Comparative 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 the spikelet number per panicle. This study investigated the mechanism involved in heat-induced damage to panicle development and spikelet formation in rice cultivars that differ in heat tolerance. Transcriptome data from developing panicles grown at 40°C or 32°C were compared for two 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-resistant-cultivar-related genes (RHR), 707 heat-susceptible-cultivar-related genes (SHR), and 1,675 common heat stress-responsive genes. A Gene ontology (GO) analysis showed that the DEGs in the RHR category 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. A Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that the DEGs in the RHR and SHR categories were enriched in 15 and 11 significant metabolic pathways, respectively. Improved signal transduction capabilities of endogenous hormones under high temperature seemed to promote heat tolerance, while impaired starch and sucrose metabolism under high temperature might have inhibited young panicle development. Our transcriptome analysis provides insights into the different molecular mechanisms of heat stress tolerance in developing rice.

35 Key words: Rice; Panicle development; Heat stress; Transcriptome analysis

Introduction

39   Climate change is predicted to increase the average global temperatures by 0.3-4.8°C by the
40 end of the 21st century (Stocher *et al*. 2013). Unusually high temperatures occur frequently during
41 the rice growing season (Dwivedi *et al*. 2015; Tao *et al*. 2013), and cause reductions in the yield
42 and quality in several rice producing regions, including China, India, and Japan (Anand *et al*. 2018;
43 Morita *et al*. 2016; Wang *et al*. 2019). The primary cause of rice yield reductions is a reduction in
44 spikelet fertility due to high temperatures during the flowering period (Espe *et al*. 2017). Rice
45 quality is also influenced by high temperature, which causes carbohydrate metabolism disorders
46 (Yamakawa & Hakata 2010). As climate change has intensified, extremely high temperatures
47 above 40°C have become more frequent. Such high temperatures inhibit rice panicle development,
48 reduce the spikelet number by 5%-15%, and aggravate rice yield losses (Wang *et al*. 2017).

49   High temperatures adversely affect floral development by reducing the antioxidant capacity,
50 inhibiting nutrient accumulation, and degenerating tapetal cells (Prasad *et al*. 2017). A previous
51 study showed that high temperature (39°C) conditions downregulated certain genes related to
52 tapetum function, pollen adhesion, and germination, including *OsINV4* and *OsMST8*, which
53 influenced spikelet fertilization (Endo *et al*. 2009). In addition, sugar and endogenous hormone
54 metabolism under high temperature reportedly plays an important role in pollen formation in both
55 rice and cotton (Islam *et al*. 2018; Min *et al*. 2014). At the rice ripening stage, high temperature
56 induces early termination of grain filling (Kim *et al*. 2011). Grain chalkiness increases under a
57 mean temperature greater than 32°C, resulting in the deterioration of eating and cooking quality,
58 which are both closely linked to starch and sucrose metabolism (Zhong *et al*. 2010). Transcriptome
59 analysis has shown that high temperatures influence the expression of genes involved in the
60 inhibition of sucrose degradation and starch biosynthesis while promoting starch degradation and
61 storage proteins synthesis (Yamakawa & Hakata 2010; Yamakawa *et al*. 2007). Takehara *et al*.
62 (2018) reported that the upregulation of *OsSUS3*, which encodes sucrose synthase, improved high-
63 temperature tolerance.

64   The panicle initiation stage is an important period of spikelet proliferation. Dry matter
65 accumulation is essential for panicle development; however, the pathway for carbohydrate
66 accumulation during spikelet formation under heat stress remains vague. The reduction in spikelet
67 number that occurs under high temperature conditions has been associated with heat-induced
68 phytohormone changes, especially enhanced cytokinin degradation (Wu *et al*. 2017; Wu *et al*.

69 2016). The number of spikelets per panicle is determined by spikelet differentiation and
70 degeneration. Spikelet differentiation is correlated with dry matter accumulation and influenced
71 by environmental factors (Liu *et al.* 2005). Ding *et al.* (2016) reported that hormone metabolism,
72 the stress response, carbohydrate metabolism and transport, and protein degradation were
73 regulated to influence panicle initiation. Additionally, certain genes, such as MADS-box genes,
74 are related to panicle initiation (Kang *et al.* 2013; Kobayashi *et al.* 2012). Quantitative trait loci
75 for spikelet degeneration have been identified (Yamagishi *et al.* 2004), and the genes *SP1*, *ASP1*,
76 *TUT1*, *PAA2*, and *OsALMT7* have been found to control spikelet degeneration (Bai *et al.* 2015;
77 Heng *et al.* 2018; Li *et al.* 2010). However, the mechanism of panicle development under high
78 temperature conditions is still unclear. In this study, an RNA-Seq analysis was used to explore the
79 mechanism of heat tolerance during panicle development. Huanghuazhan (HHZ) is a heat-tolerant
80 rice cultivar widely grown in the middle and lower reaches of the Yangtze River in China (Cao *et*
81 *al.* 2009; Zhou *et al.* 2012). IR36 is a heat-susceptible cultivar (Fang *et al.* 2006) and a parental
82 line of HHZ. In the current study, we investigated transcriptome differences between these two
83 cultivars exposed to different temperatures 40°C and 32°C, during the spikelet differentiation
84 stage. We identified differentially expressed genes (DEGs) in young panicles of the two cultivars
85 under the two temperature treatments and performed Gene Ontology (GO) enrichment and the
86 Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. This work improves our
87 understanding of the molecular mechanism underlying the heat-induced inhibition of spikelet
88 development and provides important insights into rice breeding.
89

Materials and methods

Plant materials and heat stress treatments

92   We used the rice cultivars HHZ and IR36 in this study. Pre-germinated seeds were sown in
93 seed trays filled with a mixture of vermiculite (20%), charcoal (30%), soil (40%), and slow-release
94 fertilizer (10%). After 20 days, the seedlings were transplanted into pots with four seedlings per
95 pot. Each pot (24 cm length × 22.5 cm width × 21.5 cm height) contained 10 kg air-dried paddy
96 soil. Pots were kept under natural environmental conditions (the average temperature was 30-
97 35°C).
98   Before seedlings were transplanted into pots, fertilizer was applied to each pot based on a
99 field application rate of 14 kg nitrogen per 666.7 m^2. Before transplanting into the pots, 3.5 g
100 compound fertilizer (nitrogen: phosphorus: potassium = 15%: 15%: 15%) was applied to each pot.
101 At the tillering stage, 0.6 g urea was supplemented in each pot. At panicle initiation, 0.6 g urea and
102 0.5 g potassium chloride were also applied to each pot. Pests, diseases, and weeds were intensively
103 controlled.

104   Automatic growth chambers (Qiushi Environment Corporation, Hangzhou, China) were used
105   to conduct the temperature treatments. Plants were moved to the growth chambers on the
106   approximate date of spikelet differentiation when the panicle length was approximately 0.2 cm
107   (around 60-70 d after seed sowing). The high-temperature (40°C) and control temperature (32°C)
108   treatments were implemented for eight hours each day from 9:30 to 17:30 h (the setting details are
109   shown in Table S1) for nine days. The humidity in the chambers was maintained at 75-80%. Rice
110   plants were grown under natural ambient conditions during all growth stages before and after the
111   temperature treatments. Each treatment contained three replicates with 20 pots per replicate.
112

113 Panicle and spikelet morphology

114   Ten main tillers were sampled per replicate on day 9 of treatment at 40°C or 32°C to
115   investigate the development of young panicles at high temperature.

116   Spikelet differentiation or degeneration of the main tiller panicles was determined at the
117   heading stage. The number of degenerated spikelets was calculated by counting the vestiges
118   present on the panicles. The number of differentiated spikelets was the sum of the surviving and
119   degenerated spikelets. The proportion of degenerated spikelets was then calculated.

120   Spikelet morphology was observed under a stereomicroscope (Olympus SZX7, Olympus
121   Corporation, Tokyo, Japan) and the glume length and width (mm)were measured at 0.63x and 2.5x
122   using the microscale in the Image Pro-Plus 5.1 image processing software (Olympus SZX7,
123   Olympus Corporation, Tokyo, Japan). Fifteen spikelets were collected from the upper, middle and
124   lower parts of each panicle, with five panicles sampled for each replicate.
125

126 RNA extraction, transcriptome sequencing, and mapping

127   After nine days at the 40 °C or 32 °C treatment, young panicles from 20 main tillers were
128   collected for each replicate at 12:00-13:00 and immediately frozen in liquid nitrogen. In quick
129   succession, TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from
130   the young panicles according to the manufacturer's instructions. A TruSeq RNA Sample
131   Preparation Kit (Illumina Inc., CA, USA) was used to generate 12 sequencing libraries according
132   to the manufacturer's instructions. The sequencing libraries were then sequenced on a HiSeq
133   platform (Illumina, Inc., CA, USA). High-quality sequence reads were obtained by filtering the
134   raw   data   and   then   compared   to   the   9311-reference   genome
135   (Oryza_indica.ASM465v1.dna.toplevel. fa) obtained from http://www.ensembl.org/. The raw
136   RNA sequence data were submitted to the NCBI Sequence Read Archive with accession number
137   PRJNA508820.
138

139 Gene expression level and differential expression analysis

140   We used HTSeq (0.9.1) to statistically compare the read count values of each gene, which
141   represent the original expression of each gene. Fragments per kilobase of transcript per million
142   mapped reads (FPKM) was used to standardize the expression. Next, we used DESeq (1.30.0) to
143   analyze the differential expression of genes with the following screening conditions: an expression
144   difference of |log2foldChange| > 1 and a significant *P-value* < 0.05.
145

GO and KEGG enrichment analysis of DEGs

147   For the GO enrichment analysis of DEGs, we used the Singular Enrichment Analysis tool in
148   AgriGO (http://bioinfo.cau.edu.cn/agriGO/analysis.php) with the default parameters, and a *P-*
149   value≤0.05 indicated significant enrichment. The KEGG enrichment analysis of DEGs was

150   performed using KOBAS software with default parameters and a *P*-value $\leqslant$ 0.05 indicated
151   significant pathway enrichment.
152

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

154   First-strand cDNA was synthesized using ReverTra Ace qPCR RT Master Mix with gDNA
155   Remover (Toyobo, Osaka, Japan) according to the manufacturer's instructions. The qRT-PCR
156   analyses were performed using an Applied Biosystems 7500 Real-Time PCR system with Power
157   SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). The primers used for
158   qRT-PCR are listed in Supplementary Table S2. The *OsUBQ* gene was used as an internal control.
159   Relative gene expression levels were determined from the equation $2^{-\Delta\Delta CT}$ (Czechowski *et al*.
160   2004), where ΔΔCT represents ΔCT (target gene of interest)–ΔCT (control gene).
161

Statistical analyses

163   Microsoft Excel 2016 (Microsoft Inc., Redmond, WA, USA) was employed for data
164   collection. The panicle and spikelet morphological data collected for the 40°C and 32°C treatments
165   (mean of three replicates) were statistically analyzed by Student's t-test (*P* < 0.05). Graphs were
166   created using Origin 9.1 (Ver. 9.1, OriginLab, Northampton, MA, USA).
167

Results

Spikelet development at high temperature

170   A preliminary experiment showed a significant difference in panicle development, which was
171   measured as spike differentiation, after nine days of the high-temperature treatment. The results
172   reported in the current study are consistent with these preliminary findings. Spikelet differentiation
173   inhibited young panicle growth after nine days of the high temperature treatment (Fig. 1). After

174   the temperature treatments, the panicles required an additional 15-20 days to complete growth.

175   Compared to the control temperature treatment, the high temperature treatment reduced

176   spikelet survival by 22.3% ($P < 0.05$) for HHZ and 53.6% ($P < 0.05$) for IR36. With high

177   temperature, the number of differentiated spikelets decreased by 9.6% and 33.2% ($P < 0.05$) for

178   HHZ and IR36, respectively, and the proportion of degenerated spikelets significantly increased

179   by 32.3% ($P < 0.05$) and 67.4% ($P < 0.05$), respectively. In addition, the heat treatment reduced

180   the glume length by 10.3% ($P < 0.05$) for HHZ and by 16.0% ($P < 0.05$) for IR36 and reduced the

181   glume width by 12.0% ($P < 0.0.5$) and 8.0% ($P < 0.05$), respectively. The reductions in spikelet

182   number and size led to reductions in panicle weight of 33.2% ($P < 0.05$) for HHZ and 67.7% ($P <$

183   0.05) for IR36. The larger reduction in panicle weight in IR36 suggests that high temperature has

184   a greater effect on young panicle development in heat susceptible cultivars (Table 1).

185

186   **Transcriptome analysis**

187   Under the 32°C control temperature, a total of 44.2 million and 48.9 million raw reads were

188   obtained from HHZ (referred to as HHZ_32) and IR36 (referred to as IR36_32), respectively.

189   Under the 40°C treatment, a total of 45.5 million raw reads were obtained from both HHZ

190   (HHZ_40) and IR36 (IR36_40) (Table 2 and Table S3). More than 99.0% clean reads were

191   obtained for the downstream analysis. The results of RNA sequence mapping indicated that 85.8-

192   88.0% of the clean reads could be mapped onto the reference genome and most were uniquely

193   mapped (Table 2).

194

195   **Identification of DEGs**

196   To compare the differences between the two cultivars at 40°C and 32°C, we used four

197   comparison groups: HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and

198   IR36_32 vs HHZ_32. DEGs for the four groups were restricted to those with a |log2fold change|

199   > 1 and a *P-value* < 0.05. With these criteria, 3,342, 2,469, 2,949, and 2,461 DEGs were detected

200   for HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and IR36_32 vs HHZ_32,

201   respectively (Fig. 2). Significantly different gene expression was observed both between cultivars

202   and between treatments. For HHZ, 1,794 genes were upregulated and 1,548 genes were

203   downregulated in the 40°C treatment compared with the 32°C treatment (Fig. 2). Furthermore,

204   1,140 genes were upregulated and 1,329 genes were downregulated in IR36 under the 40°C

205   treatment compared with the 32 °C treatment (Fig. 2). For comparisons within treatments, 1,408

206   genes were upregulated and 1,541 were downregulated in the IR36_40 vs HHZ_40 group and 893

207   genes were upregulated and 1,751 genes were downregulated in the IR36_32 vs HHZ_32 group

208   (Fig.2c and 2d).

209

Classification of DEGs

210

211 In all four groups, a total of 5,533 unique DEGs were identified, and they could be divided

212 into 15 disjointed subgroups (Fig. 3). Among the 15 subgroups, eight from the IR36_32 vs

213 HHZ_32 group were excluded from the analysis because they were not influenced by high

214 temperature. In addition, 1,157, 603, 524, and 402 DEGs were uniquely identified in the HHZ_32

215 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and IR36_32 vs HHZ_32 groups,

216 respectively. The DEGs in groups that were responsive to high temperature could be further sorted

217 into three categories: heat-tolerance-cultivar-related genes (RHR, 1,688 genes), heat-susceptible-

218 cultivar-related genes (SHR, 707 genes), and common heat stress-response genes (CHR, 1,675

219 genes) (Table 3 and Table S4). The DEGs in the RHR category might have played an important

220 role in heat tolerance, whereas the DEGs in the SHR category might be associated with heat

221 injuries in the heat-susceptible cultivar.

222

Analysis of GO annotation

223

224 The purpose of the GO enrichment analysis was to obtain GO functional terms with

225 significant enrichment of DEGs and thus reveal the possible functions of the DEGs. Of all DEGs,

226 2,307 (69.0%), 1,680 (68.0%), 1,832 (62.1%), and 1,472 (59.8%) DEGs were enriched in GO

227 terms in HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and IR36_32 vs

228 HHZ_32 groups, respectively. There were 75, 11, 13, and 31 significant GO terms observed in

229 HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, IR36_40 vs HHZ_40, and IR36_32 vs HHZ_32,

230 respectively (Fig. 4). The maximum number of DEGs was observed for the heterocycle

231 biosynthetic process in the IR36_40 vs HHZ_40 group. In IR36_32 vs IR36_40 and HHZ_32 vs

232 HHZ_40, the DEGs were enriched in the terms response to stimulus, response to temperature

233 stimulus, and response to heat in the biological process category. Within the cellular component

234 category, the DEGs were commonly enriched in the terms chromatin, DNA packaging complex,

235 and nucleosome in the IR36_32 vs IR36_40 and HHZ_32 vs HHZ_40 groups. However, there

236 were no common GO terms in the category of molecular function in the IR36_32 vs IR36_40 and

237 HHZ_32 vs HHZ_40 groups.

238 We further identified GO term categories for DEGs in the RHR, SHR, and CHR categories

239 (Fig. 5 and Table S5). Among the 1,689 DEGs in RHR, 54 significant GO terms were detected.

240 However, no significant GO terms were observed among the 707 DEGs in SHR. In CHR, 30

241 significant GO terms were detected. In the CHR group, eight significant GO terms were observed

242 in the biological process category, including response to stimulus, response to temperature

243 stimulus, and response to heat; 17 GO terms were in the cellular component category; and two

244 significant GO terms were in the molecular function category. In the RHR group, 30, 14 and 10

245 significant GO terms were in the biological process, cellular component, and molecular function

246   categories, respectively. The most significant GO terms, in decreasing order, were RNA

247   biosynthetic process, nucleus, and DNA binding. In the molecular function category, 50 DEGs

248   were specifically assigned to DNA-binding transcription factor activity, which may play an

249   important role in heat stress tolerance.

250   The 50 DEGs of DNA-binding transcription factor activity could be divided into 11

251   transcription factor (TF) families, including HSF (1), WRKY (6), MADS (12), HD-ZIP (7), GATA

252   (3), ERF (12), ABAI (1), b-ZIP (4), ARR-B (2), E2F (1), and NF-YA (1). Expression of the genes

253   *BGIOSGA006348* of HSF, *BGIOSGA010835* of ABAI, *BGIOSGA010142* of HAP, and

254   *BGIOSGA000303* and *BGIOSGA000304* of ARR-B was significantly upregulated. In addition,

255   five genes in WRKY, eight genes in MADS, two genes in HD-ZIP, two genes in GATA, six genes

256   in ERF, and two genes in b-ZIP were also upregulated (Table S6). These results suggest that, these

257   30 TF genes may play important roles in heat stress resistance.

258

Analysis of KEGG pathway enrichment

260   In the KEGG analysis, 1,158 DEGs were classified into 225, 191, 239, and 211 functional

261   pathways in HHZ_32 vs HHZ_40; 838 DEGs in IR36_32 vs IR36_40; 732 DEGs in IR36_40 vs

262   HHZ_40; and 539 DEGs in IR36_32 vs HHZ_32. A total of 79 pathways were significant (*P-value*

263   < 0.05) (Fig. 6). Among these pathways, the phenylpropanoid biosynthesis pathway was common

264   in HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, and IR36_40 vs HHZ_40, which suggests that heat

265   stress impaired phenylpropanoid biosynthesis.

266   Based on further analysis of the three categories with different heat-stress responses, 146

267   DEGs in RHR were involved in 15 overrepresented pathways, including purine metabolism,

268   pyrimidine metabolism, and amino sugar and nucleotide sugar metabolism; 45 DEGs in SHR were

269   involved in 11 overrepresented pathways, including arginine biosynthesis, starch and sucrose

270   metabolism, and polyketide sugar unit biosynthesis; and 184 DEGs in CHR were involved in 29

271   overrepresented pathways (Fig. 7 and Table S7).

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

273   the 15 KEGG pathways in RHR, 21 DEGs were involved in plant hormone signal transduction, of

274   which 14 DEGs were upregulated in HHZ; three DEGs were involved in cytochrome P450

275   metabolism, which plays a role in brassinosteroid (BR) biosynthesis; and two were upregulated

276   (Table 4).

277   In SHR and CHR, there were three common pathways: the starch and sucrose metabolism

278   pathway, the NOD-like receptor signaling pathway, and the estrogen signaling pathway.

279   Carbohydrate accumulation was essential for panicle development. In the KEGG analysis, seven

280   DEGs involved in starch and sucrose metabolism were observed in SHR and 18 DEGs involved

281   in starch and sucrose metabolism were observed in CHR. In SHR, the genes in HHZ were not

282   different between HHZ_40 and HHZ_32. However, genes *BGIOSGA010570* and
283   *BGIOSGA026140* encoding sucrose synthase (EC 2.4.1.13), genes *BGIOSGA026976*,
284   *BGIOSGA009181*, and *BGIOSGA030796* encoding trehalose-6-phosphate synthase (EC 2.4.1.15),
285   and gene *BGIOSGA000509* encoding trehalose-6-phosphate phosphatase (EC 3.1.3.12) were
286   significantly downregulated in IR36_40 compared with IR36_32. However, gene
287   *BGIOSGA031385* encoding beta-amylase (EC 3.2.1.2) was significantly upregulated in IR36_40
288   compared with IR36_32 (Table 5).
289
290   **qRT-PCR verification**
291   To confirm the accuracy of the RNA-Seq results, ten representative DEGs each from the
292   HHZ_32 vs HHZ_40 (a) and IR36_32 vs IR36_40 (b) groups, as well as five DEGs each from the
293   IR36_40 vs HHZ_40 (c) and IR36_32 vs HHZ_32 (d) groups were chosen to determine relative
294   expression. Of the ten DEGs from the HHZ_32 vs HHZ_40 group, five were in RHR:
295   *BGIOSGA022020* is related to BR synthesis, *BGIOSGA006348* encodes a heat shock factor (Hsf),
296   *BGIOSGA017088* is involved in the ETH TF family, *BGIOSGA006285* participates in ethylene
297   responsive regulation, and *BGIOSGA024710* is an auxin-responsive gene involved in plant
298   hormone transduction. Among the ten DEGs from the IR36_32 vs IR36_40 group, five were in
299   SHR and encoded cytokinin oxidase/dehydrogenase (*BGIOSGA005140*), sucrose synthase
300   (*BGIOSGA026140*), trehalose-6-phosphate synthase (*BGIOSGA026976*), trehalose-6-phosphate
301   phosphatase (*BGIOSGA000509*), and catalase (*BGIOSGA007252*). Four DEGs were in CHR from
302   the HHZ_32 vs HHZ_40 and IR36_32 vs IR36_40 groups and two common genes,
303   *BGIOSGA032653* and *BGIOSGA015767*, were validated. *BGIOSGA032653* is involved in
304   phenylpropanoid biosynthesis and *BGIOSGA015676* encodes a heat shock protein (HSP). The
305   qRT-PCR results for the DEGs were all consistent with the RNA-Seq data (Fig.8).
306

Discussion

308   The exposure of rice plants to high temperature growing conditions during spikelet
309   differentiation inhibited panicle initiation and reduced spikelet number per panicle (Fig. 1).
310   Previous studies have shown that the genes *SP1*, *ASP1*, *TUT1*, *PAA2*, and *OsALMT7* are closely
311   related to branch and spikelet development in rice (Bai *et al.* 2015; Heng *et al.* 2018; Li *et al.*
312   2010). However, in the current study, we observed no significant difference in the expression of
313   these genes between the 40°C treatment and the 32°C control treatment in either rice cultivar,
314   which indicates that the expression of these genes might not be inhibited in young panicles exposed
315   to high temperature.
316   In general, the upregulation of HSPs contributes to the heat stress response in plants (Guan *et*

317   *al.* 2010; Jagadish *et al.* 2010; Jung *et al.* 2013). Moon *et al.* (2014) reported that heterologous
318   overexpression of *OsHSP1* (*BGIOSGA015767*, encoding a HSP) increased heat tolerance in
319   Arabidopsis. However, in the current study, *BGIOSGA15767* expression was upregulated in both
320   HHZ (log2 (HHZ_40/HHZ_32) = 5.7, *P*-value = 0) and IR36 (log2 (IR36_40/IR36_32) = 5.0, *P*-
321   value = 0). In addition, there was no gene expression difference in the GO term of HSPs between
322   cultivars, which demonstrates that the heat stress reaction is common to both rice cultivars when
323   exposed to high temperature. The GO enrichment analysis revealed that the DEGs for the CHR
324   group were commonly enriched in response to GO terms representing heat, stress, and temperature
325   stimuli in the biological process category (Fig. 5). These results demonstrate that the heat stress
326   response did not directly inhibit panicle development but rather may disrupt physiological
327   processes related to panicle development.

328   An important factor determining heat tolerance is antioxidant capacity (Lan *et al.* 2016). Buer
329   *et al.* (2010) reported that flavonoids can positively regulate reactive oxygen species (ROS), which
330   can affect the transport of plant hormones and influence pollen development. The flavonoid
331   synthesis pathway was overrepresented in the IR36_32 vs IR36_40 group. Specifically, five genes
332   involved in flavonoid synthesis were downregulated at 40°C, which might indicate a reduction in
333   the antioxidant capacity of IR36 under heat stress. In addition, 14 DEGs in the IR36_32 vs IR36_40
334   group were enriched in the peroxisome pathway. Among these, 10 DEGs were significantly
335   downregulated and four DEGs were significantly upregulated. However, the peroxisome pathway
336   was not significant in the KEGG analysis of HHZ_32 vs HHZ_40 (Fig. 6). BGIOSGA007252 and
337   BGIOSGA011520, which encode catalase (EC:1.11.1.6), were significantly downregulated in
338   IR36 at 40°C compared with 32°C, whereas no expression differences were observed in HHZ_32
339   vs HHZ_40. This finding suggests that high temperature had a greater negative effect on the
340   antioxidant capacity of IR36 than of HHZ, which provides a primary explanation for the greater
341   heat injury observed in the young IR36 panicles than in those of HHZ.

342   Regulation of endogenous hormones affects the development of young panicles. Wu *et al.*
343   (2017) reported that a lower spikelet number under high temperature growing conditions was
344   associated with cytokinin degradation. In the current study, BGIOSGA001314, which encodes a
345   cytokinin-activity enzyme, did not differ between the 40°C and 32°C treatments in HHZ (log2
346   (HHZ_40/HHZ_32) = -0.41) or IR36 (log2 (IR36_40/IR36_32) = -0.38). However, the gene
347   BGIOSGA005140, which encodes cytokinin oxidase/dehydrogenase, was significantly
348   upregulated in the IR36_32 vs IR36_40 group (log2 fold change = 1.67, *P-value* = 0.004), but was
349   not different in the HHZ_32 vs HHZ_40 group (log2 fold change = 0.86, *P-value* = 0.088). These
350   results are consistent with those of Wu et al. (2016) and suggest that spikelet formation is
351   associated with cytokinin degradation and greater degradation occurs at high temperatures in the
352   heat-susceptible cultivar than in the heat-tolerant cultivar.

353   The DEGs in RHR were enriched in 54 GO terms (Fig. 5). The GO term analysis revealed
354   biological processes promoting resistance to heat stress in the heat-tolerant cultivar HHZ.
355   Downregulation of *BGIOSGA022020* in the heterocycle biosynthetic process induces GRAS
356   protein reduction, which promotes BR synthesis to enhance heat tolerance (Vriet *et al*. 2012). In
357   the molecular function category for RHR, 50 DEGs were involved in DNA-binding transcription
358   factor activity. *BGIOSGA006348* encoded an HSF TF and was upregulated in the HHZ_32 vs
359   HHZ_40 group, although differences were not observed in the IR36_32 vs IR36_40 group. Wang
360   *et al.* (2009) reported that the higher expression of heat shock TFs contributed to high temperature
361   tolerance. WRKY genes encode TFs that play important roles in abiotic stress responses (Chen *et*
362   *al.* 2010), especially to abscisic acid (ABA) (Zhen *et al.* 2005). In this study, six DEGs were
363   WRKY TFs, namely, *BGIOSGA003134*, *BGIOSGA017063*, *BGIOSGA029574*,
364   *BGIOSGA005924*, *BGIOSGA024948*, and *BGIOSGA033505*, which might promote young panicle
365   development associated with sucrose consumption mediated by ABA under high temperature
366   (Feng *et al.* 2018). However, few studies have reported the relationship between the WRKY family
367   and heat resistance, which should be further studied. *BGIOSGA029574* is a general stress-response
368   gene, which has putative functions in distinct cellular processes, such as transcription regulation,
369   stress response, and sugar metabolism under Fe-excess-induced, dark-induced, and drought-
370   induced stress (Ricachenevsky *et al.* 2010). Among the six WRKY genes, BGIOSGA017063 was
371   downregulated while the other five genes were upregulated, although the gene has not been cloned
372   for the gene function analysis and therefore requires further study. Of the 10 DEGs in the ETH
373   family, five genes were downregulated and the downregulation of *BGIOSGA017088* reduced the
374   ABA content and promoted gibberellin (GA) signal transduction, which is beneficial for rice plant
375   growth (Yaish *et al.* 2010). The upregulation of *BGIOSGA006285*, *BGIOSGA010867*,
376   *BGIOSGA030019*, *BGIOSGA005915*, and *BGIOSGA012535* plays an important role in ethylene
377   response regulation. Cao *et al.* (2006) reported that the upregulation of *BGIOSGA005915*
378   enhanced tolerance to salt, cold, drought, and wounding, and the current study reveals that this
379   gene might also contribute to the improvement of high-temperature stress resistance.
380   *BGIOSGA000303* and *BGIOSGA000304* are genes in the cytokinin receptor family, and the
381   upregulation of these two genes promotes cytokinin activation (Ito & Kurata 2006). The MADs
382   box gene is related to flower development (Kobayashi *et al.* 2012) and the upregulation of the
383   MAD genes in RHR indicated that the MAD family might enhance heat stress tolerance. The HZ-
384   ZIP TF family might have a similar function.

385   In the RHR category, the DEGs enriched in the KEGG pathways appear beneficial for heat-
386   stress tolerance, including plant hormone signal transduction and BR biosynthesis. Twenty-one
387   DEGs were involved in plant hormone signal transduction, of which 14 DEGs were upregulated,
388   including the auxin-responsive genes *BGIOSGA024710*, *BGIOSGA001585*, *BGIOSGA019301*,

389   and *BGIOSGA037837*, which facilitate rice plant growth (Hagen & Guilfoyle 2002). In BR

390   biosynthesis, *BGIOSGA002945,* which encodes *D2/CYP90D2*, a gene that catalyzes the steps from

391   6-deoxoteasterone to 3-dehydro-6-deoxoteasterone and from teasterone to 3-dehydroteasterone,

392   was upregulated to promote BR synthesis in the latter pathway (Hong *et al.* 2003), and

393   *BGIOSGA001585* was downregulated to promote BR activity (Sakamoto *et al.* 2011). The genes

394   related to hormone signal transduction and BR biosynthesis might contribute to young panicle

395   development under high temperature. Seven DEGs involved in plant hormone signal transduction

396   were downregulated, and among these, *BGIOSGA036617*, *BGIOSGA034767*, and

397   *BGIOSGA010559* have not been cloned for functional analysis while *BGIOSGA034772* plays a

398   more important role in organismal development. The genes *BGIOSGA024374*, *BGIOSGA023368*,

399   *BGIOSGA000304* and *BGIOSGA005312* are A-type response regulated genes (Jain *et al.* 2006).

400   However, it is unclear whether the downregulation of *BGIOSGA024374* and *BGIOSGA023368*

401   contributes to improved heat tolerance in rice varieties. In addition, the downregulated gene,

402   *BGIOSGA010919*, is an ABA receptor. Tian *et al.* (2015) reported that ABA accumulation

403   upregulates gene expression. In the current study, downregulation of *BGIOSGA010919* may

404   contribute to excessive ABA accumulation. The role of ABA in panicle development requires

405   further study. *BGIOSGA014915*, which participates in BR synthesis, was downregulated in RHR.

406   Previous reports have found that BRs can modulate the metabolic responses of plants to abiotic

407   environmental stresses (Vriet *et al.* 2012; Wang *et al.* 2018). BR accumulation reportedly reduces

408   spikelet degeneration under nitrogen application (Zhang, 2018). *BGIOSGA002945* and

409   *BGIOSGA014915* participate in different BR biosynthesis pathways (Shi, 2015), but

410   *BGIOSGA002945* may play a more important role in modulating spikelet development under high

411   temperature.

412   Carbohydrate storage and utilization are essential for panicle initiation (Tian et al. 2016). The

413   KEGG analysis showed that the phenylpropanoid biosynthesis pathway was commonly

414   overrepresented in HHZ_32 vs HHZ_40, IR36_32 vs IR36_40, and IR36_40 vs HHZ_40. The

415   phenylpropanoid biosynthesis pathway is involved in lignin synthesis, which suggests that high

416   temperature inhibits lignin synthesis; however, phenylpropanoid biosynthesis was not associated

417   with heat tolerance in our heat resistant cultivar (Fig. 6). In the SHR category, seven DEGs were

418   enriched in the starch and sucrose metabolism pathway (Fig.7b). The gene *BGIOSGA031385*,

419   which encodes beta-amylase, was significantly upregulated in IR36_32 vs IR36_40, suggesting

420   that it promoted starch hydrolysis and reduced carbohydrate storage. The genes *BGIOSGA010570*

421   and *BGIOSGA026140*, which encode sucrose synthesis, were significantly downregulated in the

422   IR36_32 vs IR36_40 group, whereas no difference in expression was observed in the HHZ_32 vs

423   HHZ_40 group. Sucrose degrades into uridine 5'-diphosphoglucose and fructose, which are major

424   forms of carbon used for energy. Impairment of sucrose synthase activity reportedly reduced

425   resistance to heat stress (Hirose *et al.* 2008; Takehara *et al.* 2018). The results of the current study

426   suggest that impaired carbohydrate metabolism in the heat-susceptible cultivar aggravated spikelet

427   reduction. The starch and sucrose pathway genes were also highly represented in the CHR group

428   (Fig.7c). Such genes are involved in the downregulation of genes encoding beta-

429   fructofuranosidase, fructokinase, beta-glucosidase, trehalose-6-phosphate phosphatase, alpha-

430   trehalase, and others. Trehalose-6-phosphate synthase, trehalose-6-phosphate phosphatase, and

431   alpha-trehalase are involved in trehalose synthesis. Trehalose plays an important role in abiotic

432   stress resistance, and trehalose-6-phosphate, an intermediate product of trehalose synthesis

433   participates in sucrose signal transduction (Lunn *et al*. 2006; Ruan 2014). Nunes *et al*. (2013)

434   reported that trehalose-6-phosphate served as a sugar signal that could induce the expression of

435   genes associated with the alleviation of abiotic stress injury. In this study, certain DEGs in the

436   CHR group were also upregulated to promote trehalose-6-phosphate synthesis, and the

437   upregulation of *BGIOSGA026976*, *BGIOSGA009181*, and *BGIOSGA030796* promoted trehalose-

438   6-phosphate synthesis in SHR. These findings indicate that trehalose-6-phosphate synthesis may

439   be a normal response of young rice panicles to high temperature and that the heat-sensitive rice

440   cultivar synthesizes trehalose-6-phosphate more readily than the heat-tolerant cultivar in response

441   to heat stress. However, the gene encoding trehalose-6-phosphate phosphatase, *BGIOSGA000509*,

442   was significantly downregulated in IR36 at 40°C compared with that at 32°C, which might cause

443   a decrease in trehalose content and in turn disrupt carbohydrate distribution. Our results suggest

444   that trehalose-6-phosphate metabolism was disordered under the high temperature condition and

445   that the effects were more severe in the heat-susceptible cultivar than in the heat-tolerant cultivar.

446   A close relationship is observed between endogenous hormones and carbohydrate

447   accumulation, which may suggest that the regulation of endogenous hormones in heat-tolerant

448   varieties promotes carbohydrate utilization. The identification of DEGs in this study could improve

449   understanding of the molecular mechanisms of heat resistance in young panicles. In the practice

450   of rice production and breeding, DEGs associated with hormone metabolism in the RHR category

451   and DEGs associated with starch and metabolism in the SHR category under high temperature

452   could be used to quickly identify heat tolerant cultivars.

453

Conclusions

454

455   In summary, heat stress-responsive DEGs in young panicles were identified by a

456   transcriptome analysis of a heat-tolerant rice cultivar and a heat-susceptible rice cultivar grown at

457   high temperature (40°C) and a control temperature (32°C). The statistical analysis of 5,533 DEGs

458   revealed three categories of genes (RHR, SHR, and CHR) containing a total of 4,070 DEGs. We

459   highlighted the differential expression of a group of DNA-binding TFs that was significantly

460 enriched in the RHR category as well as the differential expression of genes involved in the starch
461 and sucrose metabolism pathway that were overrepresented in the SHR category. Overall, DEGs
462 related to plant hormones and signal transduction might be specifically beneficial for young
463 panicle development at high temperature. Heat-tolerant cultivars seem to increase endogenous
464 hormones and maintain a stable carbohydrate metabolism pathway under high temperature.
465 However, certain metabolic pathways, including starch and sucrose metabolism, were much more
466 damaged in the heat susceptible cultivars under high temperatures, and this damage might have
467 inhibited the of panicle development.
468

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622

623   **Figure legends:**

624

625   **Figure 1.** Effects of high temperature on panicle development. (a) young panicle morphologies

626   after 9 d of high-temperature treatment; and (b) panicle morphologies at the heading stage

627   after high-temperature treatment. Bars = 0.5 cm in (a) and 3 cm in (b).

628

629   **Figure 2.** Gene expression in the four comparison groups. (a) HHZ_32 vs HHZ_40, (b) IR36_32

630   vs IR36_40, (c) IR36_40 vs HHZ_40, and (d) IR36_32 vs HHZ_32. Red (upregulated) and

631   blue (downregulated) dots indicate significant differences in gene expression, whereas gray

632   dots represent genes with no significant difference in expression.

633

634   **Figure 3.** Venn diagrams for DEGs in the four comparison groups. (a) HHZ_32 vs HHZ_40, (b)

635   IR36_32 vs IR36_40, (c) IR36_40 vs HHZ_40, and (d) IR36_32 vs HHZ_32.

636

637

638   **Figure 4.** Enriched GO terms ($P<0.05$) of all DEGs. (a) biological process, (b) cellular

639   component, and (c) molecular function.

640

641   **Figure 5.** Enriched GO terms ($P<0.05$) of the DEGs in the RHR and CHR groups. (a) biological

642   process, (b) cellular component, and (c) molecular function.

643

644   **Figure 6.** KEGG enrichment analysis of all DEGs. (a) HHZ_32 vs HHZ_40, (b) IR36_32 vs

645   IR36_40, (c) IR36_40 vs HHZ_40, and (d) IR36_32 vs HHZ_32.

646

647   **Figure 7.** KEGG enrichment analysis for heat stress responsive genes from the three categories.

648   (a) RHR, (b) SHR, and (c) CHR.

649

650   **Figure 8.** Gene expression levels determined by RNA-Seq and qRT-PCR. (a) HHZ_32 vs

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

652

Panicle characters after high temperature treatment

1 **Table 1 Panicle characters after high temperature treatment**

Cultivars	Treatment	Panicle weight(g)	Spikelet number	The number of differentiated spikelet	The proportion of degenerated spikelet (%)	Spikelet fertility (%)	Grain weight(mg)	Glume length (mm)	Glume width (mm)
HHZ	32℃	3.6±0.4	235.0±20.0	335.3±20.5	30.0±1.7	83.0±2.0	18.5±0.3	8.7±0.2	2.5±0.0
	40℃	2.4±0.3**	182.7±11.2*	303.3±10.1	39.7±5.3	78.5±1.3**	17.0±0.3**	7.8±0.1**	2.2±0.1**
IR36	32℃	3.1±0.4	183.3±7.6	264.7±13.8	30.7±1.5	81.5±1.8	20.5±0.3	8.1±0.3	2.5±0.0
	40℃	1.0±0.1**	85.0±13.5**	176.7±17.6**	51.4±9.3**	73.5±1.1**	16.0±0.1**	6.8±0.2**	2.3±0.1**

2 * and ** indicate significance differences between the control (32°C) ang high (°C) temperature treatments (one-tailed Student's t-test): $^*P<0.05$; $^{**}P<0.01$.

Table 2. RNA sequencing results

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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%)

4 Note: HHZ_32: The sample of HHZ treated with 32°C; HHZ_40: The sample of HHZ treated with 40°C; IR36_32:
5 The sample of IR36 treated with 32°C; IR36_40: The sample of IR36 treated with 40°C.
6

Table 3. Classification of three categories of DEGs

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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

5 Note: RHR, heat-resistant-cultivar-related genes; SHR, heat-susceptible-cultivar-related genes;

6 CHR, common heat stress-response genes.

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

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

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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.

(a) young panicle morphologies after 9 d of high-temperature treatment; and (b) panicle morphologies at the heading stage after high-temperature treatment. Bars = 0.5 cm in (a) and 3 cm in (b).
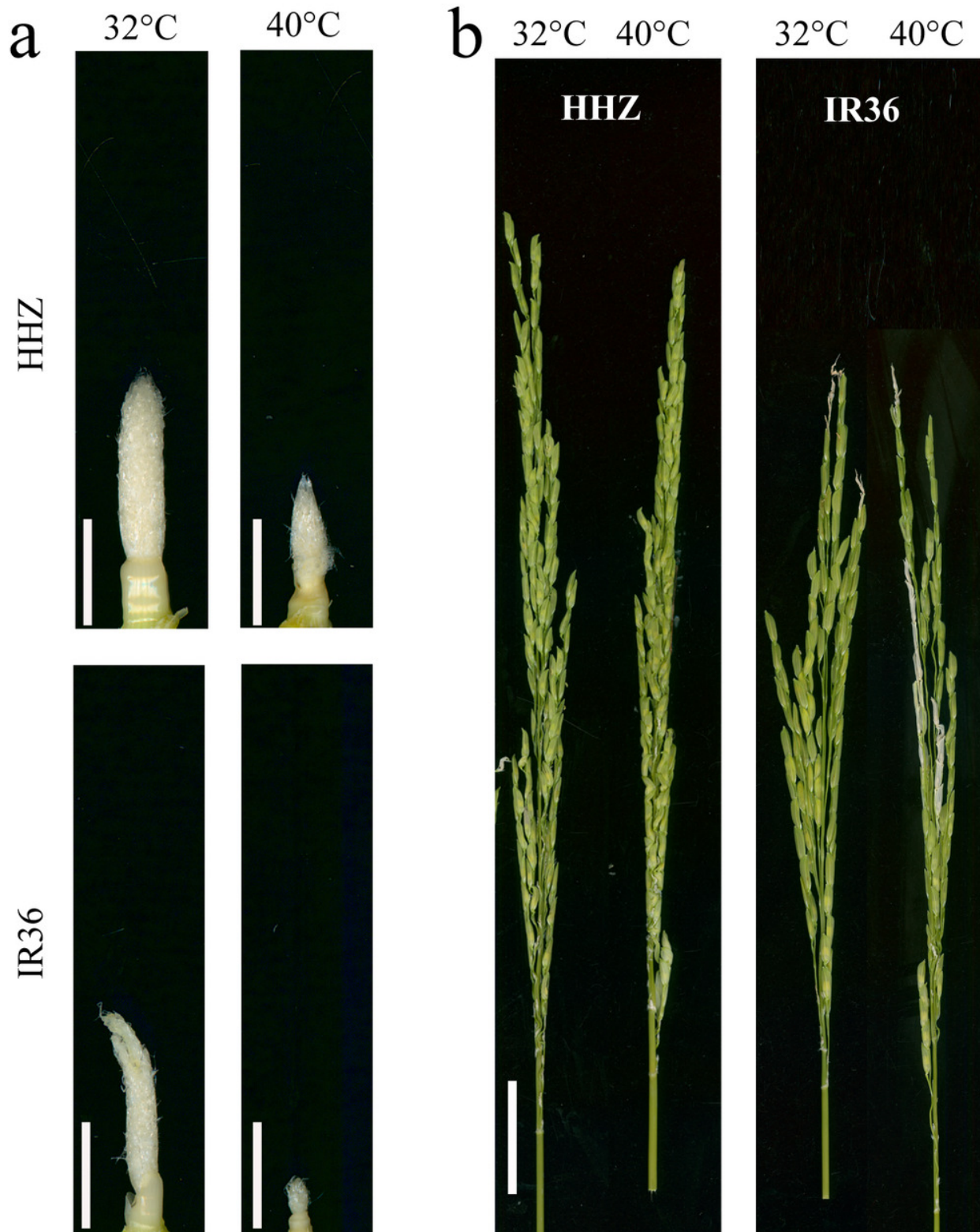
Figure 2

Figure 2. 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 significant differences in gene expression, whereas gray dots represent genes with no significant difference in expression.
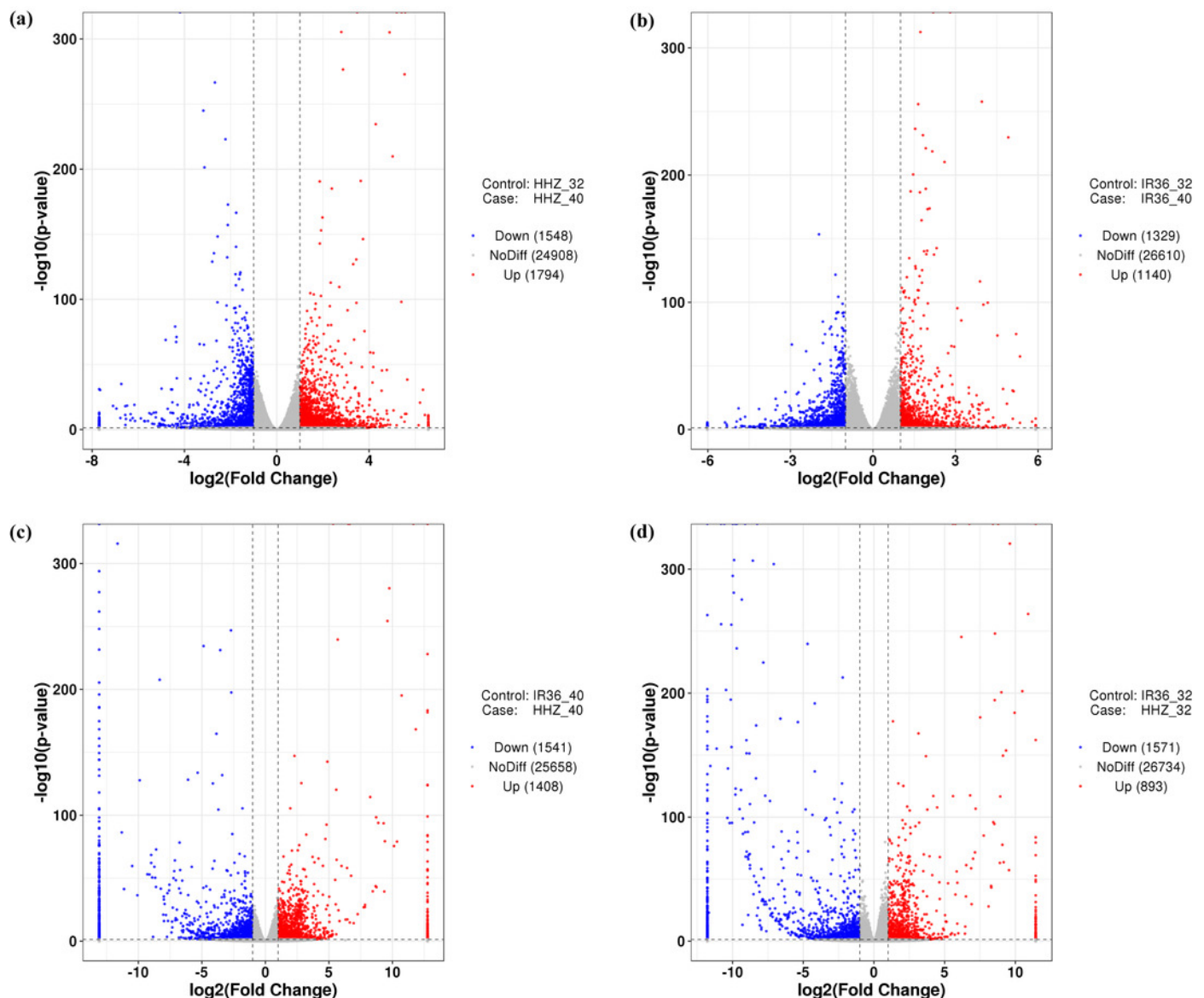
Figure 3

Figure 3. 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. 2.
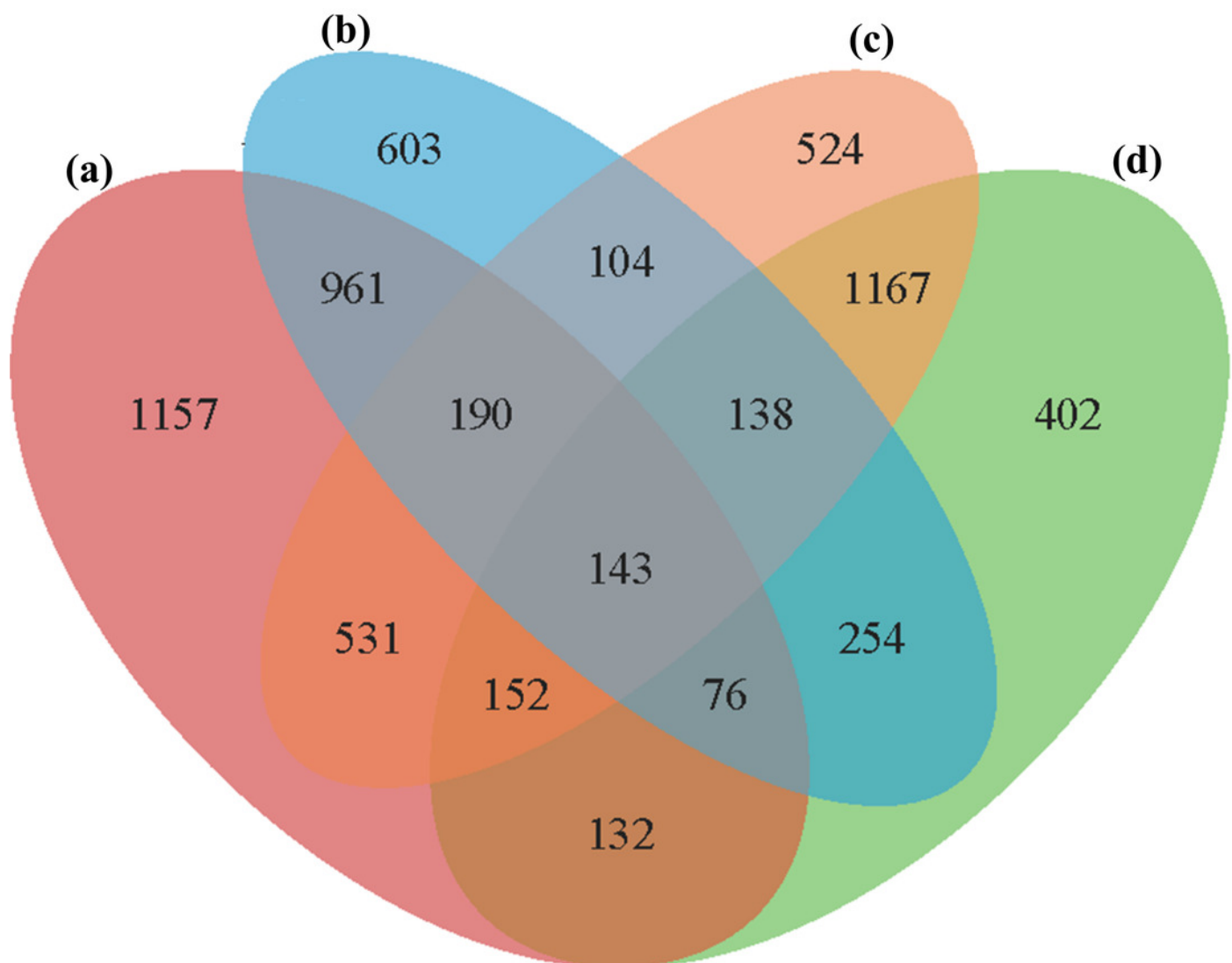
Figure 4

Figure 4. Enriched GO terms (*P*<0.05) of all DEGs.

(a) biological process, (b) cellular component, and (c) molecular function.
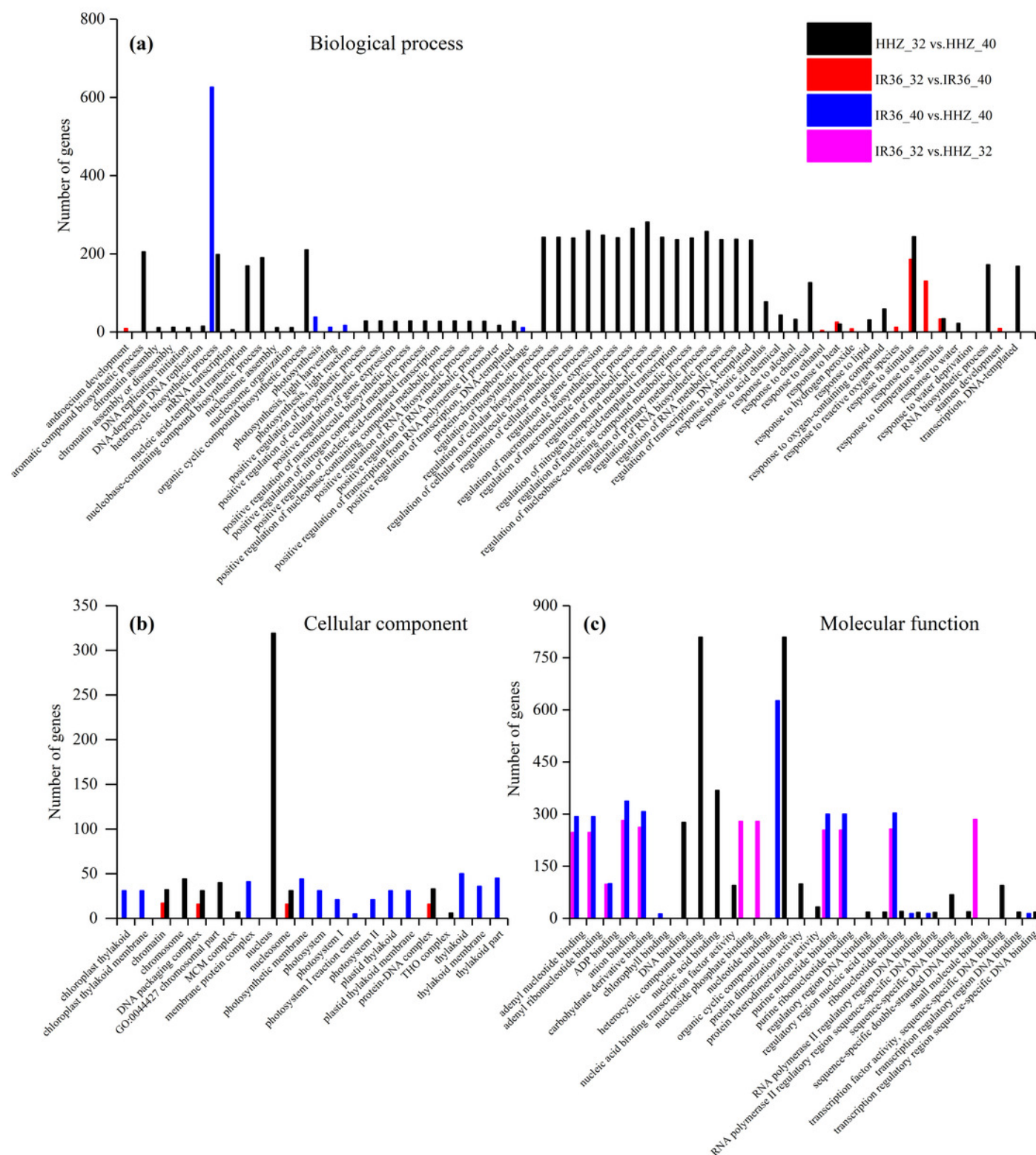
Figure 5

Figure 5. Enriched GO terms (*P*<0.05) of DEGs in RHR and CHR.

(a) biological process, (b) cellular component, and (c) molecular function.

Figure 6

Figure 6. KEGG enrichment analysis of all DEGs.

(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.
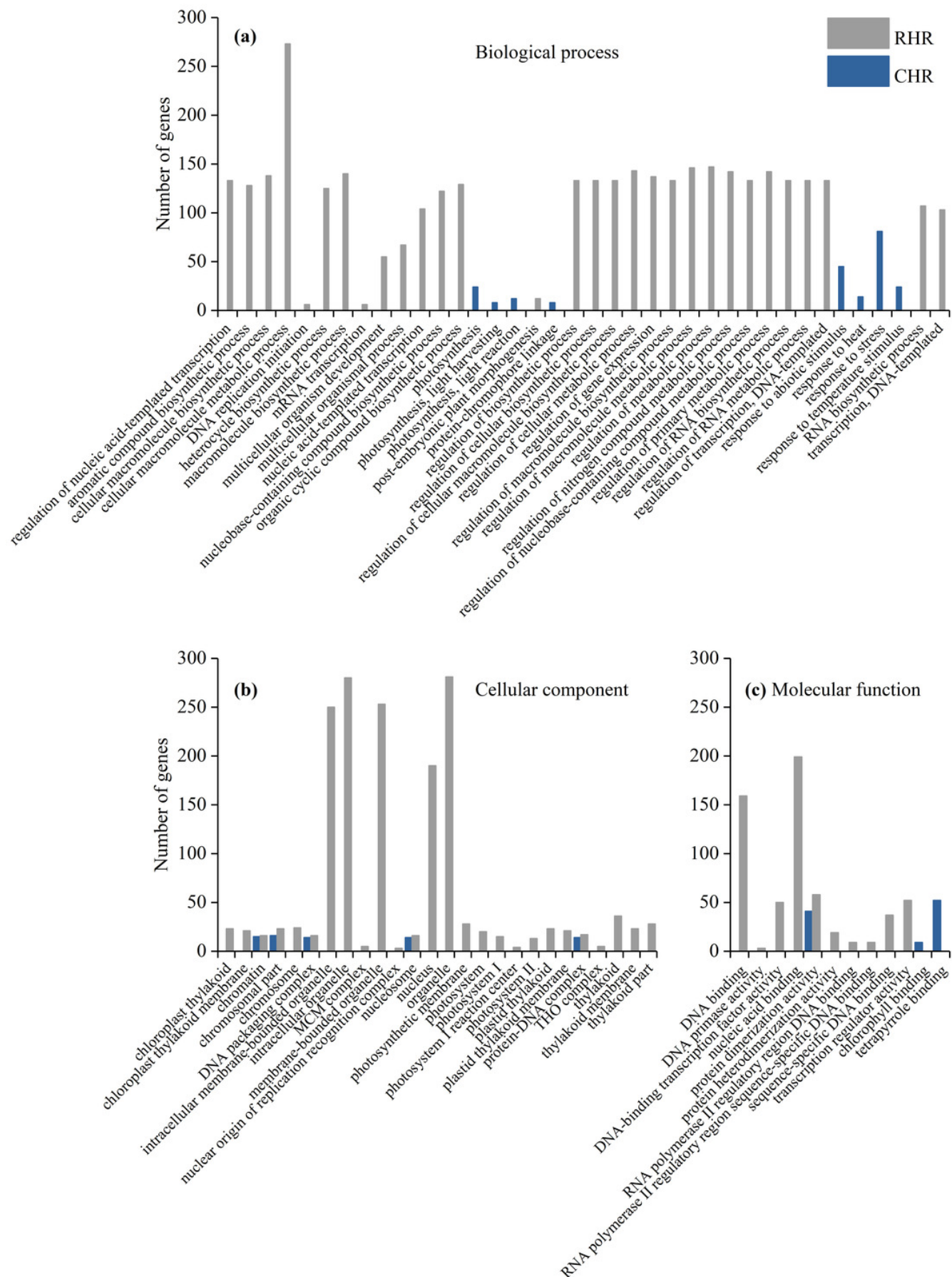
Figure 7

Figure 7. KEGG enrichment analysis for heat stress responsive genes from the three categories.

(a) RHR, (b) SHR, and (c) CHR.
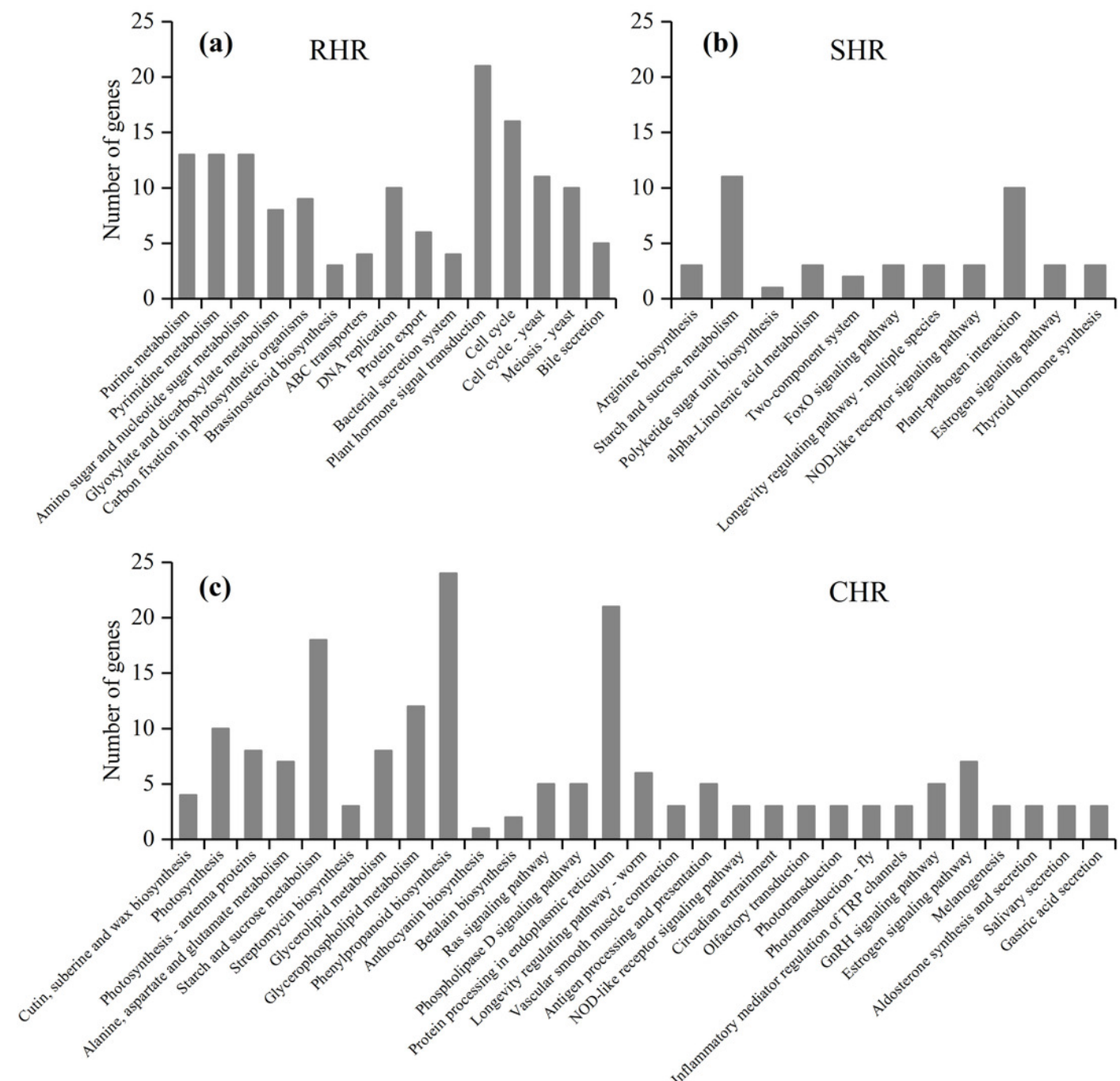
Figure 8

Figure 8. Gene expression levels determined by RNA-Seq and qRT-PCR.

(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.