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Five pectinase gene expressions highly responding to heat stress in rice floral organs revealed by RNA-seq analysis

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ABSTRACT

Heat stress hurts rice, and floral organs are mostly sensitive to heat stress. We aimed to unravel molecular responses to heat stress in rice floral organs using Illumina/Solexa sequencing technology for addressing the increasing concern of globle warming. At meiophase of the pollen mother cell (pulvinus flat), the plants were stressed for 3 d at 38 C, and RNA was extracted from the stressed pistil and stamen for RNA-Seq sequencing to build the heat stress transcriptom library. A total of 7178 defferentially expressed genes (DEGs) between the normal and heat stress libraries were significant, 61% up-regulated and 39% down-regulated. The 7178 DEGs were significantly classified to 34 gene ontology (GO) categories, and 11 of the GO categories were significantly enriched. The GO:0016787 for hydrolase activity of molecular function was mostly enriched with the least probability, and included 11 DEGs named *Hy1* – *Hy11*. Expression levels of five DEGs, *Hy4* – *Hy6* and *Hy9* – *Hy10* for starch and sucrose metablism via pectinase, increased 12 – 14 times in response to the heat stress. Further investigation of the five DEGs for pectin metabolism and association with reported heat responsive genes may help develop a molecular strategy to remedy heat damage in rice.

1. Introduction

Rice is a staple food for nearly half of the world's population, and rice production is directly related to global food security and internationally political stability [1]. With the accelerated processes of global warming due to growing industrialization in recent years, heat stress damage has become one of the major concerns to rice production in the world [2–4]. As a result, a large number of studies have investigated the effects and mechanisms of high temperature stress on rice growth, development, grain yield and quality [3,5,6].

High temperature stress or heat stress is defined as the temperature beyond a cretical threshhold for a peroid of time sufficiently to cause an irreversable damage to plant growth and development [7]. It is well known that both the booting (10 d before heading) and heading (from heading to 10 d after) stages are extremely sensitive to heat stress [8,9]. A direct impact of heat stress during both the stages is on pollen development, pollen germination on stigma, and effective fertilization, which ultimately brings about grainless plants and yieldless crop [10,11]. As a result, heat stress during both the stages is the most damaging stress, and our study aimed to address the heat stress at the booting stage treated with 38 C/30 C (day/night) for 3 days.

Transcriptomics deals with the transcribed regions throughout the genome for various functions of our interest [12,13]. Transcriptomic knowledge is essential to unveil functional elements in the genome and interprete phenotypic variation associated with gene expression. Transcriptome sequencing analysis of RNA based on high-throughput next-generation sequencing technologies (RNA-seq) is a sensitive, effective, and reliable method to study gene expression. RNA-seq technology studys the direct transcript profiling without compromise and potential bias, thus improves the profiling sensitivity and accuracy to resemble the transcriptomes in the biological functions thoughout the genome [14,15]. However, RNA-Seq technology has not been used to specifically analyze floral organs stressed by high temperature in rice.

Abbreviations: CAT, catalase; COG, Clusters of Orthologous Groups; DEGs, differentially expressed genes; FDR, false discovery rate; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PE, pectinesterases; PL, pectinlyase; POD, peroxidase; qRT-PCR, Quantitative Real Time PCR; RHG, rhamnogalacturonases; RNA-seq, RNA sequencing; RPKM, Reads Per Kilo bases per Million reads; SOD, the superoxiode dismutase.

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In this paper, we performed Digital Gene Expression Profiling via Illumina/Solexa sequencing technology to parallelally sequence the floral transcriptomes of a rice line that was heat stressed at the booting stage with the normal check. After both transcriptome libraries of the heat stress and the normal were constructed, we conducted quality assessment of the transcriptome data and analyzed all high-quality clean sequence tags using the analytical model of the reference genome and transcriptome of Nipponbare. Comparative analysis between the two libraries by gene annotation for functional gene ontology (GO) and metabolic pathways (KEGG) resulted in DEGs responding to the heat stress, and verified the differential expressions of the most significant DEGs. The results would help understand the functional dynamics of the transcriptoms responding to heat stress and develop an effective strategy to remedy the damage at the booting stage in rice.

2. Materials and methods

2.1. Material preparation and heat treatment

Oryza sativa L. ssp. *japonica* heat-sensitive cultivar 'Ningjing 4' was used in this study. Ningjing 4 has been widely grown from the middle to lower districts of the Yangz River. Rice plants of Ningjing 4 were grown in 2013 at the experiemntal farm of Anhui Agricultural University. At 10 d before the heat stress at 38 °C was applied, individual plants at similar developmental stage were selected and transplanted to plastic pots sized 40 × 40 × 30 cm^3. At meiophase of the pollen mother cell (pulvinus flat), the plants were moved to an artificial climate chamber for optimal and high temperature treatments for 3d. The optimal chamber (control) was set as day 08:00–18:00, 32 °C and night 18:00–08:00, 25 °C, and the high temperature chamber was set as day 08:00–18:00, 38 °C and night 18:00–08:00, 30 °C. Both chambers had same light intensity 800 umol m^{-2} s^{-1}, and same humidity >75%. Individual stems at same growth stage were marked before treatment. At 3 d after high temperature treatment, the marked panicles were randomly sampled, immediately frozen in liquid nitrogen and consistently stored at −80 °C for the RNA-seq experiments. From our previous experience, 38 °C/30 °C (day/night) for 3 d is a severe stress to cause nearly a complete spikelet fertility.

2.2. RNA extraction from floral organs and RNA quality tests

The RNAprep Pure Plant Total RNA extraction kit purchased from TIANGEN company was used to extract total RNA of floral organs for both control (A) and heat stress treatment (B). Flower organs from five sampled panicles were pooled as a technical replicate for RNA extraction, two replicates for each temperature treatment. The floral organs included only the pistil and stamen after removal of the glumes using a dissecting needle. After the extraction, electrophoresis in 1% agarose gel was used to estimate the purity and integrity of the total RNA, and the Agilent 2100 Bioanalyzer was used to measure RNA concentration and the A260/OD280, A260/OD230 ratios. The resultant RNA samples were then used for RNA-seq and qRT-PCR analysis.

2.3. RNA-seq library construction and sequencing

cDNA libraries for RNA-Seq sequencing were prepared with the mRNA-Seq Sample Preparation Kit (Illumina) according to the protocol of the manufacturer, and sequencing of the RNA-Seq libraries was performed by Beijing Genomics Institute (BGI) using the 10G Illumina Genome Analyzer. The Illumina Genome Analyzer system was used to statisticaly analyze the transcriptome sequencing results of RNA isolated from floral organs under normal (A) and heat stress (B) treatments comparatively.

2.4. Sequencing assessment and assembly of raw data

The short read alignment software SOAPaligner/soap was used to map the clean reads of both parallele libraries to Nopponbare reference genome and reference gene sequences for evaluating the relative randomness of the read distribution in genes. The Illumina paired-end (PE) sequencing method was used for the rice transcriptome, and SOAPdenovo software was used to assemble the original sequences *de novo*.

2.5. Screening and significant test for differentially-expressed genes (DEGs)

Differential expression analysis was used to identify the DEGs between the normal (A) and heat stress (B) transcriptome libraries. Using the method of digital gene expression profiling published by Audic and Claverie (1997) [16] and DESeq software, the DEGs between the two libraries in response to the heat stress were screened in accordance with strict algorithms. A random sampling model for hypothesis testing was then used to analyze the significance of differential expression genes. FDR $\leq$ 0.01 and |log2 Ratio| $\geq$1 for *p*-values of the DEGs were used as the thresholds to claim the statistical significance of differential gene expression between the two libraries.

2.6. Gene ontology (GO) and KEGG pathway enrichment analysis of the DEGs

GO functional enrichment analysis for the statisticaly siginificant DEGs was carried out using Blast2GO (version 2.3.5) (http://www.blast2go.org/). Analyses of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for the DEGs were performed using Cytoscape software (version 2.6.2) (http://www.cytoscape.org/) with the Clue GO plugin (http://www.ici.upmc.fr/cluego/cluegoDownload.shtml) [17].

2.7. Fluorescent quantitative PCR analysis

Using the total RNA from rice floral organs of both the normal (A) and heat stress (B) treatments, the first-strand cDNA was synthesized using the PrimeScript RT reagent Kit (Perfect real time) (TaKaRa Company). The UltraSYBR Mixture (with ROX) kit (CWBIO company) was used for qRT-PCR assays on a quantitative PCR instrument (Bio-Rad) based on the fluorescence of SYBR green I dye. The 18 S rDNA gene that is stably expressed in rice was used as an internal expression control, and relative quantification by the $2^{-\Delta\Delta C}$ method was used for analysis. Three biological replicates were performed for the qRT-PCR assays, and standard deviation of gene expression was estimated for each of the following five DEGs for starch and sucrose metablism via pectinase. Primer 3.0 software was used to design the primer sequences for the five DEGs in the qRT-PCR assays: *Hy4* as F:5′-GACCTGCAGTGTTCGATT-3′ and R:5′-ACCGATCAATTGGAGCTC-3′, *Hy5* as F:5′-CAAGGCAACATCCATC-3′ and R:5′-GCACCAAACAAAGCAA-3′, *Hy6* as F:5′-ACTCAGGGTCTTCC-3′ and R:5′-AGGTTGACTACAAGTTTCC-3′, *Hy9* as F:5′-ATTC-TATCGTCGCCAC-3′ and R:5′-GCATACATACGAATCAAAT-3′, and *Hy10* as F:5′-CCCTTCTTCTGCTGAGACA-3′ and R:5′-CACTACTCATTGGGCT CAT-3′.

3. Results

3.1. Normal rice had more sequencing reads but less nucleotides than the heat stressed rice

After low-quality sequences including impurities and 3′ linker sequences were removed from the raw sequence reads, the normal library (A) contained 660,800 or 4.2% more clean reads than the heat stress library (B) (Table 1). However, the data size was smaller in A library than it in B library, in which A had 14,541,433 or 0.8% less nucleotides than B. The average read length was ~130 bp, and the Q20 ratio (sequencing error rate <1%) of sequences was 100% in the two libraries. The data size and Q20 ratio demonstrated a high quality of sequencing data in both A and B libraries with a completion transcriptome sequencing.

3.2. Normal rice had more mapped reads but less multi-position mapped reads than the heat stressed rice

After completly mapping the sequencing reads to the reference genome of Nipponbare, the totally mapped reads were more in the normal libarary (A) than in the heat stress library (B), where A had 664,021 or 4.3% more totally mapped reads than B (Table 2). Similarly, the uniquely mapped reads were more in the normal library (A) than in the heat stress library (B), where A had 688,522 or 4.6% more uniquely mapped reads than B. Uniquely Mapped Reads refers to the number of reads that had no mismatches or InDels. Adversely, the multi-position mapped reads were less in the normal library (A) than in the heat stress library (B), where A had 24,501 or 4.5% less multi-position mapped reads than B. These results demonstrated a higher level of homology to Nipponbare genome in the normal library (A) than in the heat stress library (B).

3.3. Normal rice had less transcripts than the heat stressed rice

Coverage analysis of cDNAs transcriptome sequencing resulted in a total of 28,234 transcripts in the normal RNA-seq library (A) that had 2.36 less transcripts than the heat stress library (B) (Fig. 1). The transcript differences between A and B were the most for 90–100% coverage where A had 1584 less transcripts than B, followed by 80–90% coverage where A had 590 less transcripts than B, 0–10% coverage where A had 212 less transcripts than B, and 70–80% coverage where A had 122 less transcripts than B. Adversely, A had 162 more transcripts than B for both 30–40% and 50–60% coverages, respectively.

3.4. There were 7178 differentially expressed genes (DEGs) responding to heat stress in the rice floral organs

The values of the Reads Per Kilo bases per Million reads (RPKM) for differentially expressed genes (DEGs) in the normal library (A) and heat stress library (B) passed the repeating correlation detection (Fig. 2), which sufficiently validated the subsequent analysis of DEGs. Using DESeq software [26] to inspect the scatter plots of all the genes expressed in the rice floral organs of A and B identified a total of 7178 DEGs. The differential expressions due to heat stress treatment between A and B for the identified genes were highly significant at a probability of p_value $\leq$0.001, FDR (false discovery rate) $\leq$0.001, and corrected value (Q_value) $\leq$0.01, |Log2 (A/B)|$\geq$1. Among those significant DEGs, 4355 or 61% of DEGs showed up-regulated expressions, while other 2823 or 39% of DEGs showed down-regulated expression.

Table 1
Summary of Illumina sequence assembly data on rice floral organ in normal (A) and heat stress (B) libraries.

Library	ReadsNum	Nucleotides	Cycle Q20(%)
A	16,352,564	1,909,977,256	100
B	15,691,764	1,924,518,689	100

Table 2
Statistics for Illumina RNA-seq reads of the floral organs between the normal (A) and heat stress (B) library mapped to the reference genome of Nipponbare.

	A		B	
Totally mapped reads	16,261,370	99.44%	15,597,349	99.40%
Uniquely mapped reads	15,737,556	96.24%	15,049,034	95.90%
Multi-position mapped reads	523,814	3.20%	548,315	3.49%
Total unmapped reads	911,94	0.56%	944,15	0.60%

3.5. Functional classification and gene ontology enrichment analysis of the DEGs

The database for Clusters of Orthologous Groups (COG) of proteins contains various groups of proteins from the genomes of bacteria, algae, and eukaryotes that share common evolutionary histories. Gene ontology (GO) deals with the orthologs and consistently describes gene products across various species. Functional classification annotation and functional significant enrichment analysis for blastx (e-value $\leq$ le-5) alignments via the GO database (http://www.geneontology.org/) classified the 7178 DEGs responding to heat stress into 34 GO categories (Fig. 3).

The 34 GO categories belonged to three fuctional domains, biological process domain with 16 including 2906 DEGs, cellular component domain with 9 including 3368 DEGs and molecular function domain with 9 including 904 DEGs (Fig. 3). In the biological process domain, a great majority of DEGs (44.6%) were involved in metabolic processes category, followed by cellular processes (32.2%) and stress process in response to stimulus (10.9%). In the cellular component domain, those DEGs were mainly involved in functions of the cell (45.6%), cell part (33.7%), and organelles (15.3%). In the molecular function domain, the DEGs were mainly involved in functions of the binding (42.2%) and catalytic activity (39%). These GO mapping results indicated that the majority of DEGs responding to heat stress were involved in metabolic processes, cells and catalytic activity, and enzymatic activity. These affected activities suggest that heat stress treatment mainly brought about functional changes in physiological metabolism and cell differentiation.

Based on mapping the DEGs to each GO category, a hypergeometric test was used to compare the DEGs with the whole genome background for identification of significantly enriched GO categories. The test resulted in 11 GO categories which DEGs satisfied the corrected P-value <0.05 and error rate FDR <0.01, showing that those DEGs were significantly enriched for their specific biological functions (Table 3). The 11 significantly enriched GO categories belonged to three functional classes, most in biological process with 5, followed by cellular component with 4, and molecular function with 2. Based on the Corrected-P value, top five of the enriched GO categories were more significant than the others, and their fuctional descriptions were hydrolase activity, catalytic activity, extracellular region, external encapsulating structure, and cell wall. Among the top five enriched GO categories, the hydrolase activity of molecular function with GO ID of GO:0016787 was mostly significant at much lower probability than any others.

The mostly significant hydrolase activity annotated by the GO enrichment category (GO: 0016787) is widely found in higher plants, and its function is closely related to the growth and

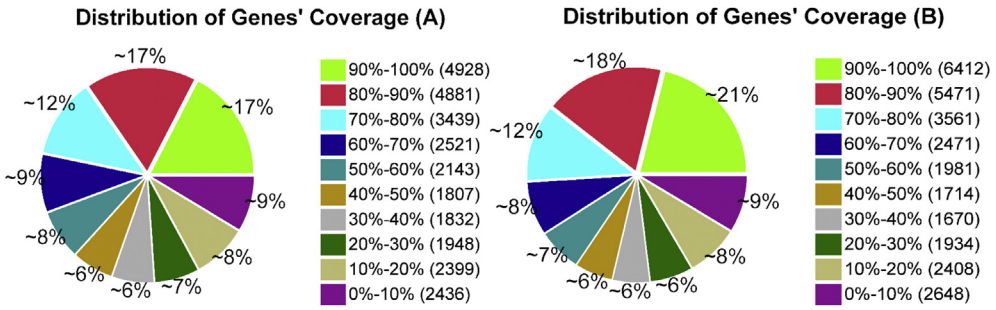

Fig. 1. Relative distribution of gene coverages between the normal (A) and heat stress (B) RNA-seq libraries of rice floral organs.

development of plants and plant aging [18]. Analysis of the GO: 0016787 resulted in 11 hydrolase activity DEGs (Table 4).

The alignment and analysis of the 11 DEGs in the GO: 0016787 category failed to result in GO familiar names, so that we named the 11 DEGs as *Hy1* - *Hy11* (Table 4). The 11 DEGs were all up-regulated. *Hy1, Hy2* and *Hy5* has the same functional description, invertase/pectin methylesterase inhibitor family protein. Both *Hy6* and *Hy9* had the same function as pectinase. Four: *Hy4, Hy7, Hy8* and *Hy10* were pectinesterase with slightly different functions. The remaining two functioned individually, *Hy3* as glyceryl phosphoryl choline phosphodiesterase family protein and *Hy11* as pheophorbidase.

3.6. Pathway enrichment analysis of DEGs, five pentinase DEGs expressed 12–14 times more to heat stress

Pathway enrichment analysis determines the main biochemical metabolic pathways and signal transduction pathways in which the DEGS are involved. Using a probability of 0.05 for both the *P* and *Q* values, 14 pathways were found to be significantly enriched in response to heat stress treatment (Table 5). In the 14 significant pathways, a total of 1753 DEGs were pathway-annotated. The ko04626 for Plant-pathogen interaction had the most DEGs as 492, followed by ko04075 for Plant honene signal transduction as 257, ko00500 for Starch and sucrose metabolism as 170, ko00230 for Purine metabolism as 162, and ko00940 for Phenylpropanoid biosynthesis as 124. The numbers of DEGs were less than 100 for the rest of metabolic pathways.

The metabolism of starch and sucrose is cretical for pollen development [19,20]. Thus, we investigated five DEGs that were significantly enriched for starch and sucrose metabolism pathways via pectinase using real-time fluorescent quantitative PCR. Theese DEGs were on three chromosomes (Chr): *Hy4* on Chr 3, *Hy5* and *Hy6* on Chr 4, and both *Hy9* and *Hy10* on Chr 11. When the gene expressions of the five DEGs were uniformly adjusted to 1 in the normal floral organs (A), the expressions of *Hy4, Hy6* and *Hy9* were over 15, *Hy5* over 14 and *Hy10* over 13 in the heat stressed floral organs (B) (Fig. 4)). In another word, the five DEGs in rice floral organs expressed over 13–15 times of the normal in response to the heat stress.

4. Discussion

4.1. Pectin metabolism is important for plants to respond to heat stress at booting stage

Fig. 4 shows that the expression levels of five differentially expressed genes (DEG *Hy4, Hy5, Hy6, Hy9* and *Hy10*) increased up to 12 – 14 times in response to heat stress. Both DEG *Hy6* and *Hy9* functioned as pectinas, *Hy4* as pectinesterase 67-like protein, *Hy10* as pectinesterase family protein and *Hy5* as invertase/pectin methylesterase inhibitor family proteins (Table 4). All the five DEGs were involved in starch and sucrose metabolism (Table 5) from hydrolase GO:0016787 which was mostly significantly enriched among 34 GO categories of 7178 DEGs identified in this study (Table 3 and Fig. 3). These results suggest an essential role of pectin metabolism in rice floral organs under heat stress conditions.

Pollen tube cell wall consists of an inner sheet including both callose and cellulose and covered by an outer fibrillar layer largely composed of pectin [21]. The apical pollen tube wall is almost exclusively composed of a single layer of pectin, suggesting that

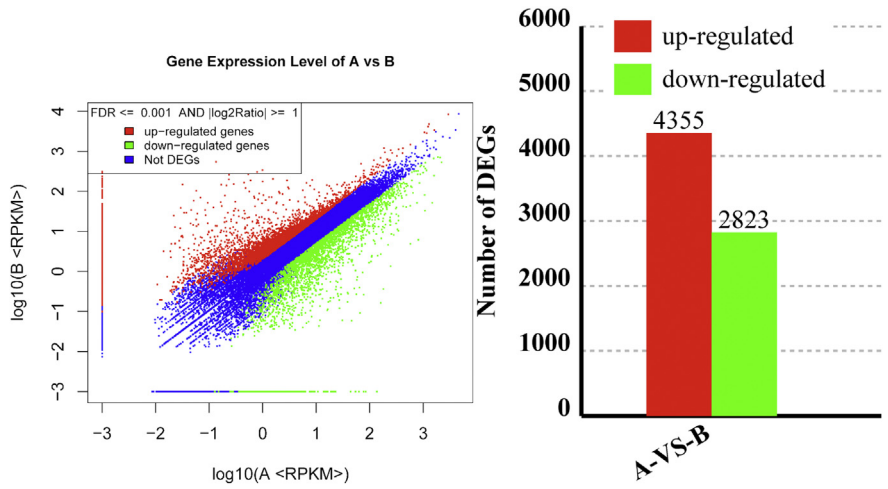

Fig. 2. Scatter plots of differentially expressed genes significant at a probability of $P \leq 0.001$ between RNA-seq normal (A) and heat stress (B) libraries in floral organs.

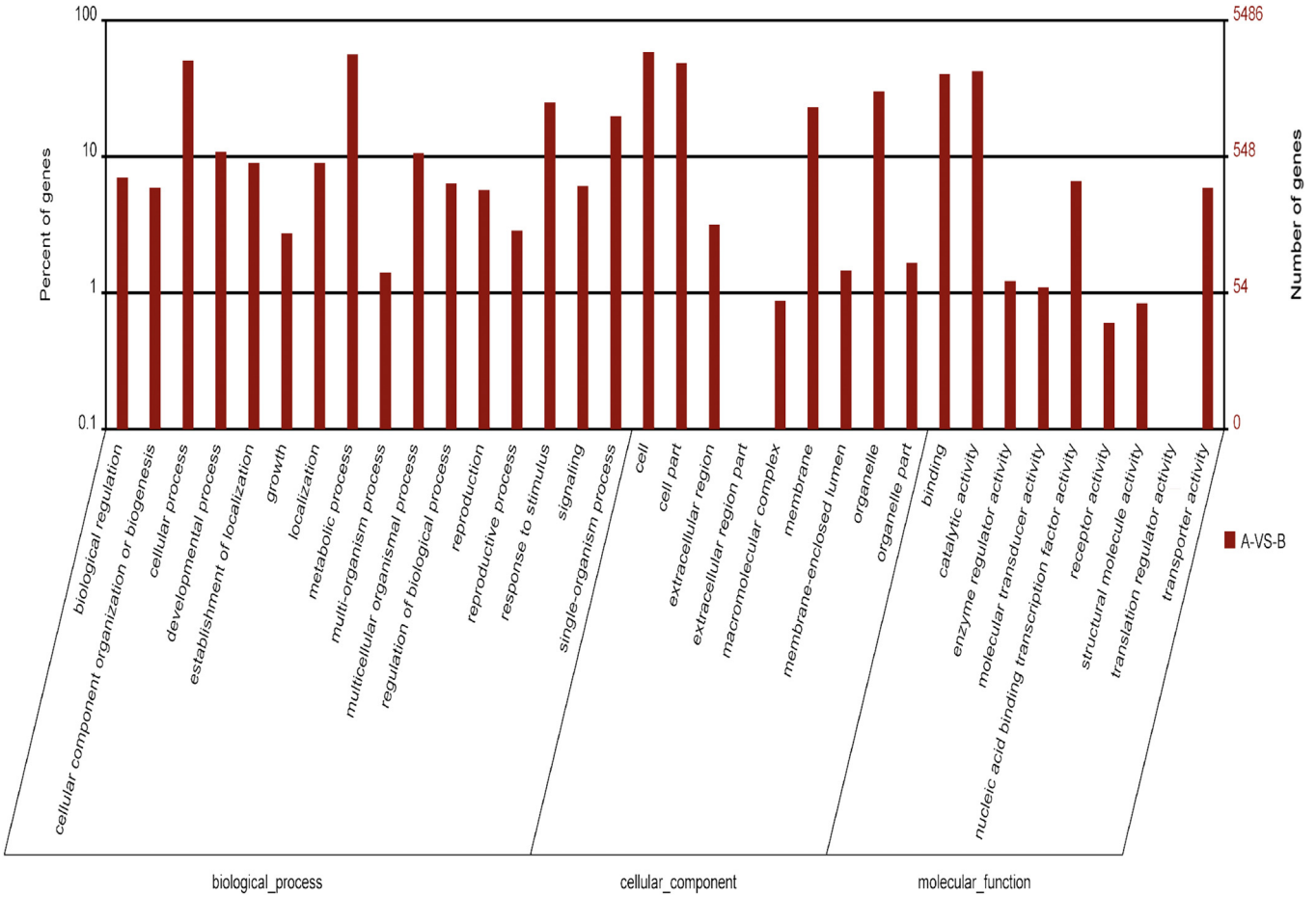

Fig. 3. Gene Ontology classification of 7178 differentially expressed genes (DEGs) significant between the normal (A) and heat stress (B) RNA-seq libraries in rice floral organs.

Table 3
Significantly enriched GO categories from the analysis of DEGs responding to heat stress at heading stage of rice.

GO ID	GO category	Description	Corrected-P value
GO:0016787	Molecular Function	hydrolase activity	1.28e-10
GO:0003824	Molecular Function	catalytic activity	1.98e-03
GO:0005576	Cellular Component	extracellular region	1.16e-03
GO:0030312	Cellular Component	external encapsulating structure	2.44e-03
GO:0005618	Cellular Component	cell wall	4.67e-03
GO:0006950	Biological Process	response to stress	0.0049
GO:0005975	Biological Process	carbohydrate metabolic process	0.0136
GO:0071944	Cellular Component	cell periphery	0.0206
GO:0050896	Biological Process	response to stimulus	0.0606
GO:0008219	Biological Process	cell death	0.1256
GO:0016265	Biological Process	death	0.1256

Table 4
Eleven hydrolase activity DEGs for GO enrichment category: 0016787 responding to heat stress at heading stage of rice.

Gene name	GI accession	Functional description
Hy1	LOC_Os01g14940	invertase/pectin methylesterase inhibitor family protein
Hy2	LOC_Os01g50810	invertase/pectin methylesterase inhibitor family protein
Hy3	LOC_Os02g09450	glyceryl phosphoryl choline phosphodiesterase family protein
Hy4	LOC_Os03g19610	pectinesterase 67-like protein
Hy5	LOC_Os04g38560	invertase/pectin methylesterase inhibitor family proteins
Hy6	LOC_Os04g54850	pectinase
Hy7	LOC_Os05g20570	pectinesterase inhibitor domain containing protein
Hy8	LOC_Os11g08760	pectinesterase family protein invertase/pectin methylesterase inhibitor family proteins
Hy9	LOC_Os11g45730	pectinase
Hy10	LOC_Os11g45720	Pectinesterase family protein
Hy11	LOC_Os12g02510	pheophorbidase

Table 5
Metabolic pathways in which DEGs were significantly enriched at Q value < 0.05 responding to heat stress in rice floral organs at heading stage.

Metabolic pathway	DEGs with pathway annotation	All genes with pathway annotation	P-value	Q_value	Pathway identity
Plant-pathogen interaction	492	1823	4.91E-24	6.28E-22	ko04626
Starch and sucrose metabolism	170	617	1.04E-10	4.42E-09	ko00500
Phenylalanine metabolism	80	225	1.53E-09	9.79E-08	ko00360
Amino sugar and nucleotide sugar metabolism	82	243	1.64E-05	5.24E-05	ko00520
Phenylpropanoid biosynthesis	124	449	1.59E-04	4.07E-04	ko00940
Carotenoid biosynthesis	65	203	6.62E-04	1.11E-03	ko00906
Plant honene signal transduction	257	1160	7.21E-04	1.32E-02	ko04075
ABC transporters	59	208	8.16E-04	1.69E-02	ko02010
Limonene and pinene degradation	61	217	1.19E-03	3.69E-02	ko00903
Other glycan degradation	44	152	2.02E-03	6.42E-02	ko00511
Purine metabolism	162	718	2.37E-03	7.41E-03	ko00230
Cutin, suberine and wax biosynthesis	46	163	2.91E-2	1.28E-2	ko00073
Ascorbate and aldarate metabolism	31	99	3.52E-2	2.79E-02	ko00053
Stilbenoid,diarylheptanoid and gingerol biosynthesis	80	321	4.32E-2	3.993-02	ko00945

pectin metabolism plays a central role in pollen tube development and growth [22,23]. Our expression study on the DEGs in response to heat stress used the floral organs including the pistil and stamen. The highly responsive DEGs all being pectinases suggests that the pectin metabolism may be highly responsive to heat stress at heading stage, and grain fertility damaged by the heat stress may be caused by the responsive pectin metabolism.

Pectin, a polysaccharide polymer of galacturonic acid with different degrees of esterification via an α-1, 4-glycosidic bond, is primarily deposited in the cell wall and cell interlayer in almost all the higher plants [22]. Pectinases are all the enzymes invloved in the pectin metabolism, including pectinhydrolases, pectin methylesterases (PME), pectin lyases (PL), pectin esterases (PE), and protopectinases [24,25]. Pectinases are endogenously regulated by pectin content in plant cell walls and cell interlayer to hydrolyze the glycosidic bond of poly-lactobionic acid for cell separation and organ abscission [22].Zhang et al. (2012a) [26] analyzed two PME genes from Arabidopsis, *At2g47550* and *At4g02330*. *At2g47550* is mainly expressed in pollen grains and microtubule tissues for development of pollen grains and pollen tubes, while *At4g02330* was expressed in abscission zone, stigmatic surface, pollen grains and microtubule tissues for pectin degradation in cell walls to cause cell separation and floral organ abscission. However, none of the PME genes have been isolated from rice, and the study of the rice PME genes would be important to clarify the molecular mechanism of grain fertility in response to heat stress in rice.

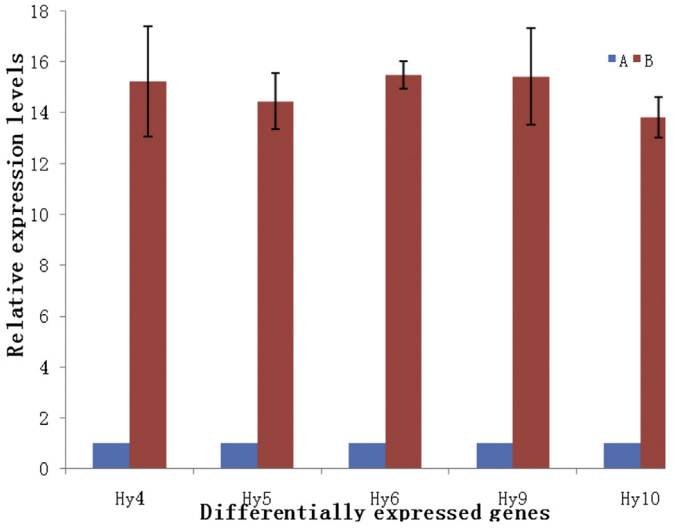

Fig. 4. Expressions of five differentially expressed pectinase genes (DEG) between the normal (A) and heat stressed (B) floral organs revealed by real-time fluorescent quantitative PCR.

Heat stress universally induces the synthesis of heat shock proteins (HSP) in living things [3]. HSPs act as molecular chaperons to remedy the proteins damaged by heat stress and protect the cellular tissues from harmful effects [27]. Zhang et al. (2012b) [28] identified 2449 heat responsive (HR) genes during heat stress of 40 C at the time point of 20 min, 60 min, 2 h, 4 h and 8 h using microarray technology. Most of those HRs function as transcription factors belonging to heat shock factor (*Hsf*) families, such as *HsfA* sub-family and *HsfB* sub-family.

According to the definition of previous studies, *Hy4, Hy5, Hy6, Hy9* and *Hy10* should be similar to the heat responsive genes in Zhang et al. (2012b) [28] study, and their translational proteins should be similar to the heat shock proteins in Zou et al. (2011) [3] study. All the five DEGs identified in our study were pectinases, implying that all their resultant heat shock proteins are associated with pectin metabolism. As the heat shock proteins function as a protective mechanism for plants during heat stress [3], biological functions of the pectin proteins resulting from *Hy4, Hy5, Hy6, Hy9* and *Hy10* are worth of further investigation.

There are 25 heat shock factor (*Hsf*) members in rice genome, 21 *Hsf* in rice microarray, and they are divided three conserved classes: A, B and C [3,29]. The relationship of the *Hy4, Hy5, Hy6, Hy9* and *Hy10* identified by RNA-seq technology in our study with the *Hsf* families is worth of further investigation. The further investigations would help clarify the molecular mechaism for rice to react to heat stress, so that an effective strategy could be developed to remedy heat damage for meeting with the global warming threat.

4.2. Functional coincidences between RNA-seq profiling and microarray profiling

Our RNA-seq prifiling of rice floral organs stressed with 38 C for 3 d resulted in 7178 differentially expressed genes (DEG) belonging to 34 functional gene ontology (GO) categories in three domains, 16 in biological process, and 9 each in cellular component and molecular function (Fig. 3). Top three GO categories with the most DEGs in the biological process domain were cellular process, metabolic process and response to stimulus; top three GO categories in the cellular component domain were cell, cell part and organelle; and top two GO categories in the molecular function domain were binding and catalytic activity.

The coincidence adds the reliability of differentially expressed gene analysis for heat stress using either RNA-seq prifiling or microarray profiling at the booting stage of rice, and provides cross

support for those dominating GO categories to play dominant roles in rice to react under heat stress at the booting stage. Therefore, those dominating GO categories should receive dominant attentions in the molecular study of heat stress during productive stage of rice.

Conflict of interest

Liquan Wu, Taohua Zhou, Wenbin Gui, Lisen Xu, Juan Li and YanFeng Ding declare that they have no competing interests.

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