Comparison transcriptome analysis of panicle development under heat stress in two rice (*Oryza sativa* L.) cultivars differing in heat tolerance

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Abstract

Heat stress inhibits rice panicle development and reduces spikelet number per panicle. This study investigated the mechanism involved in heat-induced damage to panicle development and spikelet formation in rice cultivars differing in heat tolerance. Transcriptome data from developing panicles grown at 40°C or 32°C were compared for two different rice cultivars: heat-tolerant Huanghuazhan and heat-susceptible IR36. Of the differentially expressed genes (DEGs), 4,070 heat stress-responsive genes were identified, including 1,688 heat-tolerance-cultivar-related genes (RHR), 707 heat-susceptible-cultivar-related genes (SHR), and 1,675 common heat stress-responsive genes. Gene ontology analysis showed that the DEGs in the RHR set of genes were significantly enriched in 54 gene ontology terms, some of which improved heat tolerance, including those in the WRKY, HD-ZIP, ERF, and MADS transcription factor families. DEGs in the RHR group were enriched in 15 significant metabolic pathways and some DEGs were

regulated to resist heat stress, including theplant hormone signal transduction pathway. The regulation of some DEGs in the SHR group was affected by heat stress, including those in the starch and sucrose metabolism pathway. Transcriptome analysis provides insight into different molecular mechanisms of heat stress tolerance in developing rice.

Introduction

Climate change is predicted to increase average global temperatures by 0.3-4.8°C by the end of the 21st century (Stocher *et al*. 2013). Unusually high temperatures occur frequently during the summer rice planting season (Dwivedi *et al*. 2015; Tao *et al*. 2013) and cause reductions in rice yields and quality in certain rice production regions, including China, India, and Japan. The primary cause of rice yield reduction is a reduction in spikelet fertility due to high temperatures during the flowering period (Espe *et al*. 2017). Rice quality is also influenced by high temperature, which causes carbohydrate metabolism disorders (Yamakawa & Hakata 2010). As climate change has intensified, extremely high temperatures above 40°C have become more frequent. Such temperatures inhibit rice panicle development and reduce spikelet number by 5%-15%, thereby aggravating rice yield losses (Wang *et al*. 2017).

High temperatures adversely affect floral organ development by reducing antioxidant capacity, inhibiting nutrition accumulation, and degenerating tapetal cells (Prasad *et al*. 2017). A previous study showed that high temperature (39°C) downregulated certain genes related to tapetum function, pollen adhesion, and germination, including *OsINV4* and *OsMST8*, which influenced spikelet fertilization (Endo *et al*. 2009). In addition, sugar and endogenous hormone metabolism under high temperatures reportedly plays an important role in pollen formation (Islam *et al*. 2018; Min *et al*. 2014). At the rice ripening stage, high temperature induces early termination of grain filling (Kim *et al*. 2011). Grain chalkiness increases under a mean temperature greater than 32°C, resulting in the deterioration of eating and cooking quality, which are both closely linked to starch and sucrose metabolism (Zhong *et al*. 2010). Transcriptome analysis has shown that high temperatures influence the expression of genes involved in the inhibition of sucrose degradation and starch biosynthesis while promoting starch degradation and the synthesis of storage proteins (Yamakawa & Hakata 2010; Yamakawa *et al*. 2007), Takehara *et al*. (2018) reported that upregulation of *OsSUS3*, which encodes sucrose synthase, improved high-temperature tolerance.

The panicle initiation stage is an important period for spikelet proloferation.
Dry matter accumulation is essential for panicle development; however, the pathway for carbohydrate accumulation during spikelet formation under heat stress remains vague. The

reduction in spikelet number that occurs under high temperature conditions has been associated with heat-induced phytohormone changes, especially enhanced cytokinin degradation (Wu *et al*. 2017; Wu *et al*. 2016). The number of spikelets per panicle is determined by spikelet differentiation and degeneration. Spikelet differentiation is correlated with dry matter accumulation and influenced by environmental factors (Liu *et al.* 2005). Ding *et al*. (2016) reported that hormone metabolism, stress response, carbohydrate metabolism and transport, and protein degradation were regulated to influence panicle initiation. Additionally, certain genes, such as MADS-box genes, are related to panicle initiation (Kang *et al*. 2013; Kobayashi *et al*. 2012). Quantitative trait loci for spikelet degeneration have been identified (Yamagishi *et al*. 2004), and the genes *SP1*, *ASP1*, *TUT1*, *PAA2*, and *OsALMT7* have been found to control spikelet degeneration (Bai *et al*. 2015; Heng *et al*. 2018; Li *et al*. 2010).

However, the mechanism of panicle development under high temperature conditions is still unclear. In this study, RNA-Seq analysis was used to explore the mechanism of heat damage to panicle development. Huanghuazhan(HHZ) is a heat-tolerant rice(*Oryza sativa* L.) cultivar that, is widely grown in the middle and lower reaches of the Yangtze River in China (Cao *et al*. 2009; Zhou *et al*. 2012). The inbred *indica* cultivar IR36 is a heat-susceptible cultivar (Fang *et al*. 2006), and was an original parent in the breeding pedigree of HHZ. These two rice cultivars were used in the current study to ascertain the transcriptome differences between a heat-tolerant rice cultivar and a heat-susceptible cultivar grown at 40°C and 32°C begining at the spikelet differentiation stage. The differentially expressed genes (DEGs) of young panicles in the two cultivars under the two temperature treatments were identified by Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to improve our understanding of the molecular mechanism of heat-induced inhibition of spikelet development and to provide an important reference for rice breeding.

Materials and methods

Plant materials and heat stress treatments

We used the inbred *indica* rice (*Oryza sativa* L.) cultivars HHZ (heat-tolerant) and IR36 (heat-susceptible) in this study. Pregerminated seeds were sown in seed trays filled with a matrix consisting of vermiculite, charcoal, soil, and slow-release fertilizer. After 20 days, the seedlings were transplanted into pots (four seedlings per pot). Each pot (24 cm length × 22.5 cm width × 21.5 cm height) contained 10 kg air-dried paddy soil. The potted rice plants were kept under natural environmental conditions.

The amount of fertilizer applied to each pot was based on fertilizer used in field rice production, which 14 kg nitrogen per 666.7m^2. Before transplanting into the pots, 3.5 g compound

fertilizer (nitrogen: phosphorus: potassium = 15%:15%:15%) was applied to each pot. At the tillering stage, 0.6 g urea was supplemented in each pot. At panicle initiation, 0.6 g urea and 0.5 g potassium chloride were also applied in each pot. Pests, diseases, and weeds were intensively controlled.

Automatic growth chambers were used to control the two temperature treatments used on the study. The high-temperature (40°C) and control temperature (32°C) treatments were imposed for eight hours each day from 9:30 to 17:30 h; the temperature setting details are shown in Table 1. The humidity in the chambers was maintained at 75-80%. Rice plants were grown under natural ambient conditions during all the growth stages before and after the high temperature and control temperature treatments were applied.

Rice plants were exposed to the different temperature treatment for nine days at spikelet differentiation stage (panicle length≈2 mm) and then returned to ambient conditions. Each treatment included three replicates (20 pots/replicate). Plants were moved to the growth chambers on the approximate date of spikelet differentiation when the panicle length was approximately 0.2 cm (60-70 d after seed sowing).

Panicle and spikelet morphology

Ten main tillers were sampled per replicate on day 9 of treatment to monitor young panicle development under 40°C and 32°C.

Spikelet differentiation or degeneration of the main tiller panicles was determined at the heading stage. The number of degenerated spikelets was calculated by counting the vestiges present on the panicles. The number of differentiated spikelets was the sum of the surviving and degenerated spikelets. The proportion of degenerated spikelets was calculated as number of degenerated spikelets per number of total differentiated spikelets × 100%.

Spikelet morphology was observed under a stereomicroscope (Olympus SZX7, Olympus Corporation, Tokyo, Japan) and glume length (mm) and glume width (mm) were measured at 0.63x and 2.5x using Image Pro-Plus 5.1(Olympus SZX7, Olympus Corporation, Tokyo, Japan).

RNA extraction, transcriptome sequencing, and mapping

After nine days at 40 °C or 32 °C treatment, young panicles of 20 main tillers were collected from each replicate at 12:00-13:00, and immediately frozen in liquid nitrogen. In quick succession, TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from the young panicles according to the manufacturer's instructions. A TruSeq RNA Sample Preparation Kit (Illumina Inc., CA, USA) was used to generate 12 sequencing libraries according to the

manufacturer's instructions. The sequencing libraries were then sequenced on a HiSeq platform (Illumina, Inc., CA, USA). High quality sequence reads were obtained after filtering from raw data and were then compared to the 9311 reference genome (Oryza_indica.ASM465v1.dna.toplevel.fa) from http://www.ensembl.org/. The raw RNA sequence data were submitted to the NCBI Sequence Read Archive with accession number PRJNA508820.

Gene expression level and differential expression analysis

We used HTSeq (0.9.1) to statistically compare the read count values of each gene as the original expression of the gene, and fragments per kilobase of transcript per million mapped reads (FPKM) was used to standardize the expression. Next, we used DESeq (1.30.0) to analyze differential expression of genes with the following screening conditions: an expression difference of |log2foldChange| > 1 and a significant *P-value* < 0.05.

GO and KEGG enrichment analysis of DEGs

For GO enrichment analysis of DEGs, we used the Singular Enrichment Analysis tool in AgriGO (http://bioinfo.cau.edu.cn/agriGO/analysis.php) with default parameters, and a *P*-value $\leqslant$ 0.05 to indicate significant enrichment. KEGG enrichment analysis of DEGs was performed using KOBAS software with default parameters and *P*-value $\leqslant$ 0.05 indicating significant pathway enrichment.

Verification of RNA-Seq by quantitative real-time PCR (qRT-PCR)

First-strand cDNA was synthesized using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan) according to the manufacturer's instructions. The qRT-PCR analyses were performed using an Applied Biosystems 7500 Real-Time PCR system with Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). The primers used for qRT-PCR are listed in Supplementary Table S1. The *OsUBQ* gene was used as an internal control. Relative gene expression levels were determined from the equation $2^{-\Delta\Delta CT}$ (Czechowski *et al.* 2004), where ΔΔCT represents ΔCT (target gene of interest)–ΔCT (control gene).

Statistical analyses

Microsoft Excel 2016 (Microsoft Inc., Redmond, WA, USA) was employed for data collection. The panicle and spikelet morphological data collected for the 40°C and 32°C treatments (mean of three replicates) were statistically analyzed by Student's t-test (P<0.05). Graphs were created using Origin 9.1 (Ver. 9.1, OriginLab, Northampton, MA, USA).

173

174 Results

175 Spikelet development at high temperature

176 A preliminary experiment showed a significant difference in panicle development measured
177 as spike differentiation after nine days of high-temperature treatment. The current results reported
178 here are consistent with these preliminary findings. High temperature for nine days during spikelet
179 differentiation inhibited young panicle growth; the 40°C treatment reduced young panicle length
180 by 37.4% in the HHZ cultivar and 84.9% in the IR36 cultivar over the 32°C control treatment (Fig.
181 1a,b). In addition, the heat treatment reduced the dry weight of young panicles by 35.8% and
182 95.1%, respectively (Fig. 1c), significantly reduced spikelet survival by 22.3% and 53.6% (Fig.
183 2a), reduced the number of differentiated spikelets by 9.6% for HHZ and 33.2% for IR36 (Fig.
184 2b), and increased the proportion of degenerated spikelets by 32.3% and 67.4%, respectively (Fig.
185 2c). After nine days of high-temperature and control temperature treatment, approximately 15-20
186 days were needed for panicles to complete growth. The heat treatment reduced glume length by
187 10.3% for HHZ and by 16.0% for IR36 (Fig. 3b), and reduced glume width by 12.0% and 8.0%,
188 respectively (Fig. 3c). The reductions in spikelet number and size led to reductions in panicle
189 weight and rice yield (TableS2); the heat-susceptible IR36 experienxed a greater reduction than
190 heat-tolerant HHZ, suggesting that high temperature had a weaker effect on young panicle
191 development in the heat-tolerant cultivar.

192

193 Transcriptome analysis

194 Under the 32°C control temperature, a total of 44.2 million and 48.9 million raw reads were
195 obtained from HHZ (referred to as HHZ_32) and IR36 (referred to as IR36_32), respectively.
196 Under the 40°C treatment, a total of 45.5 million raw reads were obtained from both HHZ
197 (HHZ_40) and IR36 (IR36_40) (Table 2 and Table S3). More than 99.0% clean reads were
198 obtained for downstream analyses. The results of RNA sequence mapping indicated that 85.8-
199 88.0% of the clean reads could be mapped onto the reference genome. On average, 84.8% of the
200 clean reads from HHZ_32 and 83.0% of the clean reads from HHZ_40 were uniquely mapped onto
201 the reference genome, and for IR36, 83.1% of the clean reads from IR36_32 and 83.4% of the
202 clean reads from IR36_40 were uniquely mapped onto the reference genome (Table S4).

203

204 Identification of DEGs

205 To compare the differences between the two cultivars at 40°C and 32°C, we used four
206 comparison groups: HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and
207 IR36_32 vs HHZ_32. DEGs for the four groups were restricted to those with a |log2fold change|

> 1 and a *P*-value<0.05. With these criteria, 3342, 2469, 2949, and 2461 DEGs were detected for HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and IR36_32 vs HHZ_32, respectively (Fig.4). Significantly different gene expression was observed both between cultivars and between treatments. For HHZ, 1,794 genes were upregulated and 1548 genes were downregulated in the 40°C treatment compared with the 32°C treatment (Fig.4a). Furthermore, 1140 genes were upregulated and 1,329 genes were downregulated in IR36 under the 40°C treatment compared with the 32 °C treatment (Fig.4b). For comparisons within treatments, 1,408 genes were upregulated and 1,541 were downregulated in the IR36_40 vs HHZ_40 and 893 genes were upregulated and 1,751 genes were downregulated in the IR36_32 vs HHZ_32 group (Fig.4c and d).

Classification of DEGs

In all four groups, a total of 5533 unique DEGs were identified, and these DEGs could be divided into 15 disjointed subgroups (Fig. 5). Among the 15 subgroups, eight from the IR36_32 vs HHZ_32 group were excluded from the analysis because they were not influenced by high temperature. In addition, 1,157, 603, 524, and 402 DEGs were specifically identified in HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and IR36_32 vs HHZ_32, respectively. The DEGs in groups responsive to high temperature could be further classified into three categories: heat-tolerance-cultivar-related genes (RHR, 1,689 genes), heat-susceptible-cultivar-related genes (SHR, 707 genes), and common heat stress-response genes (CHR, 1675 genes) (Table 3 and Table S5). The DEGs in the RHR category benefited from heat resistance, while the DEGs in the SHR category presented the specific heat injury in the heat-susceptible cultivar.

Analysis of GO annotation

The purpose of GO enrichment analysis is to obtain GO functional terms with significant enrichment of DEGs, thus revealing the possible functions of the DEGs. Of all DEGs, 2,307 (69.0%), 1,680 (68.0%), 1,832 (62.1%), and 1,472 (59.8%) DEGs were enriched in GO terms in HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and IR36_32 vs HHZ_32, respectively. There were 75, 11, 13, and 31 significant GO terms observed in HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and IR36_32 vs HHZ_32, respectively (Fig. 6). The maximum number of DEGs was observed for the heterocycle biosynthetic process in the IR36_40 vs HHZ_40 group. In IR36_32 vs IR36_40 and HHZ_32 vs HHZ_40, the DEGs were enriched in response to stimulus, in response to temperature stimulus, and in response to heat in the biological process category. Within the cellular component category, the DEGs were commonly enriched in chromatin, DNA packaging complex, and nucleosome in the IR36_32 vs IR36_40 and HHZ_32 vs HHZ_40 groups. However, there were no common GO terms in the category of molecular

function in the IR36_32 vs IR36_40 and HHZ_32 vs HHZ_40 groups.

We further identified GO term categories for DEGs in the RHR, SHR, and CHR categories (Fig. 7 and Table S6). Among the 1,689 DEGs in RHR, 54 significant GO terms were detected. However, no significant GO terms were observed among the 485 DEGs in SHR. In CHR, 30 significant GO terms were detected. In the CHR group, eight significant GO terms were in the biological process category, including response to stimulus, response to temperature stimulus, and response to heat; 17 GO terms were in the cellular component category, and two significant GO terms were in the molecular function category. In the RHR group, 30, 14 and 10 significant GO terms were in the biological process, cellular component, and molecular function categories, respectively. The most significant GO terms, in decreasing order, were RNA biosynthetic process, nucleus, and DNA binding. In the molecular function category, 50 DEGs were specifically assigned to DNA-binding transcription factor activity, which may play an important role in heat stress tolerance.

The 50 DEGs of DNA-binding transcription factor activity could be divided into 12 transcription factor (TF) families (Fig. 8), including HSF (1), WRKY (6), MADS (12), HD-ZIP (7), GATA (3), ERF (12), ABAI (1), b-ZIP (4), ARR-B (2), E2F (1), and NF-YA (1). Expression of the genes *BGIOSGA006348* of HSF, *BGIOSGA010835* of ABAI, *BGIOSGA010142* of HAP, and *BGIOSGA000303* and *BGIOSGA000304* of ARR-B were significantly upregulated. In addition, five genes in WRKY, eight genes in MADS, two genes in HD-ZIP, two genes in GATA, six genes in ERF, and two genes in b-ZIP were also upregulated (Table S7). These results suggest that, these 30 TF genes may play important roles in heat stress resistance.

Analysis of KEGG pathway enrichment

In the KEGG analysis, 1158 DEGs were classified into 225, 191, 239, and 211 functional pathways in HHZ_32 vs HHZ_40; 838 DEGs in IR36_32 vs IR36_40; 732 DEGs in IR36_40 vs HHZ_40; and 539 DEGs in IR36_32 vs HHZ_32, respectively. A total of 79 pathways were significant (P <0.05) (Fig. 9). Among these pathways, the phenylpropanoid biosynthesis pathway was common in HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, and IR36_40 vs HHZ_40, which suggests that heat stress impaired phenylpropanoid biosynthesis.

Based on further analysis of the three categories with different heat-stress responses, 146 DEGs in RHR were involved in 15 overrepresented pathways,including purine metabolism, pyrimidine metabolism, and amino sugar and nucleotide sugar metabolism; 45 DEGs in SHR were involved in 11 overrepresented pathways, including arginine biosynthesis, starch and sucrose metabolism, and polyketide sugar unit biosynthesis; and 184 DEGs in CHR were involved in 29 overrepresented pathways (Fig. 10 and Table S8).

A previous study showed that plant hormones are important for panicle development. Among

the 15 KEGG pathways in RHR, 21 DEGs were involved in plant hormone signal transduction, of which 14 DEGs were upregulated in HHZ; three DEGs were involved in cytochrome P450 metabolism, which plays a role in brassinosteroid (BR) biosynthesis, and two were upregulated (Table 4).

In SHR and CHR, there were three common pathways: the starch and sucrose metabolism pathway, the NOD-like receptor signaling pathway, and the estrogen signaling pathway. Carbohydrate accumulation was essential for panicle development. In the KEGG analysis, seven DEGs involved in starch and sucrose metabolism were observed in SHR and 18 DEGs involved in starch and sucrose metabolism were observed in CHR. In SHR, the genes in HHZ were not different between HHZ_40 and HHZ_32. However, genes (*BGIOSGA010570*, *BGIOSGA026140*) encoding sucrose synthase (EC 2.4.1.13), genes (*BGIOSGA026976*, *BGIOSGA009181*, *BGIOSGA030796*) encoding trehalose-6-phosphate synthase (EC 2.4.1.15), and a gene (*BGIOSGA000509*) encoding trehalose-6-phosphate phosphatase (EC 3.1.3.12) were significantly down-regulated in IR36_40 compared with IR36_32. However, the gene (*BGIOSGA031385*) encoding beta-amylase (EC 3.2.1.2) was significantly upregulated in IR36_40 compared with IR36_32 (Table 5).

qRT-PCR verification

To confirm the accuracy of the RNA-Seq results, ten representative DEGs in HHZ_32 vs HHZ_40 (a) and IR36_32 vs IR36_40 (b), as well as five DEGs in IR36_40 vs HHZ_40 (c) and IR36_32 vs HHZ_32 (d) were chosen to determine relative expression. Among the ten DEGs in HHZ_32 vs HHZ_40, five DEGs were in RHR: *BGIOSGA022020* is related to BR synthesis, *BGIOSGA006348* encodes a heat shock factor (Hsf), *BGIOSGA017088* is involved in the ETH TF family, *BGIOSGA006285* participates in ethylene responsive regulation, and *BGIOSGA024710* is an auxin-responsive gene involved in plant hormone transduction. Among the ten DEGs in IR36_32 vs IR36_40, five were in SHR and encoded cytokinin oxidase/dehydrogenase (*BGIOSGA005140*), sucrose synthase (*BGIOSGA026140*), trehalose-6-phosphate synthase (*BGIOSGA026976*), trehalose-6-phosphate phosphatase (*BGIOSGA000509*), and catalase (*BGIOSGA007252*). Four DEGs were in CHR for HHZ_32 vs HHZ_40 and IR36_32 vs IR36_40, and two common genes, namely, *BGIOSGA032653* and *BGIOSGA015767*, were validated. *BGIOSGA032653* is involved in phenylpropanoid biosynthesis and *BGIOSGA015676* encodes a heat shock protein (HSP). The qRT-PCR results for the DEGs were all consistent with the RNA-Seq data (Fig.11).

Discussion

Rice plants exposed to high temperature growing conditions during spikelet differentiation inhibited panicle initiation and reduced the spikelet number per panicle (Fig.1 and Fig.2). Previous studies have shown that the genes *SP1*, *ASP1*, *TUT1*, *PAA2*, and *OsALMT7* are closely related to branch and spikelet development in rice, but in the current study, we observed no significant difference in expression of these genes between the 40°C treatment and the 32°C control treatment in either rice cultivars. This indicates that the genes did not respond to high temperature in young panicles.

In general, the upregulation of HSPs contributes to the heat stress response in plants (Guan *et al*. 2010; Jagadish *et al*. 2010; Jung *et al*. 2013). Moon *et al.* (2014) reported that heterologous overexpression of *OsHSP1* (*BGIOSGA015767*, encoding a heat shock protein) increased heat tolerance in Arabidopsis. However, in the current study, *BGIOSGA15767* expression was upregulated in both HHZ (log2 fold chang (HHZ_40/HHZ_32) = 5.7, *P*-value=0) and IR36 (log2 fold change (HHZ_40/HHZ_32) = 5.0, *P*-value=0), and there was no difference in the GO terms between cultivars for HSP. GO enrichment analysis revealed that the DEGs were commonly enriched in response to heat, stress and temperature stimuli in the biological process category (Fig.7). These results demonstrate that high temperature growing conditions directly damage young panicle developmen.

An important factor determining heat tolerance is antioxidant capacity (Lan *et al.* 2016). Buer *et al.* (2010) reported that flavonoids can positively regulate reactive oxygen species, which can affect the transport of plant hormones and influence pollen development. The flavonoid synthesis pathway was overrepresented in the IR36_32 vs IR36_40 group. Specifically, five genes involved in flavonoid synthesis were downregulated at 40°C, which might indicate a reduction in the antioxidant capacity of IR36 under heat stress. In addition, 14 DEGs in the IR36_32 vs IR36_40 group were enriched in the peroxisome pathway. Among these, 10 DEGs were significantly downregulated and four DEGs were significantly upregulated. However, the peroxisome pathway was not significant in the KEGG analysis of HHZ_32 vs HHZ_40 (Fig. 8). BGIOSGA007252 and BGIOSGA011520, which encoded catalase (EC:1.11.1.6), were significantly downregulated at 40°C compared with 32°C in IR36, while no expression differences were observed in HHZ_32 vs HHZ_40. This suggested that high temperature had s greater negative effect on the antioxidant capacity of IR36 than HHZ, which provides primary explanation for the greater heat injury observed in the young IR36 panicles than in HHZ, which provides a primary explanation for the greater heat injury observed in the young IR36 than in the HHZ panicles.

Regulation of endogenous hormones has important effects on the development of young panicles. Wu *et al.* (2017) reported that a lower spikelet number under high temperature growing conditions was associated with cytokinin degradation. In the current study, BGIOSGA001314, which encodes a cytokinin-activity enzyme, did not differ between the 40°C and 32°C treatments

in HHZ (log2 (HHZ_40/HHZ_32) = -0.41) or IR36 (log2 (IR36_40/IR36_32) =-0.38). The gene BGIOSGA005140, which encodes cytokinin oxidase/dehydrogenase, was significantly upregulated in the IR36_32 vs IR36_40 group (log2 flod change=1.67, *P-value*=0.004), but was not different in the HHZ_32 vs HHZ_40 group (log2 flod change=0.86, *P-value*=0.088). These results are consistent with those of Wu et al. (2016) and suggested that spikelet formation is associated with cytokinin degradation, and that more degradation occurred at the high temperature in the heat-susceptible cultivar than in the heat-tolerant cultivar.

The DEGs in RHR were enriched in 54 GO terms (Fig.6a). GO term analysis revealed biological processes promoting resistance to heat stress in the heat-tolerant cultivar HHZ. Downregulation of *BGIOSGA022020* in the heterocycle biosynthetic process induces GRAS protein reduction, which promotes BR synthesis to enhance heat tolerance (Vriet *et al*. 2012). In the molecular function category for RHR, 50 DEGs were involved in DNA-binding transcription factor activity. *BGIOSGA006348* encoded an HSF TF and was upregulated in the HHZ_32 vs HHZ_40 group, but there was no difference in the IR36_32 vs IR36_40 group. Wang *et al.* (2009) reported that higher expression of heat shock TFs contributed to high temperature tolerance. WRKY genes encode TFs that play important roles in abiotic stress responses (Chen *et al.* 2010), especially to abscisic acid (ABA) (Zhen *et al.* 2005). In this study, five DEGs were WRKY TFs, namely, *BGIOSGA003134*, *BGIOSGA029574*, *BGIOSGA005924*, *BGIOSGA024948*, and *BGIOSGA033505*, which might promote young panicle development associated with sucrose consumption mediated by ABA under high temperature (Feng *et al.* 2018). However, few studies have reported the relationship between the WRKY family and heat resistance, which should be further studied. *BGIOSGA029574* is a general stress-response gene, which has putative functions in distinct cellular processes, such as transcription regulation, stress response, and sugar metabolism under Fe-excess-induced, dark-induced and drought-induced stress (Ricachenevsky *et al.* 2010). Of the 10 DEGs in the ETH family, five genes were downregulated and the down-regulation of *BGIOSGA017088* reduced the ABA content and promoted gibberellin (GA) signal transduction, which is beneficial for rice plant growth (Yaish *et al.* 2010). Upregulation of *BGIOSGA006285*, *BGIOSGA010867*, *BGIOSGA030019*, *BGIOSGA005915*, and *BGIOSGA012535* plays an important role in ethylene response regulation. Cao *et al.* (2006) reported that the upregulation of *BGIOSGA005915* enhanced tolerance to salt, cold, drought, and wounding and the current study revealed that the gene also contributed to improvement of high-temperature stress resistance. *BGIOSGA000303* and *BGIOSGA000304* are genes in the cytokinin receptor family and upregulation of these two genes promotes cytokinin activation (Ito & Kurata 2006). The MADs box gene is related to flower development (Kobayashi *et al*. 2012) and the upregulation of the MAD genes in RHR indicated that the MAD family might enhance heat stress tolerance. The HZ-ZIP TF family might have a similar function.

In the RHR category, the DEGs enriched in the KEGG pathways appear beneficial for heat-stress tolerance, including plant hormone signal transduction and BR biosynthesis. Twenty-one DEGs were involved in plant hormone signal transduction, of which 14 DEGs were upregulated, including the auxin-responsive genes, *BGIOSGA024710*, *BGIOSGA001585*, *BGIOSGA019301* and *BGIOSGA037837*, which facilitate rice plant growth (Hagen & Guilfoyle 2002). In BR biosynthesis, BGIOSGA002945, which encodes D2/CYP90D2 that catalyzes the steps from 6-deoxoteasterone to 3-dehydro-6-deoxoteasterone and from teasterone to 3-dehydroteasterone, was upregulated to promote BR synthesis in the latter pathway (Hong et al. 2003), and BGIOSGA001585 was downregulated to promote BR activity (Sakamoto et al. 2011). These 16 genes might contribute to young panicle development under high temperature. However, BGIOSGA014915, which participates in BR synthesis, was downregulated in RHR. Previous reports have found that BRs can modulate plant metabolic responses to environmental abiotic stresses (Vriet et al. 2012; Wang et al. 2018), but how BR metabolism modulates spikelet development under high temperature needs further study.

Carbohydrate accumulation is essential for panicle initiation (Tian et al. 2016). KEGG analysis showed that the phenylpropanoid biosynthesis pathway was commonly overrepresented in HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, and IR36_40 vs HHZ_40. The phenylpropanoid biosynthesis pathway is involved in lignin synthesis, which suggests that high temperature inhibits lignin synthesis; however, phenylpropanoid biosynthesis was not associated with heat tolerance in the different cultivars (Fig. 9).

In the SHR category, seven DEGs were enriched in the starch and sucrose metabolism pathway (Fig.9b). This pathway was also highly represented in CHR (Fig.9c). Such genes are involved in the downregulation of genes encoding beta-fructofuranosidase, fructokinase, beta-glucosidase, trehalose-6-phosphate phosphatase, alpha-trehalase, and others. Sucrose degrades into uridine 5'-diphosphoglucose and fructose, which are major forms of carbon that are utilized as energy supplements. A reduction in the activities of enzymes involved in sucrose hydrolysis inhibits sucrose utilization, which impairs panicle development. Trehalose-6-phosphate synthase, trehalose-6-phosphate phosphatase, and alpha-trehalase are involved in trehalose synthesis. Trehalose plays an important role in abiotic stress resistance, and trehalose-6-phosphate, an intermediate product of trehalose synthesis participates in sucrose signal transduction (Lunn *et al*. 2006; Ruan 2014). Nunes *et al*. (2013) reported that trehalose-6-phosphate served as a sugar signal that could induce the expression of genes associated with the alleviation of abiotic stress injury. In this study, some DEGs in CHR were also upregulated to promote trehalose-6-phosphate synthesis, and the upregulation of *BGIOSGA026976*, *BGIOSGA009181*, and *BGIOSGA030796* promoted trehalose-6-phosphate synthesis in SHR. The gene encoding trehalose-6-phosphate phosphatase, *BGIOSGA000509*, was significantly downregulated in IR36 at 40°C compared with 32°C, which

might have reduced trehalose content and in turn disrupted the carbohydrate distribution. The results suggest that trehalose-6-phosphate metabolism was disordered under the high temperature growing condition and that the heat-susceptible cultivar experienced greater inhibition than the heat-tolerant cultivar.

In SHR, seven DEGs were associated with starch and sucrose metabolism. Among these seven DEGs, the genes encoding sucrose synthesis, namely, *BGIOSGA010570* and *BGIOSGA026140*, were significantly downregulated in the IR36_32 vs IR36_40 group, while no difference in expression was observed in the HHZ_32 vs HHZ_40 group. Impairment of sucrose synthase activity reportedly reduced resistance to environmental stress, and *OsSUS3* inhibition reduced the heat tolerance of rice at the grain filling stage (Hirose *et al*. 2008; Takehara *et al*. 2018). The results of the present study suggested that sucrose impairment in the heat-susceptible cultivar aggravated spikelet reduction.

There is a close relationship between endogenous hormones and carbohydrate accumulation, which may suggest that the regulation of endogenous hormones in heat-tolerant varieties promoted the utilization of carbohydrates. Molecular marker-assisted selection can be carried out according to DEGs associated with hormone metabolism in the study of RHR.

Conclusions

In summary, heat stress-responsive DEGs in young panicles were identified by a transcriptome analysis of a heat-tolerant rice cultivar and a heat-susceptible rice cultivar grown at high temperature (40°C) and control temperature (32°C). Statistical analysis of a total of 5533 DEGs revealed three categories of genes (RHR, SHR, and CHR) containing a total of 4070 DEGs. We highlight differential expression of a DNA-binding TF that was significantly enriched in the RHR category and differential expression of genes involved in the starch and sucrose metabolism pathway that were overrepresented in the SHR category. Overall, the up-regulation of DEGs related to plant hormones and signal transduction were specifically beneficial for young panicle development grown at high temperature. In addition, certain metabolic pathways, including starch and sucrose metabolism, were specifically damaged, thus aggravating the inhibition of panicle development. The identification of DEGs improves our understanding of the molecular mechanisms of heat resistance in young panicles; heat-tolerant cultivars increase endogenous hormones and maintain a stable carbohydrate metabolism pathway under high temperature growing conditions.

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Table 1(on next page)

Table 1. Temperatures in growth chambers

1

2 **Table 1.** Temperatures in growth chambers

Period	High-temperature chamber (°C)	Normal-temperature chamber (°C)
0:00:00 – 6:29:59	33	25
6:30:00 – 9:29:59	35	27
9:30:00 – 17:29:59	40	32
17:30:00 – 22:29:59	35	27
22:30:00 – 23:59:59	33	25

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Table 2(on next page)

Table 2. Statistics of RNA sequencing results

Table 2. Statistics of RNA sequencing results

Sample	HHZ_32	HHZ_40	IR36_32	IR36_40
Raw reads	44231722	45513241	45877838	46465046
Clean reads	44032896	45256701	45580821	46252929
	(99.6%)	(99.4%)	(99.4%)	(99.5%)
Total mapped	38834391	39148950	39541858	40418126
	(87.8%)	(86.0%)	(86.2%)	(87.0%)
Uniquely mapped	37502957	37759013	38120438	38853775
	(84.8%)	(83.0%)	(83.1%)	(83.1%)
Multiply mapped	1331434	1389937	1421421	1561018
	(3.0%)	(3.1%)	(3.1%)	(3.6%)

Note: HHZ_32: The sample of HHZ treated with 32°C; HHZ_40: The sample of HHZ treated with 40°C; IR36_32: The sample of IR36 treated with 32°C; IR36_40: The sample of IR36 treated with 40°C.

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Table 3(on next page)

Table 3. Classification of three categories of DEGs

Table 3. Classification of three categories of DEGs.

Categories	Subgroups	Number of DEGs
RHR	Only HHZ_32 vs HHZ_40	1157
	HHZ_32 vs HHZ_40 ∩ IR36_40 vs HHZ_40	531
SHR	Only IR36_32 vs IR36_40	603
	IR36_32 vs IR36_40 ∩ IR36_40 vs HHZ_40	104
CHR	Only IR36_40 vs HHZ_40	524
	HHZ_32 vs HHZ_40 ∩ IR36_32 vs IR36_40, HHZ_32 vs HHZ_40 ∩ IR36_32 vs IR36_40 ∩ IR36_40 vs HHZ_40	1151

Note: RHR, heat-resistant-cultivar-related genes; SHR, heat-susceptible-cultivar-related genes; CHR, common heat stress-response genes.

Table 4(on next page)

Table 4. Gene expression of DEGs in Plant hormone signal transduction and BR biosynthesis of RHR

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2
3 **Table 4.** Gene expression of DEGs in Plant hormone signal transduction and BR biosynthesis of RHR

ID	Gene annotation	Cultivar	baseMean	32°C	40°C	log2FoldChange	pval
BGIOSGA018672	Pseudo histidine-containing phosphotransfer protein 2	HHZ	64.7	36.9	92.5	1.33	0.00
		IR36	59.4	49.2	69.7	0.50	0.06
BGIOSGA004140	Probable protein phosphatase 2C 8	HHZ	665.2	219.8	1110.7	2.34	0.00
		IR36	465.6	355.3	575.9	0.70	0.00
BGIOSGA005312	Two-component response regulator ORR3	HHZ	50.6	28.6	72.6	1.35	0.00
		IR36	26.3	24.2	28.5	0.24	0.58
BGIOSGA024710	Auxin-responsive protein IAA24	HHZ	807.3	458.2	1156.3	1.34	0.00
		IR36	828.1	653.4	1002.7	0.62	0.00
BGIOSGA010835	ABSCISIC ACID-INSENSITIVE 5-like protein 2	HHZ	146.5	85.8	207.2	1.27	0.00
		IR36	83.4	74.0	92.8	0.33	0.26
BGIOSGA011032	Probable protein phosphatase 2C 30	HHZ	102.9	53.4	152.4	1.51	0.00
		IR36	113.6	108.0	119.3	0.14	0.69
BGIOSGA015611	Probable protein phosphatase 2C 37	HHZ	86.1	44.3	127.8	1.53	0.00
		IR36	75.8	52.0	99.6	0.94	0.00
BGIOSGA019301	Auxin-responsive protein IAA16	HHZ	97.4	56.6	138.3	1.29	0.00
		IR36	79.7	76.6	82.8	0.11	0.61
BGIOSGA008704	Auxin-responsive protein SAUR36	HHZ	36.6	22.9	50.3	1.14	0.00
		IR36	23.1	22.5	23.6	0.07	0.91
BGIOSGA012535	ARATH Protein ETHYLENE INSENSITIVE 3	HHZ	2890.1	1268.4	4511.8	1.83	0.00
		IR36	2293.2	1543.8	3042.6	0.98	0.00
BGIOSGA037772	ARATH Transcription factor PIF1	HHZ	27.5	14.2	40.8	1.52	0.00
		IR36	17.3	13.3	21.4	0.69	0.24
BGIOSGA000304	Two-component response regulator ORR26	HHZ	143.8	92.5	195.0	1.08	0.00
		IR36	110.4	85.9	134.9	0.65	0.00

BGIOSGA004789	Probable protein phosphatase 2C	HHZ	522.2	301.9	742.5	1.30	0.00
		IR36	623.2	501.8	744.5	0.57	0.02
BGIOSGA037837	Auxin-responsive protein SAUR72	HHZ	3.5	1.0	6.0	2.55	0.04
		IR36	0.8	1.3	0.3	-2.07	0.67
BGIOSGA024374	Two-component response regulator ORR7	HHZ	17.8	29.5	6.2	-2.26	0.00
		IR36	49.3	58.2	40.4	-0.53	0.10
BGIOSGA036617	Transcription factor TGAL11	HHZ	308.0	423.3	192.8	-1.13	0.00
		IR36	572.4	700.8	444.0	-0.66	0.00
BGIOSGA034772	BTB/POZ domain and ankyrin repeat-containing protein NH5.1	HHZ	1148.1	1629.2	667.0	-1.29	0.00
		IR36	1335.2	1698.8	971.6	-0.81	0.00
BGIOSGA010559	Protein TIFY 10a	HHZ	339.2	465.1	213.4	-1.12	0.00
		IR36	374.0	492.0	256.0	-0.94	0.00
BGIOSGA010919	Abscisic acid receptor PYL5	HHZ	34.8	55.0	14.5	-1.92	0.00
		IR36	52.9	58.0	47.8	-0.28	0.33
BGIOSGA023368	Two-component response regulator ORR25	HHZ	4.4	8.8	0.0	-Inf	0.00
		IR36	3.2	5.5	1.0	-2.49	0.15
BGIOSGA034767	BTB/POZ domain and ankyrin repeat-containing protein NH5.2	HHZ	1147.8	1623.6	672.0	-1.27	0.00
		IR36	1304.2	1737.3	871.0	-1.00	0.00
BGIOSGA002945	Cytochrome P450 90D2	HHZ	178.2	118.0	238.4	1.01	0.00
		IR36	184.9	164.3	205.5	0.32	0.05
BGIOSGA014915	Cytochrome P450 724B1	HHZ	1872.9	2570.7	1175.2	-1.13	0.00
		IR36	1251.4	1482.6	1020.2	-0.54	0.00
BGIOSGA001585	Cytochrome P450 734A6	HHZ	123.1	178.3	67.9	-1.39	0.00
		IR36	202.3	267.6	136.9	-0.97	0.00

Table 5(on next page)

Table 5. Gene expression of DEGs in starch and sucrose metabolism in SHR

Table 5. Gene expression of DEGs in starch and sucrose metabolism in SHR

ID	Gene annotation	Cultivar	baseMean	IR36_32	IR36_40	log2FoldChange	P-value
BGIOSGA010570	Sucrose synthase	HHZ	14318.1	18545.9	10090.4	-0.88	0.00
		IR36	13352.6	18616.3	8088.8	-1.20	0.00
BGIOSGA026140	Sucrose synthase	HHZ	13.5	16.5	10.4	-0.67	0.24
		IR36	16.8	24.7	8.9	-1.47	0.01
BGIOSGA026976	trehalose-6-phosphate synthase, putative, expressed	HHZ	651.9	572.0	731.7	0.36	0.12
		IR36	691.3	399.7	982.9	1.30	0.00
BGIOSGA009181	trehalose-6-phosphate synthase, putative, expressed	HHZ	731.2	562.7	899.8	0.68	0.01
		IR36	988.8	578.3	1399.3	1.28	0.00
BGIOSGA030796	trehalose-6-phosphate synthase, putative, expressed	HHZ	2.2	1.9	2.4	0.38	0.98
		IR36	4.3	0.0	8.6	Inf	0.00
BGIOSGA000509	Trehalose-6-phosphate phosphatase	HHZ	175.0	229.9	120.2	-0.94	0.00
		IR36	179.2	268.6	89.9	-1.58	0.00
BGIOSGA031385	beta-amylase, putative, expressed	HHZ	19.3	17.7	20.8	0.23	0.65
		IR36	28.0	17.4	38.5	1.15	0.01

Figure 1

Figure 1. Effects of high temperature on panicle development of HHZ and IR36 after 9 d of high-temperature treatment.

(a) young panicle morphologies , (b) young panicle length, (c) dry weight of young panicles. Bars = 0.5 cm in (a). Values are presented as the mean ± S.D. (n=3). Significance of the difference between NT and HT (one-tailed Student's t-test): *, $P<0.05$; **, $P<0.01$.

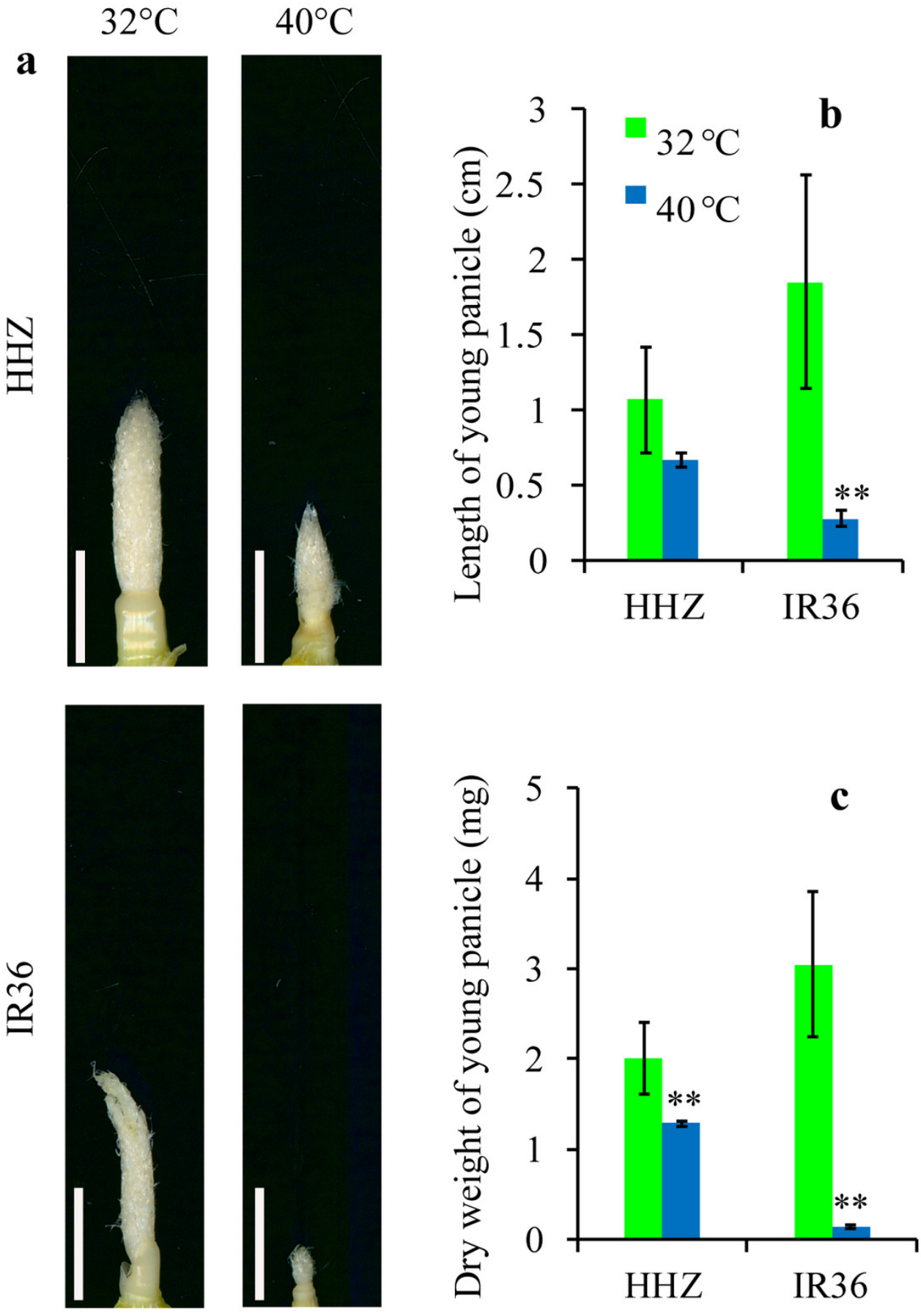

32°C
40°C
a
HHZ
IR36
b
32 °C
40 °C
Length of young panicle (cm)
3
2.5
2
1.5
1
0.5
0
**
HHZ
IR36
c
Dry weight of young panicle (mg)
5
4
3
2
1
0
**
**
HHZ
IR36

Figure 2

Figure 2. Effects of high temperature on the number of surviving spikelets of HHZ and IR36 after high-temperature treatment.

(a) panicle morphologies, (b) the number of differentiated spikelets, (c) the proportion of degeneration spikelets. Bars = 3 cm in (a). Values are presented as the mean ± S.D. (n=3). Significance of the difference between NT and HT (one-tailed Student's t-test): *, $P<0.05$; **, $P<0.01$.

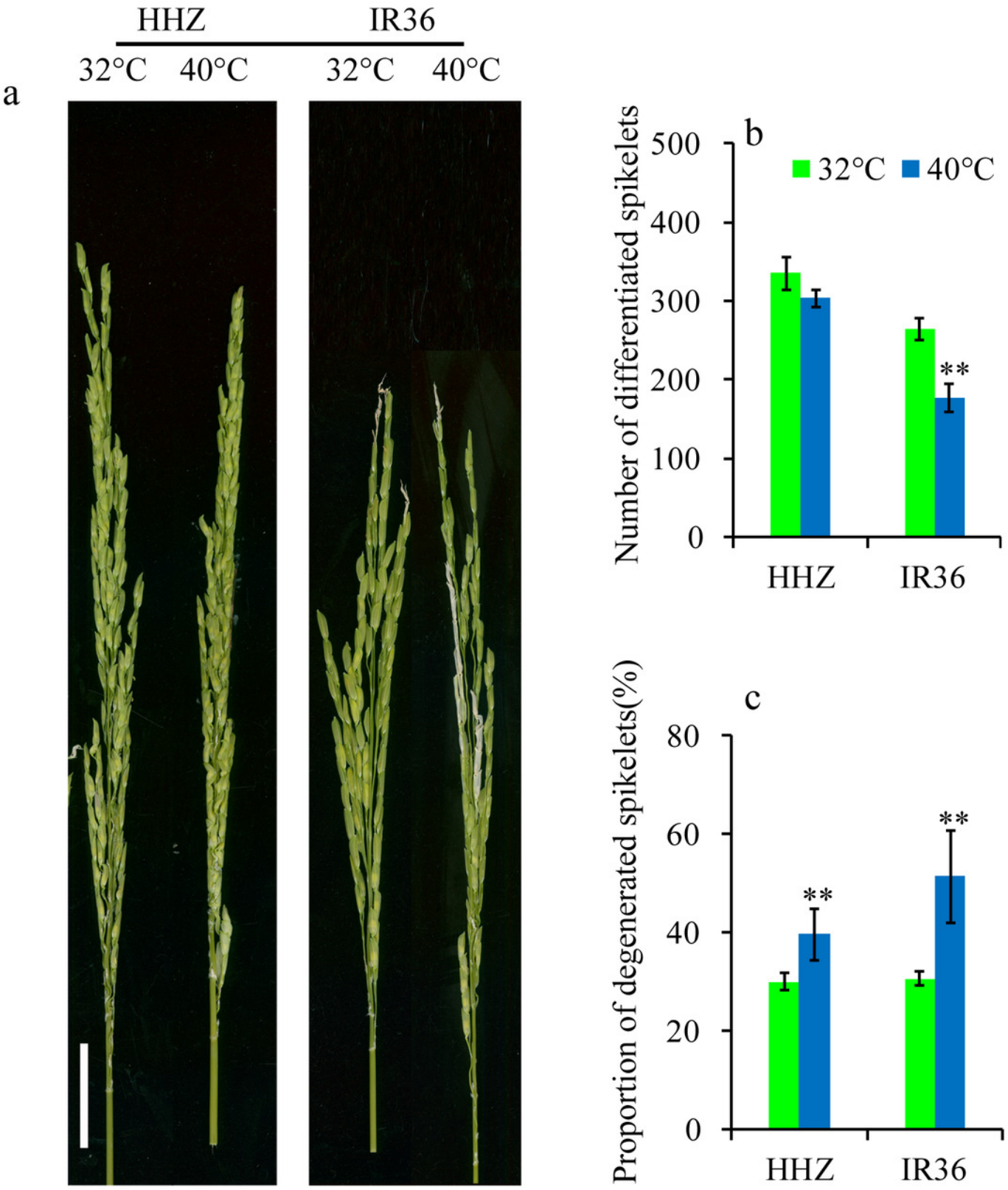

a
HHZ
IR36
32°C
40°C
32°C
40°C
b
32°C
40°C
Number of differentiated spikelets
500
400
300
200
100
0
**
HHZ
IR36
c
Proportion of degenerated spikelets(%)
80
60
40
20
0
**
**
HHZ
IR36

Figure 3

Figure 3. Effects of high temperature on spikelet size of HHZ and IR36 after high-temperature treatment.

(a) spikelet morphologies, (b) length of glumes, (c) width of glumes. Bars = 1 cm in (a). Values are presented as the mean ± S.D. (n=3). Significance of the difference between NT and HT (one-tailed Student's t-test): *, $P<0.05$; **, $P<0.01$.

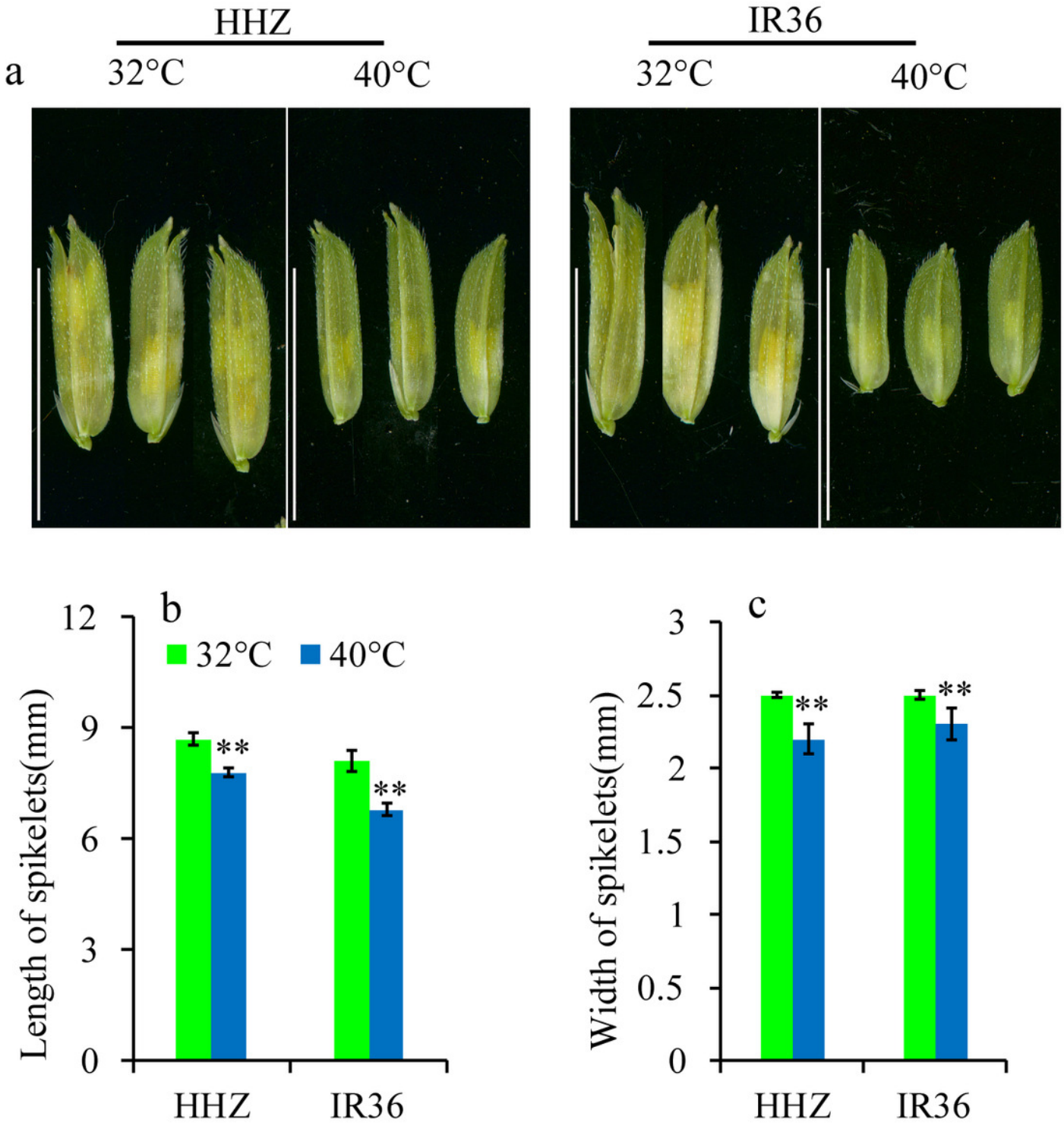

a
HHZ
32°C
40°C
IR36
32°C
40°C
b
32°C
40°C
Length of spikelets(mm)
12
9
6
3
0
**
**
HHZ
IR36
c
Width of spikelets(mm)
3
2.5
2
1.5
1
0.5
0
**
**
HHZ
IR36

Figure 4

Figure 4. Gene expression in the four comparison groups.

(a) HHZ_32 vs HHZ_40 **(b)** IR36_32 vs IR36_40, **(c)** IR36_40 vs HHZ_40, and **(d)** IR36_32 vs HHZ_32. Red (upregulated) and blue (downregulated) dots indicate that the genes had significant differences, while the gray dots correspond to genes with no significant differences.

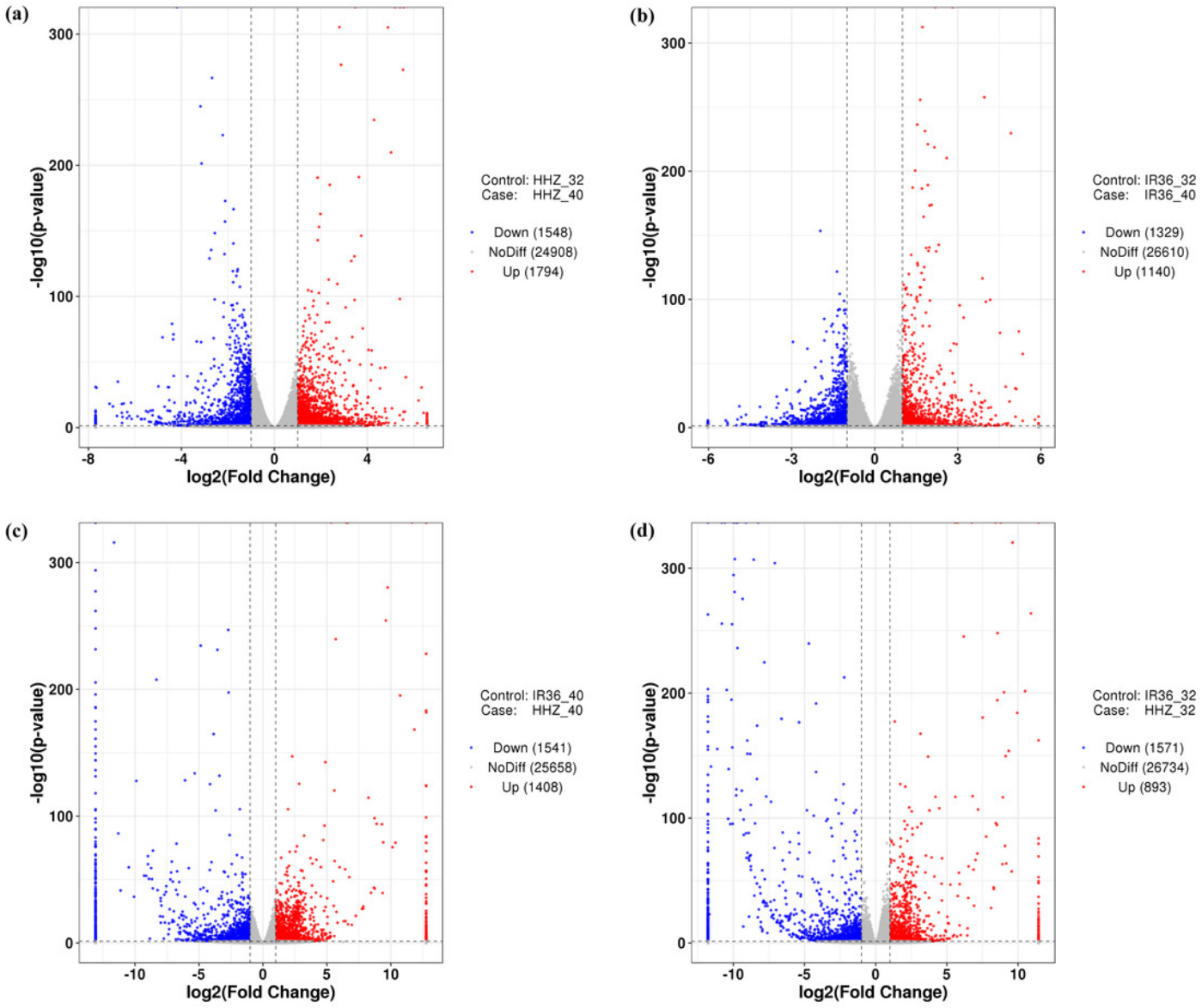


Figure 5

Figure 5. Venn diagrams for DEGs in the four comparison groups.

(a) HHZ_32 vs HHZ_40**(b)** IR36_32 vs IR36_40, **(c)** IR36_40 vs HHZ_40, and **(d)** IR36_32 vs HHZ_32.

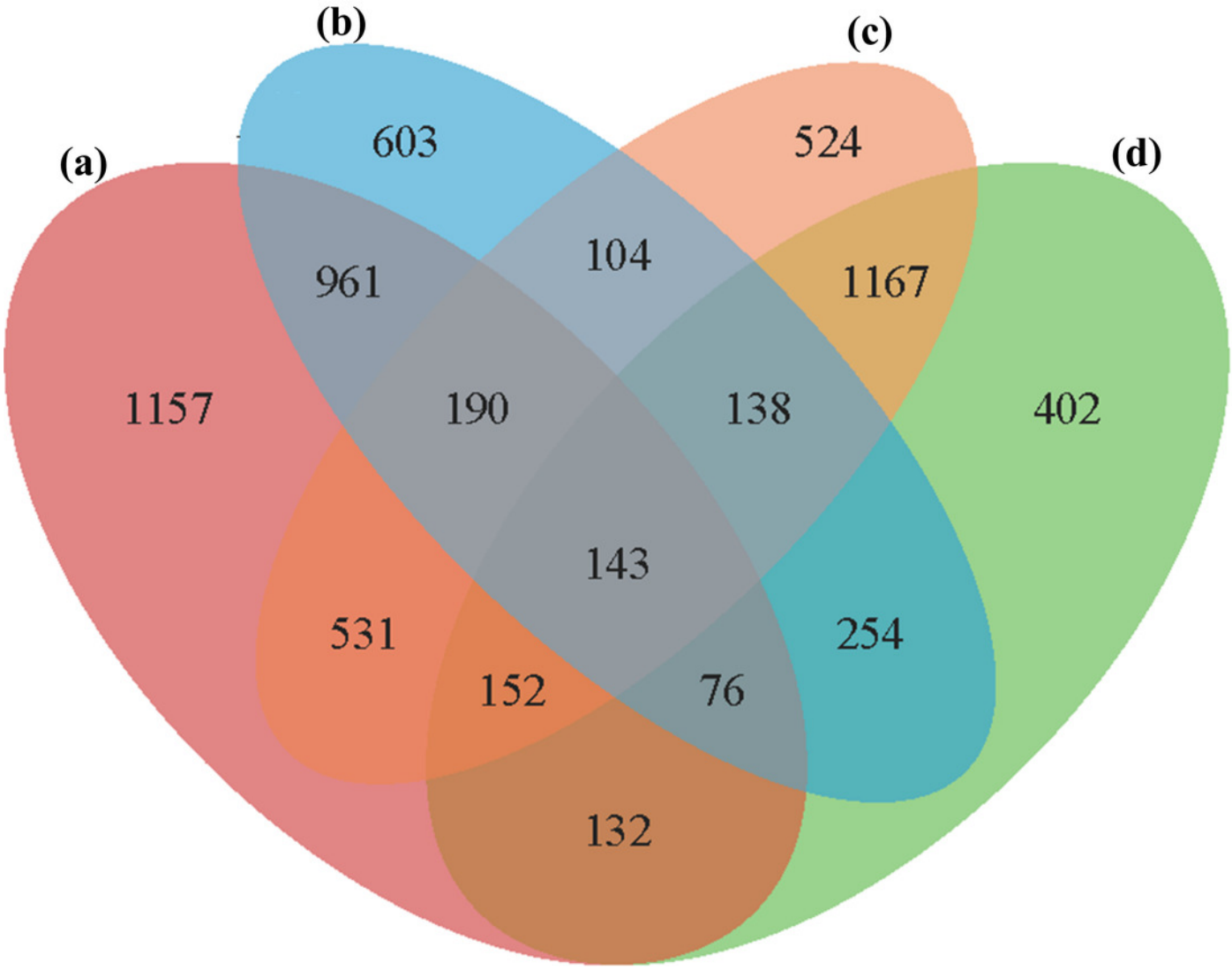


Figure 6

Figure 6. The enriched GO terms (P<0.05) of all DEGs.

(a) Biological process; (b) Cellular component; (c) Molecular function.

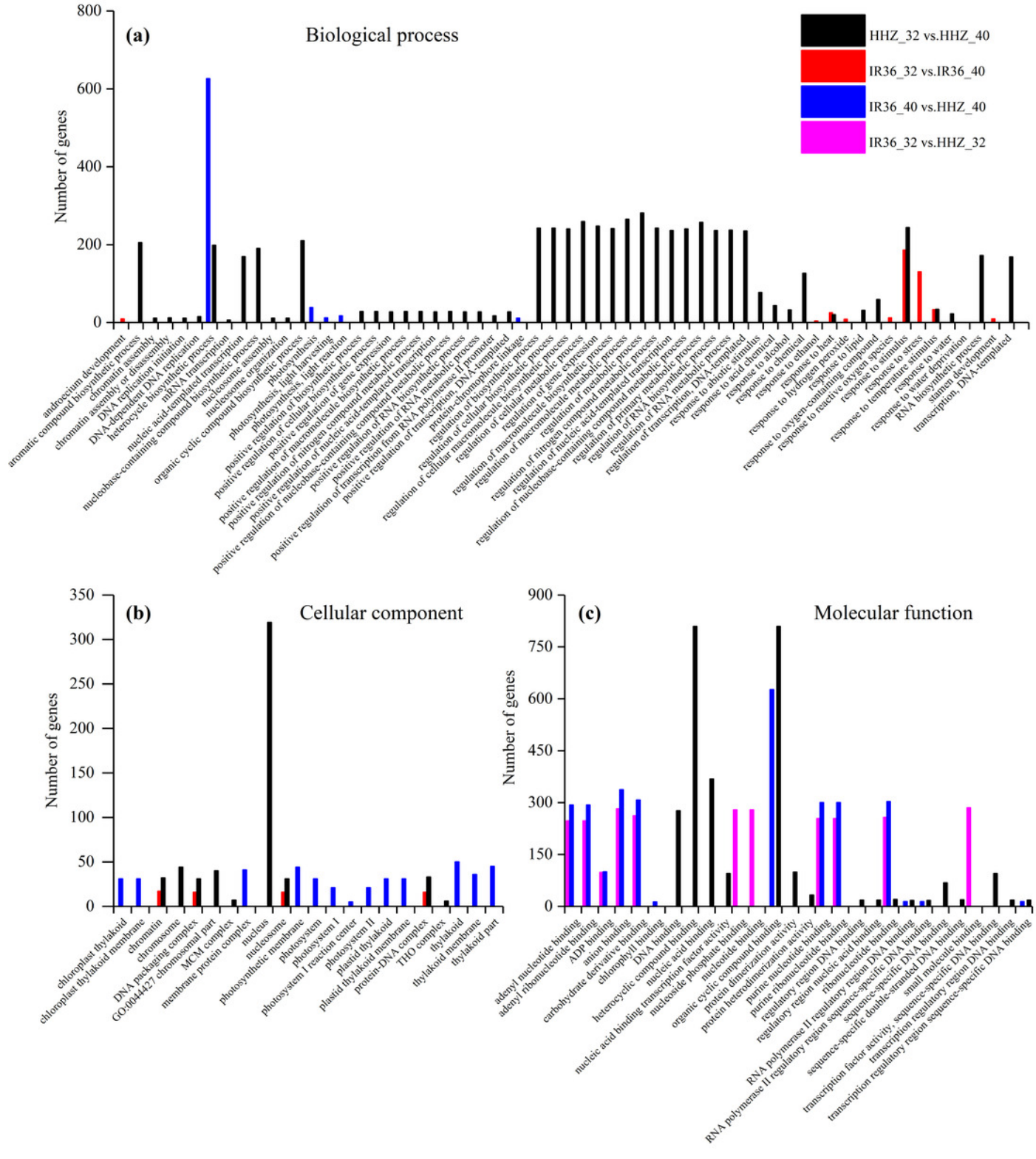

(a)
Biological process
Number of genes
HHZ_32 vs.HHZ_40
IR36_32 vs.IR36_40
IR36_40 vs.HHZ_40
IR36_32 vs.HHZ_32
androecium development
aromatic compound biosynthetic process
chromatin assembly
chromatin assembly or disassembly
DNA replication initiation
DNA-dependent DNA replication
heterocycle biosynthetic process
mRNA transcription
nucleic acid-templated transcription
nucleobase-containing compound biosynthetic process
nucleosome assembly
nucleosome organization
organic cyclic compound biosynthetic process
photosynthesis
photosynthesis, light harvesting
photosynthesis, light reaction
positive regulation of biosynthetic process
positive regulation of cellular biosynthetic process
positive regulation of gene expression
positive regulation of macromolecule biosynthetic process
positive regulation of metabolic process
positive regulation of nitrogen compound metabolic process
positive regulation of nucleic acid-templated transcription
positive regulation of nucleobase-containing compound metabolic process
positive regulation of RNA biosynthetic process
positive regulation of RNA metabolic process
positive regulation of RNA polymerase II promoter
positive regulation of transcription, DNA-templated
positive regulation of transcription from RNA polymerase II promoter
protein-chromophore linkage
regulation of biosynthetic process
regulation of cellular biosynthetic process
regulation of cellular macromolecule biosynthetic process
regulation of cellular metabolic process
regulation of gene expression
regulation of macromolecule biosynthetic process
regulation of macromolecule metabolic process
regulation of metabolic process
regulation of nitrogen compound metabolic process
regulation of nucleic acid-templated transcription
regulation of nucleobase-containing compound metabolic process
regulation of primary metabolic process
regulation of RNA biosynthetic process
regulation of RNA metabolic process
regulation of transcription, DNA-templated
response to abiotic stimulus
response to acid chemical
response to alcohol
response to chemical
response to ethanol
response to heat
response to hydrogen peroxide
response to lipid
response to oxygen-containing compound
response to reactive oxygen species
response to stimulus
response to stress
response to temperature stimulus
response to water
response to water deprivation
RNA biosynthetic process
stamen development
transcription, DNA-templated
(b)
Cellular component
Number of genes
chloroplast thylakoid
chloroplast thylakoid membrane
chromatin
chromosome
DNA packaging complex
GO:0044427 chromosomal part
MCM complex
membrane protein complex
nucleus
nucleosome
photosynthetic membrane
photosystem
photosystem I
photosystem I reaction center
photosystem II
plastid thylakoid
plastid thylakoid membrane
protein-DNA complex
THO complex
thylakoid
thylakoid membrane
thylakoid part
(c)
Molecular function
Number of genes
adenyl nucleotide binding
adenyl ribonucleotide binding
ADP binding
anion binding
carbohydrate derivative binding
chlorophyll binding
heterocyclic compound binding
DNA binding
nucleic acid binding
nucleic acid binding transcription factor activity
nucleoside phosphate binding
nucleotide binding
organic cyclic compound binding
protein dimerization activity
protein heterodimerization activity
purine nucleotide binding
purine ribonucleotide binding
regulatory region DNA binding
regulatory region nucleic acid binding
RNA polymerase II regulatory region DNA binding
RNA polymerase II regulatory region sequence-specific DNA binding
ribonucleotide binding
sequence-specific DNA binding
sequence-specific double-stranded DNA binding
transcription factor activity, sequence-specific DNA binding
small molecule binding
transcription regulatory region DNA binding
transcription regulatory region sequence-specific DNA binding

Figure 7

Figure 7. The enriched GO terms (P<0.05) of DEGs in RHR and CHR.

(a) Biological process; (b) Cellular component; (c) Molecular function.

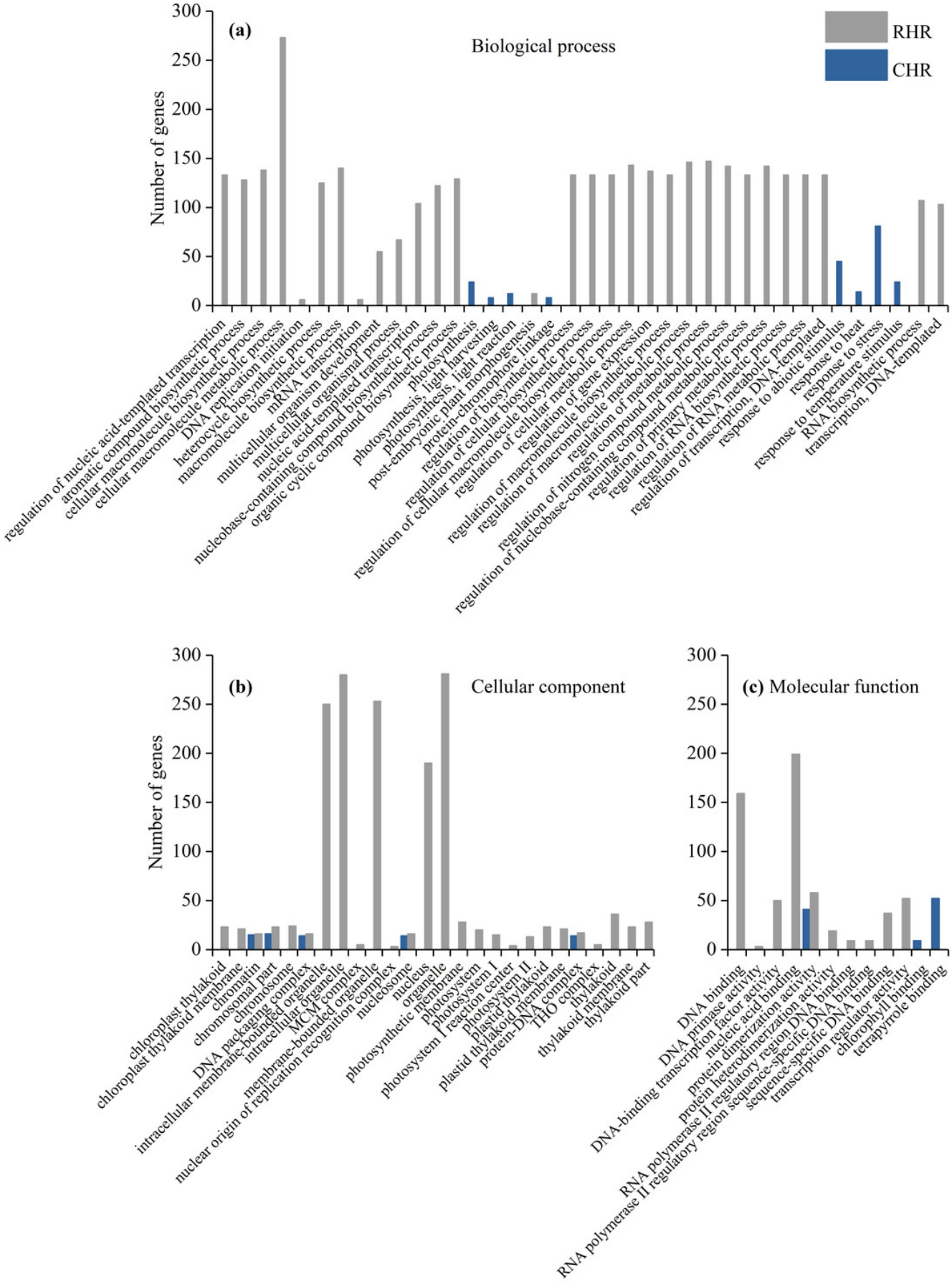

(a)
Biological process
RHR
CHR
Number of genes
300
250
200
150
100
50
0
regulation of nucleic acid-templated transcription
aromatic compound biosynthetic process
cellular macromolecule biosynthetic process
cellular macromolecule metabolic process
DNA replication initiation
heterocycle biosynthetic process
macromolecule biosynthetic process
mRNA transcription
multicellular organism development
multicellular organismal process
nucleic acid-templated transcription
nucleobase-containing compound biosynthetic process
organic cyclic compound biosynthetic process
photosynthesis
photosynthesis, light harvesting
photosynthesis, light reaction
post-embryonic plant morphogenesis
protein-chromophore linkage
regulation of cellular biosynthetic process
regulation of cellular macromolecule biosynthetic process
regulation of cellular metabolic process
regulation of gene expression
regulation of macromolecule biosynthetic process
regulation of macromolecule metabolic process
regulation of metabolic process
regulation of nitrogen compound metabolic process
regulation of nucleobase-containing compound metabolic process
regulation of primary metabolic process
regulation of RNA biosynthetic process
regulation of RNA metabolic process
regulation of transcription, DNA-templated
response to abiotic stimulus
response to heat
response to stress
response to temperature stimulus
RNA biosynthetic process
transcription, DNA-templated
(b)
Cellular component
Number of genes
300
250
200
150
100
50
0
chloroplast thylakoid
chloroplast thylakoid membrane
chromatin
chromosomal part
chromosome
DNA packaging complex
intracellular membrane-bounded organelle
intracellular organelle
MCM complex
membrane-bounded organelle
nuclear origin of replication recognition complex
nucleosome
nucleus
organelle
photosynthetic membrane
photosystem
photosystem I
photosystem I reaction center
photosystem II
plastid thylakoid
plastid thylakoid membrane
protein-DNA complex
THO complex
thylakoid
thylakoid membrane
thylakoid part
(c) Molecular function
300
250
200
150
100
50
0
DNA binding
DNA primase activity
DNA-binding transcription factor activity
nucleic acid binding
protein dimerization activity
protein heterodimerization activity
RNA polymerase II regulatory region DNA binding
RNA polymerase II regulatory region sequence-specific DNA binding
sequence-specific DNA binding
transcription regulator activity
chlorophyll binding
tetrapyrrole binding

Figure 8

Figure 8. Classification of TF families of the DEGs enriched in DNA-binding transcription factor in RHR.

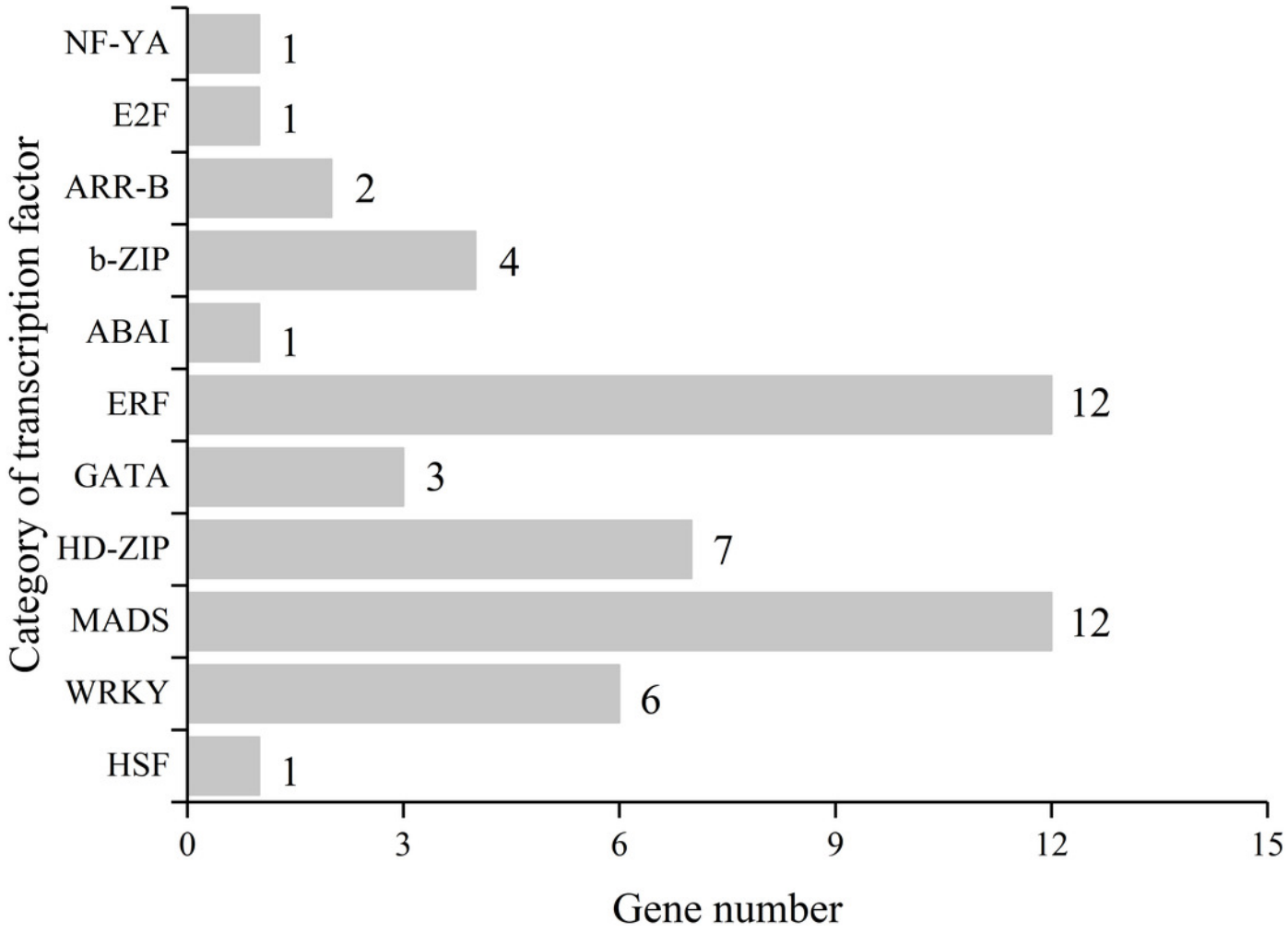


Figure 9

Figure 9. KEGG enrichment analysis of all DEGs.

(a) HHZ_32 vs HHZ_40; (b) IR36_32 vs IR36_40; (c) IR36_40 vs HHZ_40; (d) IR36_32 vs HHZ_32.

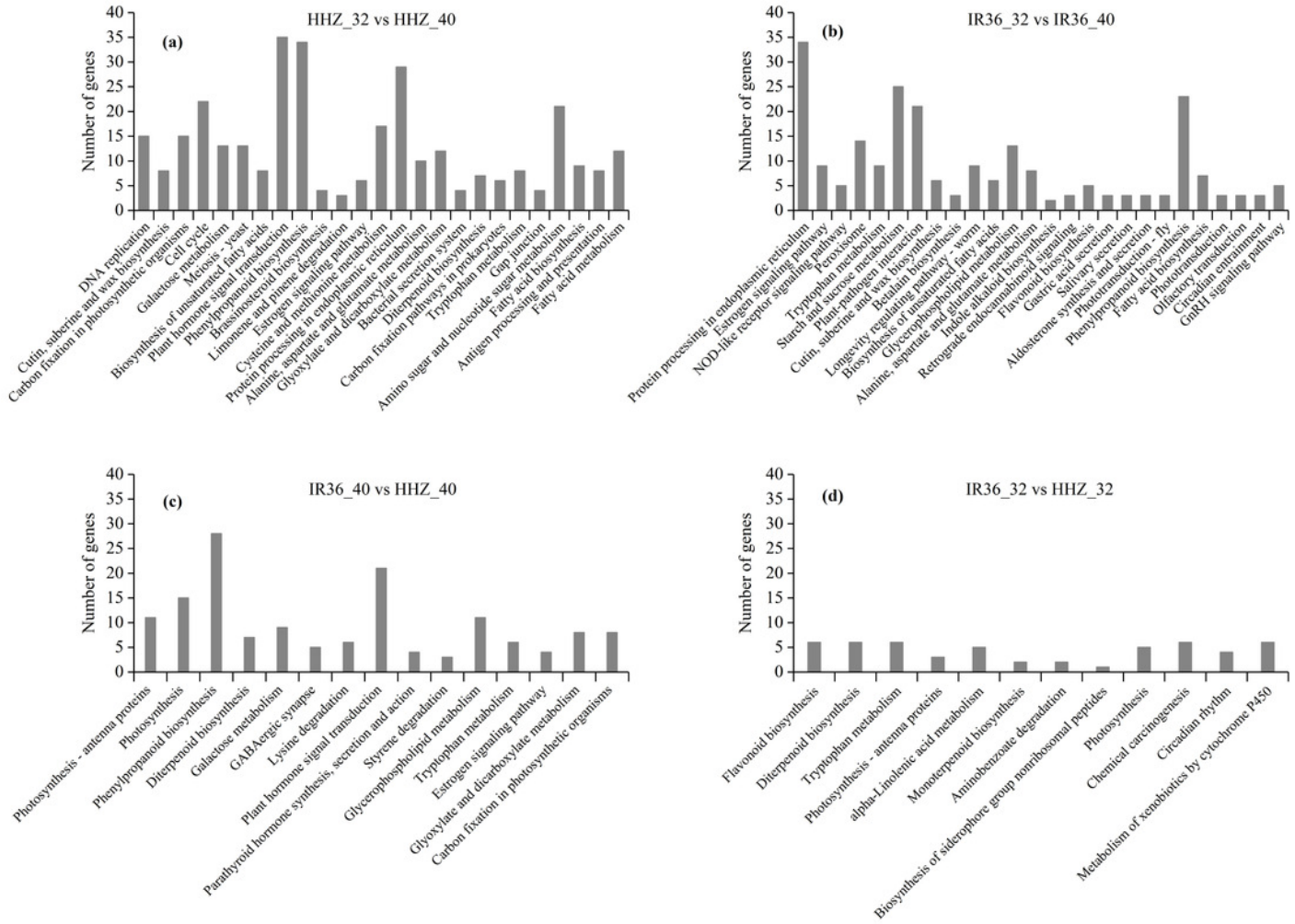


Figure 10

Figure 10. KEGG enrichment analysis for heat stress responsive genes from the three categories.

(a) RHR; (b) SHR; (c) CHR.

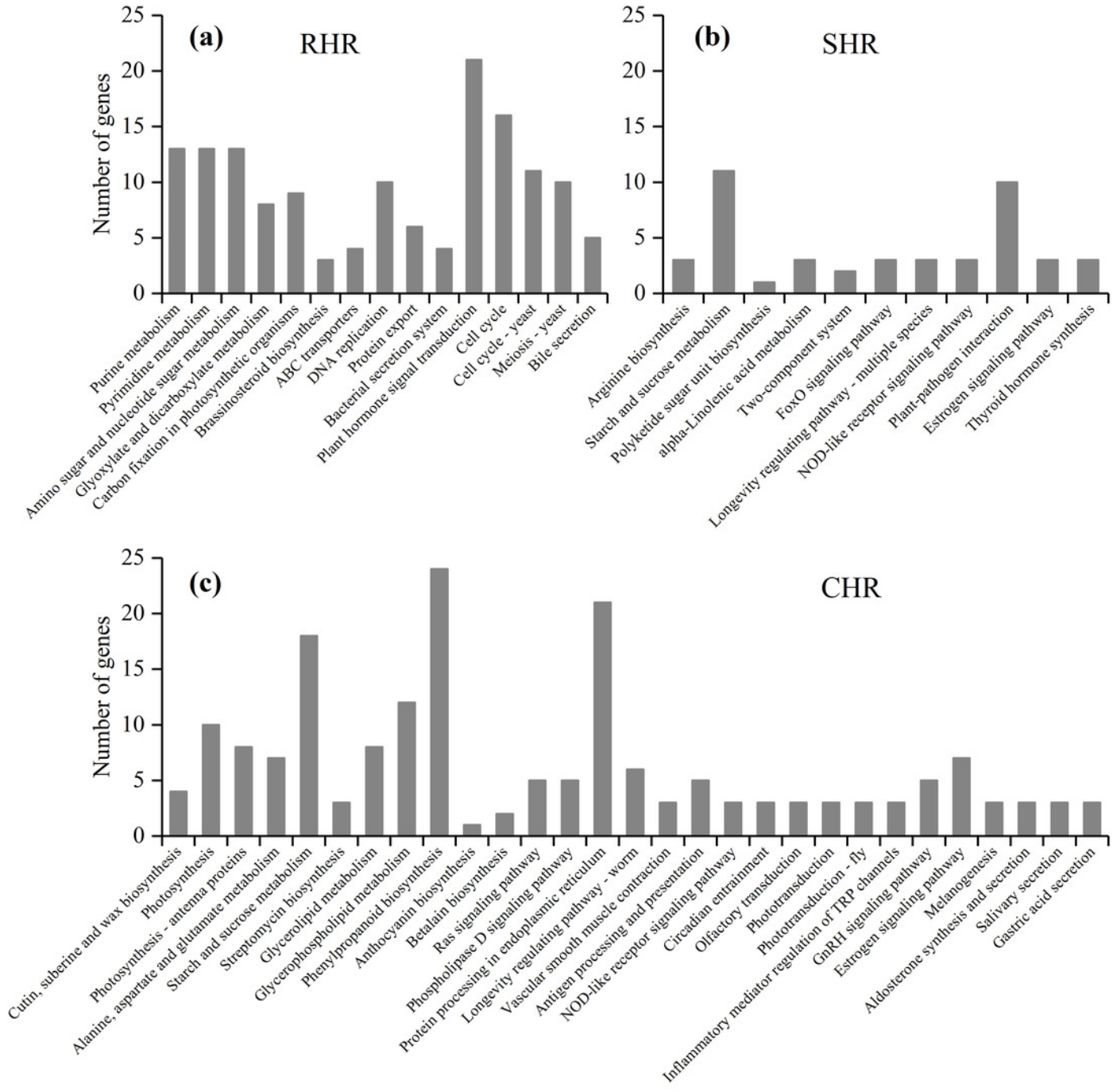
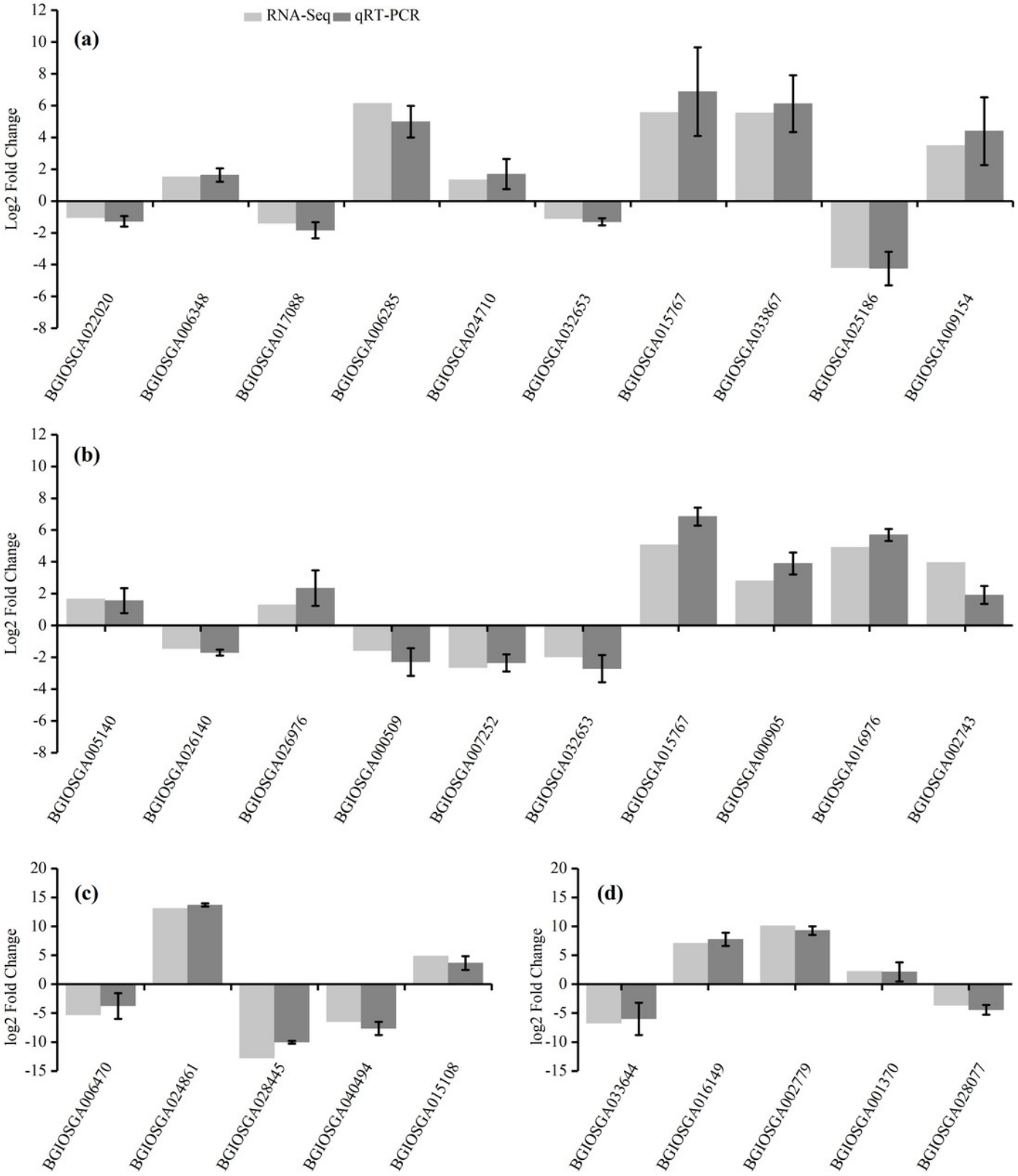


Figure 11

Figure 11. Gene expression levels of RNA-Seq results and qRT-PCR results.

(a) HHZ_32 vs HHZ_40. (b) IR36_32 vs IR36_40. (c) IR36_40 vs HHZ_40. (d) IR36_32 vs HHZ_32.


RNA-Seq
qRT-PCR
(a)
Log2 Fold Change
BGIOSGA022020
BGIOSGA006348
BGIOSGA017088
BGIOSGA006285
BGIOSGA024710
BGIOSGA032653
BGIOSGA015767
BGIOSGA033867
BGIOSGA025186
BGIOSGA009154
(b)
Log2 Fold Change
BGIOSGA005140
BGIOSGA026140
BGIOSGA026976
BGIOSGA000509
BGIOSGA007252
BGIOSGA032653
BGIOSGA015767
BGIOSGA000905
BGIOSGA016976
BGIOSGA002743
(c)
log2 Fold Change
BGIOSGA006470
BGIOSGA024861
BGIOSGA028445
BGIOSGA040494
BGIOSGA015108
(d)
log2 Fold Change
BGIOSGA033644
BGIOSGA016149
BGIOSGA002779
BGIOSGA001370
BGIOSGA028077