants. Asterisks indicate values with significant differences between two cultivars, based on Student *t* test (*$p < 0.05$, **$p < 0.01$).
CP, Chunpoong; SPAD, soil plant analysis development; YP, Yunpoong.

generate plants with fully expanded leaves under normal growth conditions (24°C, relative humidity 60%, and continuous light of 40 μmol/m^2 s). Samples of these plants were harvested as controls before heat treatment. For heat treatment, the plants were treated at 30 (±1) °C for 1 week and 3 weeks (relative humidity and light conditions were the same as the normal growth conditions). After heat treatment, sample leaves were excised, immediately frozen using liquid nitrogen (LN_2), and stored at −70°C before total RNA isolation. Three independent biological replicates were prepared, and each replicate included leaves from three or more plants.

2.2. Measurement of chlorophyll content

Chlorophyll content was measured in leaves of more than 20 plants using a chlorophyll meter [soil plant analysis development (SPAD)-502; Minolta, Japan] before and during heat treatment, as described in the study by Lee et al [16].

2.3. Total RNA isolation and RNA-Seq analysis

Total RNA was isolated from leaves using the RNeasy Plant kit (QIAGEN, Germany) and/or Hybrid-R kit (GeneAll, Korea) according to the manufacturer's instructions. After examination of its quality and quantity using a Bioanalyzer (Agilent Technologies, USA), about 2 μg of total RNA was used for the construction of RNA-Seq libraries. RNA-Seq libraries with an insert size of 300 bp were constructed independently using the TruSeq RNA Sample Preparation Kit (Illumina, California, USA) according to the manufacturer's instructions. Pooled libraries were sequenced using the Illumina HiSeq 2000 platform with paired-end reads of 101 bp at Macrogen Co. (Seoul, Korea) or the Illumina NextSeq 500 platform with paired-end read length of 150 bp at LabGenomics Co. (Seongnam, Korea). For transcriptome analysis, reads containing bacterial contaminants were removed by mapping against the available bacterial genomes using burrows-wheeler aligner (BWA) [17] followed by removal of polymerase chain reaction duplicates and ribosomal RNA reads using FastUniq [18] and SortMeRNA [19], respectively. Finally, stringent quality control and removal of adapter contamination were performed using the NGS QC Toolkit (v2.3.3) [20].

2.4. Differential gene expression analysis

The current version of the ginseng gene set (IPGA_v1.1) was retrieved from the ginseng genome database (http://ginsengdb.snu.ac.kr) [21]. Trimmed, high-quality RNA-Seq reads were mapped to the ginseng gene set to calculate Fragments Per Kilobase per Million (FPKM) using RNA-Seq by Expectation Maximization (RSEM) [22]. The sequencing library differences (due to the use of a different Illumina platform) between replicate RNA-Seq samples were normalized using Trimmed Mean of M values [23]. The bioconductor package edgeR [24] was used to identify differentially expressed (DE) transcripts between heat stress samples (control vs. 1-week vs. 3-week) of CP and YP. Genes exhibiting more than twofold changes with a significant false discovery rate of 0.001 were considered as DE. Gene ontology enrichment analysis was performed on the DE genes using Fisher's exact test with a multiple testing correction false discovery rate limit of 0.05.

2.5. Gene family annotation

Protein domains and motifs of genes in *P. ginseng* were identified using InterProScan [25] (v5.13). Genes involved in sugar metabolism and photosynthesis were identified using the KAAS server [26]. For comparative sugar metabolic gene analysis, gene sets from carrot (http://www.ncbi.nlm.nih.gov/Traces/wgs/wgsviewer.cgi?val=LNRQ01&search=LNRQ01000000&display=contigs), tomato (ftp://ftp.solgenomics.net/tomato_genome/annotation/ITAG2.4_release/), arabidopsis (ftp://ftp.arabidopsis.org/home/tair/Genes/TAIR10_genome_release/), grape (http://www.genoscope.cns.fr/externe/Download/Projets/Projet_ML/data/12X/annotation/), and pepper (ftp://ftp.solgenomics.net/genomes/Capsicum_annuum/C.annuum_cvCM334/annotation/) were used for pathway annotation by KEGG Automatic Annotation Server (KAAS) similar to

Table 1
Summary of *P. ginseng* leaf transcriptome data obtained in this study

Samples	Raw		After filtering		SRA ID
	No. of reads	Length (bp)	No. of reads	Length (bp)	
cv. Chunpoong					
Control, rep# 1 [1)]	34,200,406	3,454,241,006	28,548,966	2,883,445,566	SRR6117049
Control, rep# 2 [2)]	12,010,556	1,671,583,519	8,401,588	1,169,602,247	SRR6117050
Control, rep# 3 [2)]	14,219,204	1,971,113,105	10,112,344	1,403,615,982	SRR6117051
1-week heat treatment, rep# 1[2)]	12,797,522	1,776,036,672	9,176,372	1,403,615,982	SRR6117052
1-week heat treatment, rep# 2[2)]	14,303,250	1,985,768,203	10,266,344	1,274,623,891	SRR6117053
1-week heat treatment, rep# 3[2)]	14,795,776	2,057,976,292	10,419,368	1,427,291,425	SRR6117057
3-week heat treatment, rep#1 [1)]	40,201,970	4,060,398,970	35,188,388	1,633,028,161	SRR6117058
3-week heat treatment, rep# 2 [2)]	14,670,068	2,031,823,791	11,931,866	1,650,210,144	SRR6117059
3-week heat treatment, rep# 3 [2)]	18,163,348	2,502,843,099	14,568,344	2,004,943,384	SRR6117061
cv. Yunpoong					
Control, rep# 1 [2)]	12,468,942	1,721,266,026	11,123,218	1,528,796,322	SRR6109634
Control, rep# 2 [2)]	15,189,718	2,106,203,208	13,386,670	1,846,968,856	SRR6109657
Control, rep# 3[2)]	14,917,750	2,062,271,071	13,121,488	1,804,691,129	SRR6109670
1-week heat treatment, rep# 1[2)]	12,277,348	1,699,892,166	10,716,748	1,476,028,232	SRR6109675
1-week heat treatment, rep# 2[2)]	14,461,344	2,001,009,942	12,730,788	1,752,633,304	SRR6109678
1-week heat treatment, rep# 3[2)]	12,226,114	1,690,713,369	10,564,980	1,452,352,808	SRR6111127
3-week heat treatment, rep# 1[2)]	13,934,774	1,925,525,852	12,403,000	1,706,350,704	SRR6111131
3-week heat treatment, rep# 2[2)]	12,189,310	1,691,363,635	10,905,514	1,507,226,834	SRR6111139
3-week heat treatment, rep# 3[2)]	11,997,888	1,652,914,364	10,636,142	1,458,514,772	SRR6111142
Total	295,025,288	38,062,944,290	244,202,128	29,383,939,743	

SRA, sequencing read archive.
[1)] Sequenced using HiSeq 2000.
[2)] Sequenced using NextSeq 500, rep: replicates.

P. ginseng. The number of genes encoding enzymes in sugar metabolic pathway was manually identified based on KAAS annotation.

3. Results and discussion

3.1. P. ginseng cultivars CP and YP show different responses to heat

During the period of heat treatment, *P. ginseng* cultivars CP and YP showed different responses in both morphology and physiology. After heat treatment for 3 weeks, leaves of CP started to show damaged regions indicated by leaf burning, whereas YP did not show any visibly damaged regions (Fig. 1A). The damage in leaf tissues was confirmed by measuring chlorophyll content (SPAD values) (Fig. 1B). Although the chlorophyll content was different between the two cultivars even under normal growth conditions, the difference increased proportionally to the treatment time. The chlorophyll content was 7% higher in YP than in CP under normal growth conditions. However, the content was 8 and 13% higher in YP after treatment for 1 week and 3 weeks, respectively. This difference indicated that chlorophyll content decreased more slowly

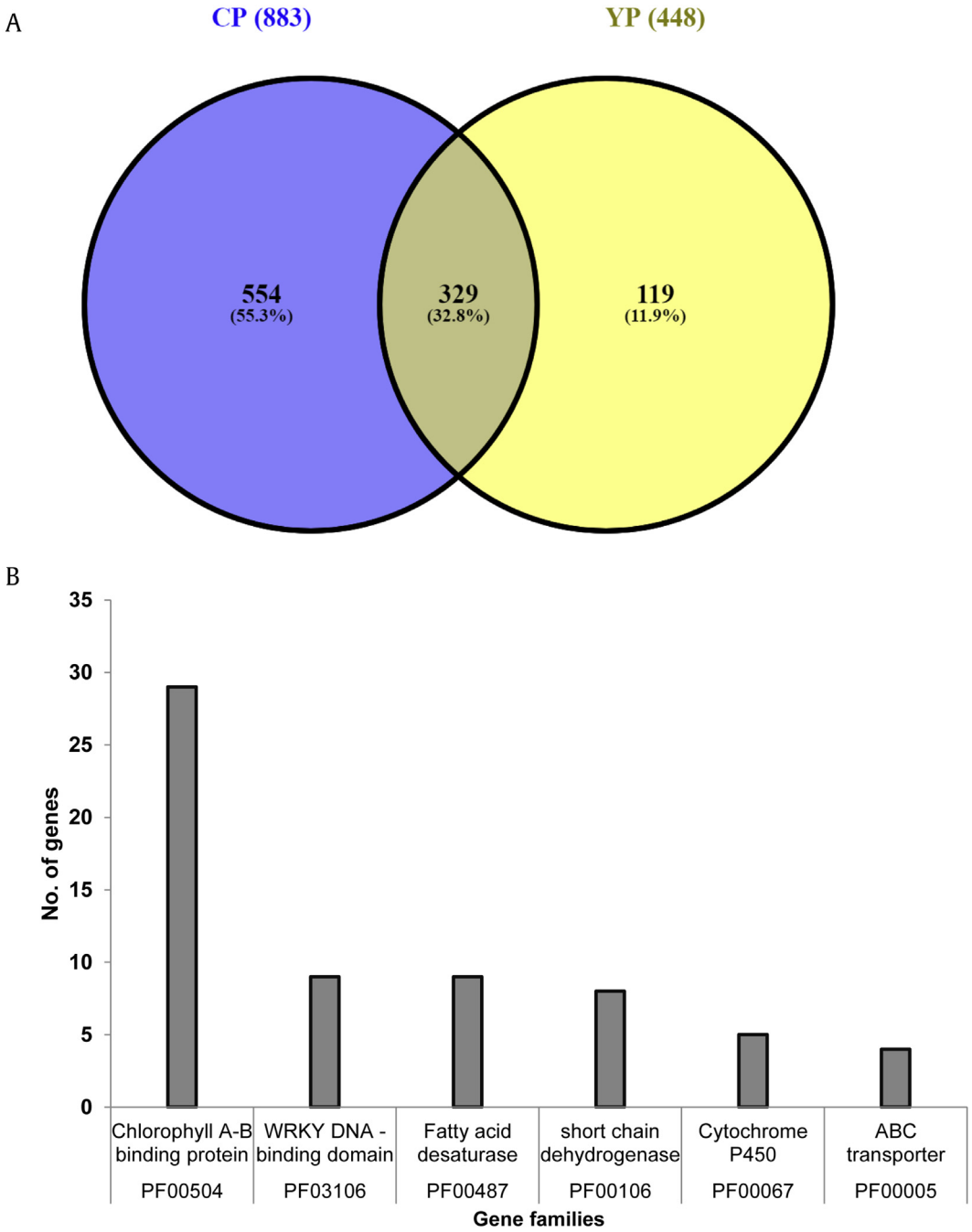

Fig. 2. Comparative transcriptome of CP and YP cultivars. (A) Venn diagram depicts number of differential expressed (DE) genes commonly found in both CP and YP cultivars. A total of 883 and 448 genes were DE during heat stress in CP and YP, respectively. A set of 329 genes showed similar expression pattern in both CP and YP cultivars during heat stress. (B) Classification of DE genes based on their Pfam domain annotated by InterProScan. All the DE genes were grouped according to the pfam domain and plotted the top six gene families showing major response against heat stress in *P. ginseng*.
CP, Chunpoong; YP, Yunpoong.

in YP than in CP under heat stress, which is in agreement with the results of a previous physiological study of the two cultivars [16].

3.2. Identification of heat-responsive genes in P. ginseng

In total, 59,352 genes were retrieved from the ginseng genome database and used as a reference for DE gene analysis. Transcriptome sequencing of three independent biological samples of both CP and YP yielded a total of 295,025,288 raw RNA-seq reads (Table 1). All raw sequencing reads were deposited into the sequencing read archive of NCBI (accession numbers are in Table 1). Before DE gene analysis, the raw RNA-Seq reads were processed and yielded 244 million high-quality reads. DE gene analysis was performed for heat-treated samples from CP and YP independently. This analysis resulted in 883 and 448 genes showing differential expression upon 1- and 3-week heat stress in CP and YP, respectively (Fig. 2A). Furthermore, a set of 329 genes was found in both CP and YP with a similar response of either upregulation or downregulation (Fig. 3) after heat stress. We considered these genes as heat-responsive genes in ginseng, irrespective of genotypes. In addition, we grouped those DE genes based on their protein domains (Fig. 2B) and found that genes belonging to the chlorophyll a/b–binding protein (CAB) family

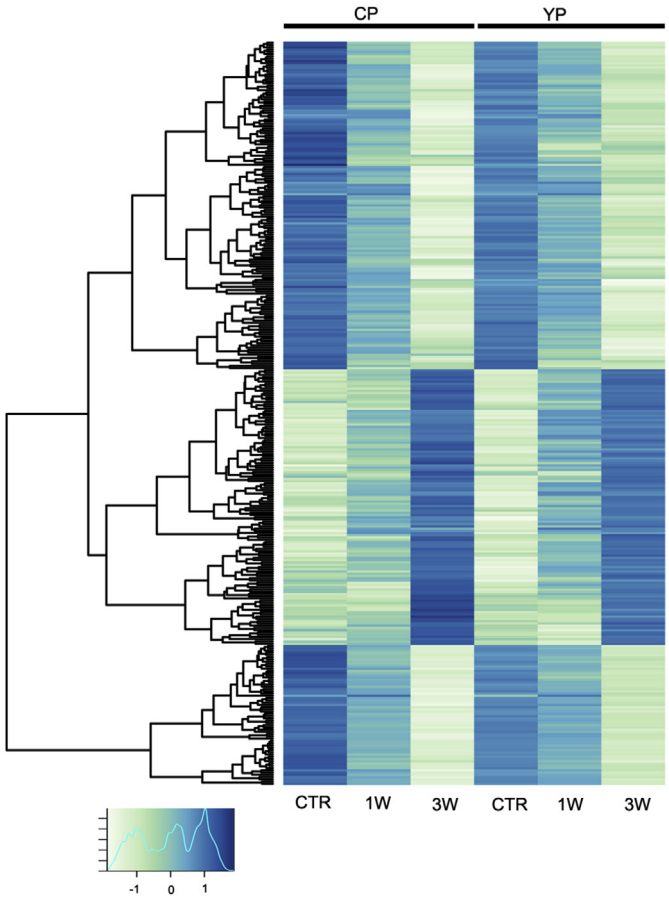

Fig. 3. Expression profiles of differentially expressed genes against time-dependent heat stress in CP and YP. A total of 329 genes showed differential expression during heat stress in both CP and YP cultivars. Heatmap shows the hierarchical clustering of average FPKM values obtained from individual normalized FPKM values of three replicates. CTR, 1W, and 3W represent control, 1-week, and 3-week heat treatment, respectively. These expression values can be seen in Appendix A. Supplementary data along with functional descriptions. Heatmap was generated using heatmap.2 function provided by the R-package gplots.
CP, Chunpoong; FPKM, Fragments Per Kilobase per Million; YP, Yunpoong.

primarily respond to heat stress. Photosynthesis is highly vulnerable to high temperature and is inhibited long before other symptoms or cell functions are impaired [1,27]. CAB genes are key components of photosynthesis, transferring light energy to the reaction centers of photosystem I (PSI) and photosystem II (PSII), where it is converted into chemical energy (i.e., nicotinamide adenine dinucleotide phosphate (NADPH) and adenoside triphosphate (ATP)). Various studies have revealed that PSII is extremely sensitive to heat, which can greatly reduce PSII activity or even stop it [28]. Our results indicate that heat might also influence the photosynthetic process in ginseng by inhibiting the light-harvesting system.

WRKY transcription factors (TFs) are one of the largest families of transcriptional regulators and play a crucial role in the defense response against various abiotic stresses [29]. WRKY39 and CaWRKY40 are the best examples of TFs that are triggered by heat in arabidopsis [30] and pepper, respectively [31]. Notably, WRKY TF genes responded strongly to heat in ginseng (Fig. 2B), suggesting their potential involvement during heat stress.

The accumulation of unsaturated fatty acids can aggravate heat damage [32], and transgenic tobacco [33] and rice [34] with silenced fatty acid desaturase (*FAD*) genes and reduced levels of trienoic fatty acid exhibit high temperature tolerance with increased photochemical efficiency of PSII. Similarly, silencing of the *LeFAD7* gene in transgenic tomato conferred tolerance to high temperature (45°C) [35]. Given these trends, it is possible that the extreme sensitivity of PSII in ginseng may be due to the effects of high temperature on chloroplast membranes. The amount of polyunsaturated fatty acid is associated with cold tolerance in plants [36,37], and a ginseng genome study has implicated an expanded *FAD* gene family in ginseng's cold adaptation [38]. The major polyunsaturated fatty acids in plant membrane lipids, such as trienoic fatty acids including hexadecatrienoic acid (16:3) and linolenic acid (18:3), are important for ensuring the maintenance of chloroplasts during plant growth under low temperatures [39]. Using KEGG (Kyoto encyclopedia of genes and genomes) analysis, we found that most of the ginseng *FAD* genes were associated with trienoic fatty acids, indicating their role in cold acclimation. We also observed that nine genes belonging to the *FAD* family showed very strong expression after 3-week heat stress. Thus, we hypothesize that silencing the *FAD* genes associated with trienoic fatty acid accumulation in ginseng might increase their tolerance to heat stress. Members of genes in short-chain dehydrogenase family are involved in cellular homeostasis including regulation of fatty acid or sugar metabolism [40]. We also noticed the differential expression of short-chain dehydrogenase genes upon heat stress in *P. ginseng* (Fig. 2B), indicating their plausible role in FAD metabolism. During drought and heat stress conditions, elevated levels of CYP450 and ABC transporter transcripts were identified in arabidopsis [41]. Similarly, we also observed high expression of genes in CYP450 and ABC (ATP-binding cassette) transporter family during heat stress, suggesting their involvement in ginseng heat defense pathways.

We performed gene ontology enrichment analysis with the DE genes to investigate their biological significance. Consistent with the previously obtained results, we found that genes involved in photosynthesis, including those involved in light harvesting, light response, cysteine biosynthesis, and sugar metabolism, were primarily affected in both cultivars of ginseng (Table 2). In the *P. ginseng* genome, *CAB*, *FAD*, and *WRKY* genes are significantly expanded, and such adaptive expansion might enable this plant to acclimate to cold and low light with high photosynthetic quantum efficiency [38]. However, based on our results, these expanded genes might also cause antagonistic effects that lead to photoinhibition [16] and increased sensitivity to heat or light, or

Table 2
Top enriched GO biological terms for common DE genes in CP and YP in response to heat stress

GO-ID	Term	Category	FDR
GO:0009768	Photosynthesis, light harvesting in photosystem I	P	2.82E-36
GO:0010196	Nonphotochemical quenching	P	4.93E-33
GO:0010114	Response to red light	P	3.99E-31
GO:0010155	Regulation of proton transport	P	5.29E-28
GO:0019344	Cysteine biosynthetic process	P	1.76E-26
GO:0010218	Response to far red light	P	9.68E-26
GO:0009637	Response to blue light	P	4.12E-24
GO:0006364	rRNA processing	P	7.58E-24
GO:0009769	Photosynthesis, light harvesting in photosystem II	P	5.60E-20
GO:0009744	Response to sucrose stimulus	P	1.18E-17
GO:0006636	Unsaturated fatty acid biosynthetic process	P	1.18E-15
GO:0018298	Protein–chromophore linkage	P	1.19E-12
GO:0030003	Cellular cation homeostasis	P	2.44E-11
GO:0009750	Response to fructose stimulus	P	4.88E-11

CP, Chunpoong; DE, differential expressed; FDR, false discovery rate; GO, gene ontology; YP, Yunpoong.

to a combination of both stresses. Clearly, more functional studies with these gene targets are needed to test these hypotheses in ginseng.

3.3. Comparative expression of genes involved in photosynthesis

Because photosynthesis was markedly influenced by heat stress in ginseng, we compared the expression of genes involved in photosynthesis between CP and YP. A total of 103 photosynthetic genes were identified using ginseng functional gene annotation information. Overall, these genes showed slightly stronger expression in CP than in YP (Fig. 4A). Notably, we observed that the expressions of electron carrier genes such as those encoding plastocyanin and ferredoxin $NADP^+$ reductase were significantly altered after 1-week and 3-week heat stress in CP. In agreement with the results obtained by Lee et al [16], this result indicated that high temperature and light might influence ginseng by exceeding its capacity for electron transfer, subsequently shutting down the electron transfer chain in a process of photoinhibition. Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is a key enzyme in CO_2 fixation and determines the photosynthetic rate in response to light intensity. We identified eleven *RuBisCO* genes and observed notably high expression of six genes encoding the large subunit of RuBisCO which is identical to chloroplast *rbcL*, suggesting the transfer of chloroplast-encoded *rbcL* in nuclear genome of ginseng in YP compared with CP under normal as well as heat stress conditions (Fig. 4B). This result corresponds with the high net photosynthetic rate of YP over CP shown in the study by Lee et al [14] and suggests that photosynthetic rate is coregulated with *RuBisCO* expression in ginseng.

3.4. Analysis of sugar metabolic genes

Sugars can serve as physiological signals that repress or activate plant genes involved in essential biological processes, including photosynthesis [42]. Furthermore, elevated sugar levels cause reduced photosynthesis activity and stunted growth in plants [42]. Intriguingly, elevated levels of soluble sugars have been identified in *P. ginseng* [43] with significant oscillation. Therefore, we performed a genome-wide scan for key genes encoding enzymes involved in sugar metabolism in ginseng including sucrose-phosphate synthase (EC 2.4.1.14), fructose 1,6-bisphosphatase (EC

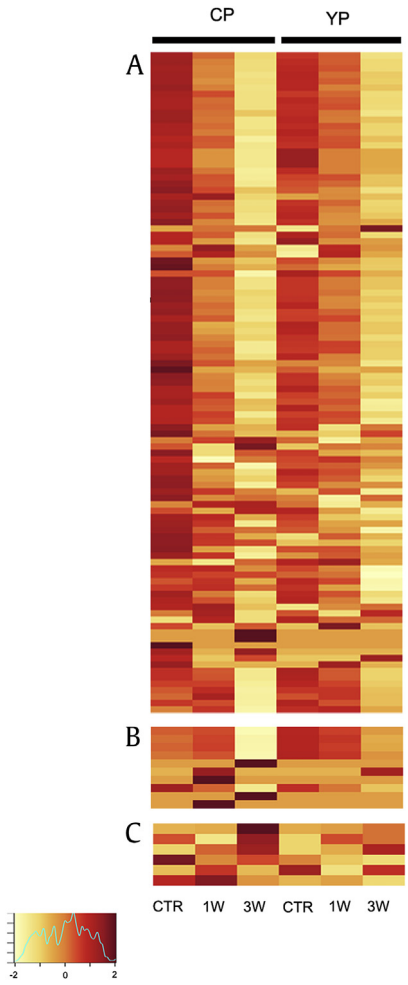

Fig. 4. Photosynthetic gene expression comparison during heat stress in CP and YP. (A) Heatmap representing the comparative expression profiling of 103 photosynthetic genes in CP and YP cultivars. The list of genes and their expression values can be found in Appendix A Supplementary Table S3. (B) Expression of 10 of 11 *RuBisCO* genes identified in cultivars of CP and YP. Detailed information can be found in Appendix A. Supplementary Table S4. (C) Heatmap showing expression patterns in CP and YP of the six sucrose transporter genes identified in *P. ginseng*. Corresponding expression values can be obtained in Appendix A. Supplementary Table S5. Each heatmap includes hierarchical clustering of average FPKM values obtained from individual normalized FPKM values of three replicates. CTR, 1W, and 3W represent control, 1-week, and 3-week heat treatment, respectively. Heatmap was generated using heatmap.2 function provided by the R-package gplots.
CP, Chunpoong; FPKM, Fragments Per Kilobase per Million; YP, Yunpoong.

3.1.3.11), sucrose-phosphate phosphatase (EC 3.1.3.24), sucrose synthase (EC 2.4.1.13), neutral invertase (EC 3.2.1.26), fructokinase (EC 2.7.1.4), hexokinase (EC 2.7.1.1), and UDP (uridine diphosphate)-glucose pyrophosphorylase (EC 2.7.7.9). When we compared these genes in *P. ginseng* with those of other plant species, we found increased gene numbers in ginseng compared with model plants and annuals endemic to sunny locales (Table 3). We also investigated the expression of those key genes and observed slightly higher expression in CP than in YP, indicating that sugar metabolic processes are more active in heat-susceptible CP.

We identified six sucrose transporter genes in ginseng. Intriguingly, two and four of the sucrose transporter genes were upregulated upon 1- and 3-week heat stress, respectively, in CP (Fig. 4C) when compared with YP. In other plant species, elevated sugar concentrations correlated negatively with *RuBisCO* transcript levels [44], which coincides with our results: CP showed strong

Table 3
Number of sugar metabolic genes in ginseng and other plant species

Species	No. of annotated genes	*SPS*[*]	*F16BPase*[*]	*SPP*[*]	*SUSY*[*]	*NI*[*]	*FK*[*]	*HK*[*]	*UGPase*[*]
Ginseng	59,352	12	15	4	27	25	13	25	15
Carrot	30,824	8	6	3	9	16	7	4	7
Tomato	34,725	6	7	2	8	19	8	6	6
Arabidopsis	27,416	8	4	4	6	12	11	6	7
Grape	26,346	7	5	2	7	12	4	7	5
Pepper	34,899	4	7	2	6	11	8	7	4

[*]SPS, sucrose-phosphate synthase; F16BPase, fructose 1,6-bisphosphatase; SPP, sucrose-phosphate phosphatase; SUSY, sucrose synthase; NI, natural invertase; FK, fructokinase; HK, hexokinase; UGPase, UTP-glucose-1-phosphate uridylyltransferase (Appendix A. Supplementary Table S6).

expression of genes involved in sugar metabolism but showed reduced transcript levels of *RuBisCO*. Therefore, elevated sugar accumulation could also be one of the components leading CP to be more susceptible than YP to heat and light stresses.

4. Conclusion

Ginseng is an obligate shade species, photosynthetic capability of which is significantly reduced by nonoptimum light intensity and temperature. In this study, we compared the transcriptome between heat injury–tolerant YP and heat-susceptible CP ginseng cultivars. Overall, CP and YP transcriptomes showed slight variation in terms of their gene expression patterns under nonstress conditions. However, our results suggest that ginseng responds to heat stress with an expanded number of *CAB*, *FAD*, and *WRKY* genes. These genes may play a major role in shade and cold adaptation. Conversely, the expansion of these gene families appears to have a significant negative impact on heat and light tolerance, which might lead to an electron overflow in the photosynthetic chain and thereby cause photoinhibition, especially in the heat-susceptible line of CP. This process might also be coregulated with elevated sugars that reduce the demand for ATP and NADPH by inhibiting the transcription level of *RuBisCO* and decreasing membrane desaturation by *FAD*. Altogether, this study provides novel and fundamental insight into ginseng heat responses which will be important for generating heat-tolerant ginseng varieties.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jgr.2018.05.007

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