BMC Genomics

RESEARCH

Open Access

Dissection of genetic basis underlying heat stress response of *Apis cerana*

Ao-Mei Li[1,2,3†], Chun-Xiu Pang[2†], Xiao-Lin Cai[2], Fu-Chang Zhuo[2], Bo Hu[2], Xue-Hua Huang[1], Jia-Xiang Huang[4*] and Yu-Ming Lu[1*]

Abstract

The honeybee *Apis cerana* as an important pollinator contributes significantly to ecological diversity. In recent years, it has been used as a common pollinator in greenhouses, but it is highly susceptible to heat stress, which affects its behavior, physiology, survival, and gene expression. Here, we conducted transcriptomic analysis to identify differentially expressed genes (DEGs) and reveal the associated biological processes in the queen head and ovary of honeybee *A. cerana* under different temperatures. Differential expression analysis revealed 116 DEGs (72 upregulated, 44 downregulated) in the head and 106 DEGs (78 upregulated, 28 downregulated) in the ovary after 24 h of heat stress. At 96 h, 29 DEGs (17 upregulated, 12 downregulated) were identified in the head, and 44 DEGs (34 upregulated, 10 downregulated) in the ovary. After 168 h, the number of DEGs increased significantly: 846 DEGs (567 upregulated, 279 downregulated) in the head, 479 DEGs (296 upregulated, 183 downregulated) in the ovary, and 582 DEGs (338 upregulated, 244 downregulated) in the thorax. DEGs associated with metabolic processes, signaling, and transport pathways were significantly altered under heat stress, potentially contributing to the reduced reproductive and growth capacity of bees. Additionally, genes related to antioxidant activity, nutrient metabolism, heat shock proteins, zinc finger proteins, and serine/threonine-protein kinases were differentially expressed across treatments. Overall, the head and ovaries of honeybee queens show a significant response to heat shock, and these responses are related to antioxidant genes, heat shock proteins, and metabolic regulation, our findings provide genetic information for the breeding of heat-resistant bee strains.

Keywords Heat stress, *Apis cerana*, Antioxidant activity, Transcriptomic, DEGs

Introduction

Globally, nearly 75% of key crops depend on animal pollinators, with bees recognized as the most significant insect pollinators. The annual economic contribution of bee pollination to global crop production surpasses $200 billion. Crops relying heavily on bee pollination contribute roughly 35% of the essential nutrients in the global human diet [1–3]. The Chinese honeybee (*Apis cerana*, commonly known as "Zhongfeng") is an indigenous species in China that plays a crucial role in maintaining ecological balance and enhancing agricultural productivity. In recent years, the application of Chinese honeybees in pollinating economically important crops

†Ao-Mei Li and Chun-Xiu Pang contributed equally to this work.

*Correspondence:
Jia-Xiang Huang
huangjiaxing@caas.cn
Yu-Ming Lu
GxaasLym@163.com
[1]Guangxi Academy of Agricultural Sciences, Nanning 530007, China
[2]Horticultural Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China
[3]Guangxi Key Laboratory of Sugarcane Genetic Improvement, Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China
[4]Key Laboratory for Insect-Pollinator Biology of the Ministry of Agriculture, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing 100093, China

within protected cultivation systems has gained increasing attention. However, the elevated temperatures in such environments pose significant challenges to bee health and pollination efficiency. Understanding the molecular mechanisms underlying the effects of heat stress on key organs of queen bees is therefore of great significance for ensuring the sustainability of bee-mediated pollination services.

Temperature is one of the abiotic factors that affects insects [4–10]. Given that climate change may affect the distribution of organisms [11]. Insects exposure to high temperatures during key developmental stages can lead to reduced reproductive output and morphological deformities [12, 13]. Honeybees, as crucial insect pollinators, are particularly susceptible to temperature fluctuations, which directly affect their reproductive capacity and colony health [14, 15]. The optimal temperature range for honeybee reproduction is between 15 ℃ and 25 ℃, beyond which the queen' s egg-laying rate declines significantly or ceases, leading to a shortage of new bees and subsequent disruptions in colony growth and foraging efficiency [16, 17]. Furthermore, elevated temperatures during larval and pupal development can result in reduced body size and structural deformities, impairing the bees' ability to forage and pollinate effectively [12, 13]. Under extreme heat conditions, the colony's thermoregulation capacity is compromised, leading to incomplete egg hatching and an increased incidence of diseases such as chronic paralysis, larval infections, septicemia, and wing deformities [16, 18–21]. During periods of intense summer heat, often accompanied by drought, nectar and pollen shortages become prevalent, further exacerbating colony stress. In large-scale apiaries, high colony densities lead to increased food consumption, raising the risk of honey starvation and cessation of egg-laying due to resource scarcity [22–24]. These challenges highlight the investigation of honeybee thermal adaptation.

Heat stress induces the excessive accumulation of reactive oxygen species (ROS) in the queen' s body, resulting in oxidative stress and potential cellular damage. In response, antioxidant defense systems, including key enzymes such as catalase (CAT), are activated to scavenge ROS and mitigate oxidative damage, thereby maintaining cellular homeostasis [25–28]. In addition to antioxidant enzymes, detoxifying enzymes such as DDTase, GST, CES, CYP450P, and AChE play critical roles in enhancing the queen' s resilience to high-temperature stress by neutralizing toxic metabolic byproducts [28]. Exposure to high temperatures often triggers multiple heat shock protein genes expression, with families such as sHsp, Hsp60, Hsp70, and Hsp90 being commonly involved in insect and honeybee thermal adaptation [29–31]. Among them, the Hsp70 family is particularly crucial in conferring heat tolerance by preventing protein denaturation and facilitating protein refolding [22, 26, 32–34].

While previous studies have explored the behavioral and physiological adaptations of honeybees to high temperatures, the molecular mechanisms remain insufficiently understood, especially transcriptome changes of *A. c. cerana* queens after exposing to heat stresss. In this study, we performed transcriptomic analysis to investigate the alteration of gene expression in *A. c. cerana* under different temperature conditions, identifying differentially expressed genes (DEGs) and associated biological processes that associated with heat stress response. Our findings lay the groundwork for future investigations into the molecular changes governing insect responses to heat stress.

Materials and methods

Sample collection and Preparation

The newly mated queen of *Apis cerana* were collected from the Guangxi Academy of Agricultural Sciences High-Quality Pollination Bee Colony and Queen Rearing Demonstration Base in Guangxi, China, and subsequently reared in the Guangxi Subtropical Crops Research Institute. Queens were collected at 72 h post-mating. Continuous temperature and humidity monitoring in both greenhouse and natural apiaries revealed that greenhouse colonies maintained an average internal temperature of 40℃±0.5℃ for 5 consecutive daylight hours, while stabilizing at34.5℃±0.5℃ during remaining periods (Fig. 1). Based on these measurements and preliminary test data, we established two experimental treatments: a control group (CK: 34.5℃±0.5℃, 75%±1% RH) and a high-temperature treatment (HT: 40℃±0.5℃, 75%±1% RH). Both groups were subjected to differential temperature and duration treatments within laboratory-controlled constant temperature and humidity chambers. One queen and 20–30 worker bees were housed together in a small cage, and both queens and workers were provided with a daily diet consisting of a mixture of water and honey in a ratio of 1:1.5. The high-temperature treatment was maintained at 40℃±0.5℃ with a temperature cycle of 5 h at this temperature followed by 19 h at 34.5℃. The control group was maintained at a constant temperature of 34.5℃±0.5℃ for 24 h daily. The control. (CK: 34.5℃±0.5℃, 75%±1% RH) and the high-temperature treatment (HT: 40℃±0.5℃, 75%±1% RH) groups were treated at one cycle (24 h), four cycles (96 h) and seven cycles (168 h). Each group sample included 60 queen individuals, and three biological replicates were sampled. After processing, the heads, thoraxes, and ovaries of all queen individuals were dissected and immediately frozen in liquid nitrogen before being stored at − 80℃for subsequent RNA extraction.

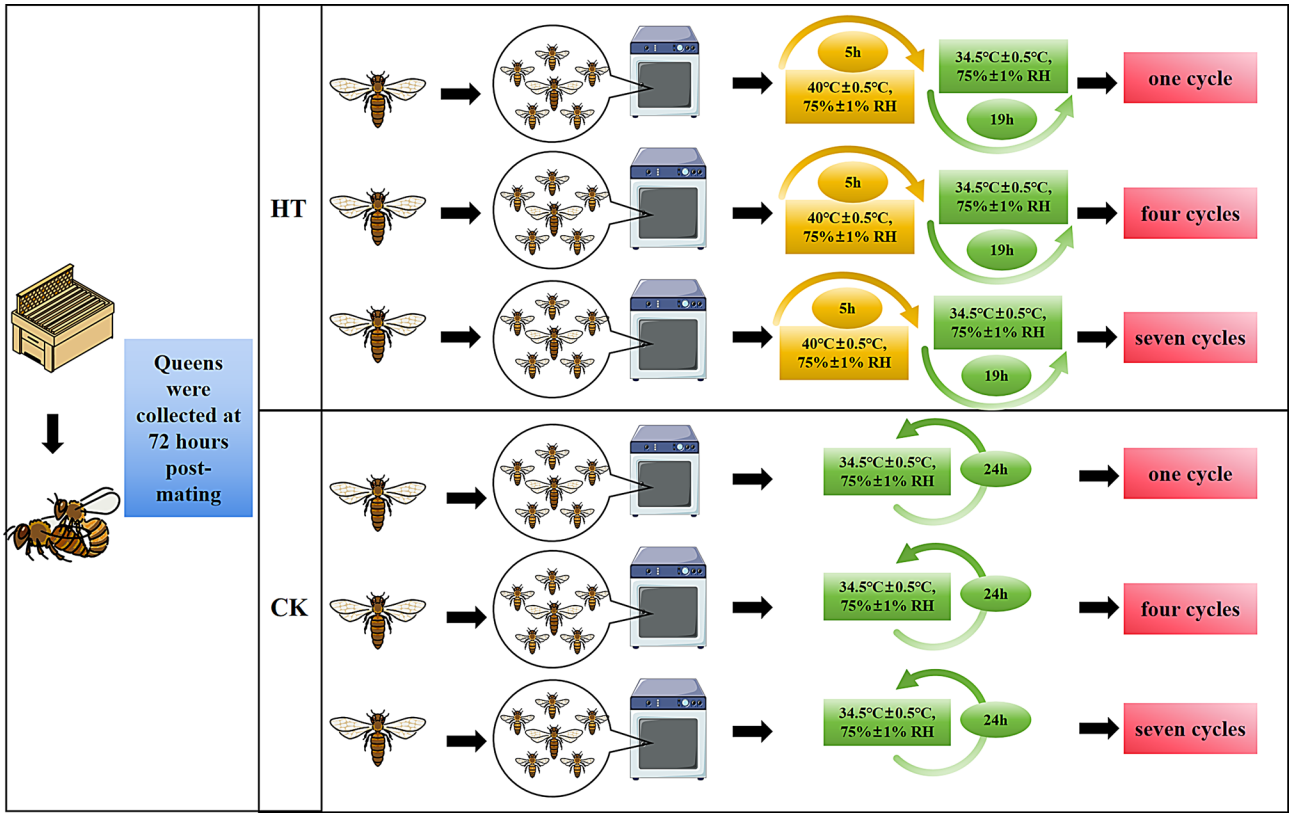

Fig. 1 Schematic representation of queens handling and sampling procedures

RNA isolation, cDNA library Preparation and sequencing

RNA quality assessment was assessed by 1% agarose gel electrophoresis. RNA purity was determined using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA), and RNA concentration was quantified with the Qubit® RNA Assay Kit on a Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was evaluated using the RNA Nano 6000 Assay Kit with the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

Sequencing libraries were prepared using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA). mRNA was isolated and fragmented, followed by first- and second-strand cDNA synthesis. Overhangs were converted to blunt ends, and adaptors were ligated. cDNA fragments were size-selected and PCR-amplified using Phusion High-Fidelity DNA Polymerase. The products were purified, and library quality was assessed on the Agilent Bioanalyzer 2100. Index-coded samples were clustered on a cBot Cluster Generation System and sequenced on an Illumina HiSeq 2500/X platform with 125/150 bp paired-end reads.

Raw fastq data were processed using in-house Perl scripts to obtain clean reads by removing adapter sequences, poly-N reads, and low-quality data. Quality metrics, including Q20, Q30, and GC content, were calculated for the clean data. All subsequent analyses were performed using the high-quality clean data.

Read mapping to the reference genome

Reference genome and annotation files were downloaded (*Apis cerana* genome, http://v2.insect-genome.com/Organism/79). The genome index was built with Bowtie v2.2.3 [35] and paired-end clean reads were aligned using TopHat v2.0.12, which generates splice junction databases for improved mapping accuracy over non-splice tools [36].

Cuffquant and Cuffnorm (v2.2.1) calculated gene FPKMs [37], summed from transcript FPKMs. FPKM represents fragments per kilobase per million mapped reads. DESeq2 calculated FPKMs based on fragment length and read counts [38].The all_sample_cluster correlation heatmap was used to evaluate the correlation among biological replicates, generated using the lattice R package.

Identification of deferentially expressed genes (DEGs)

Differential expression analysis between two conditions was performed using DESeq2 [38], which applies a negative binomial model. P-values were adjusted using the Benjamini-Hochberg method, and genes with an adjusted P-value < 0.01 were considered differentially expressed.

Gene Ontology (GO) enrichment analysis of differentially expressed genes was performed using the GOseq R package [39]. GO terms with a corrected P-value < 0.05 were considered significantly enriched. KEGG pathways were analyzed using KOBAS software [40] to assess the statistical enrichment of differentially expressed genes.

Protein-Protein interaction (PPI) network

PPI analysis of differentially expressed genes was performed using the STRING database, which includes known and predicted protein-protein interactions. For species in the database, networks were constructed directly from the target gene list. For other species, Blastx (v2.2.28) was used to align gene sequences to reference proteins, and networks were built based on known interactions of the selected reference species.

Novel transcripts prediction and alternative splicing analysis

The Cufflinks v2.1.1 RABT assembly method was used to identify known and novel transcripts from TopHat alignments. Alternative splicing events were classified into 12 types using Asprofile v1.0. The number of AS events was estimated for each sample. SNP analysis involved sorting, marking duplicates, and reordering BAM files using Picard-tools and Samtools. SNP calling was performed with GATK (v3.6) [41].

Validation of the gene expression level by qRT-PCR

Four genes associated with the heat stress response were randomly selected for expression analysis, which was quantified using qRT-PCR. The amplification conditions were as follows: initial denaturation at 95 ℃ for 3 min, followed by 45 cycles of denaturation at 95 ℃ for 10 s, annealing at 57℃ for 10 s, and extension at 72℃ for 20 s. Each reaction was performed in triplicate. The threshold cycle (CT) values were determined, and gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Results

Transcriptome analysis of the queen bee after high temperature stress

The transcriptome sequencing generated 1,405.46 million raw reads. After quality filtering, 392.37 Gb of clean data were retained (Table S1), with Q30 values ranging from 93 to 96%, and GC content between 35% and 41%. The mapping rate to the reference genome ranged from 91 to 96%. Gene annotation was identified 13,207 annotated genes (Fig. 2A). The reproducibility of the sequencing data was assessed using Pearson's correlation coefficients,

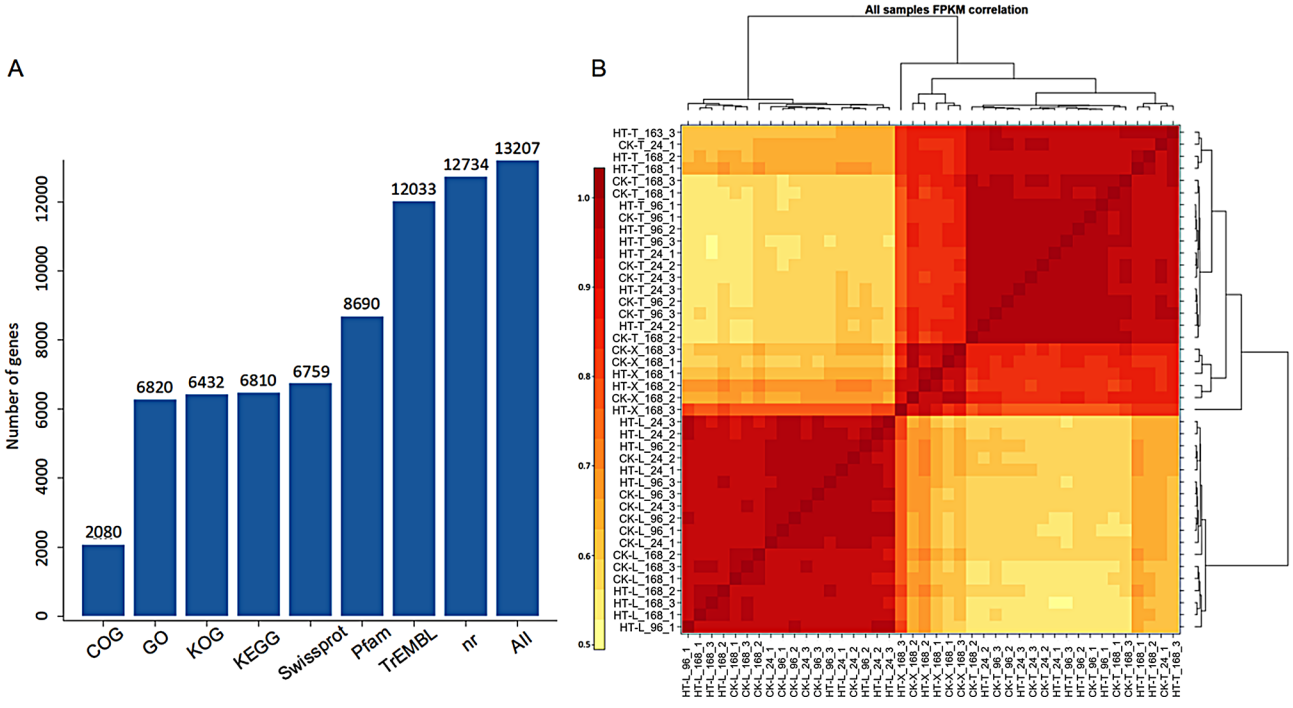

Fig. 2 Statistical analysis results of annotated databes and cluseter results of all the samples. (**A**) Statistical analysis of genes annotated in different databases. (**B**) cluseter results of gene expression in all the samples. CK_T_24 (head under normal condition at 24 h), CK_T_96 (head under normal condition at 96 h), CK_T_168 (head under normal condition at 168 h), HT_T_24 (head under heat stress at 24 h), HT_T_96 (head under heat stress at 96 h), HT_T_168 (head under heat stress at 168 h), CK_L_24 (ovary under normal condition at 24 h), CK_L_96 (ovary under normal condition at 96 h), CK_L_168 (ovary under normal condition at 168 h), HT_L_24 (ovary under heat stress at 24 h), HT_L_96 (ovary under heat stress at 96 h), HT_L_168 (ovary under heat stress at 168 h), CK_X_168 (thorax under normal condition at 168 h), and HT_X_168 (thorax under heat stress at 168 h)

with all biological replicates showing strong correlation ($R^2 \geq 0.90$) (Fig. 2B).

Differential expression after high temperature stress and gene functional classification

Compared to the normal condition, 116 DEGs (72 upregulated, 44 downregulated) were identified in the head and 106 DEGs (78 upregulated, 28 downregulated) in the ovary after 24 h of heat stress. After 96 h, 29 DEGs (17 upregulated, 12 downregulated) were identified in the head, and 44 DEGs (34 upregulated, 10 downregulated) in the ovary. After 168 h, the number of DEGs increased significantly: 846 DEGs (567 upregulated, 279 downregulated) in the head, 479 DEGs (296 upregulated, 183 downregulated) in the ovary, and 582 DEGs (338 upregulated, 244 downregulated) in the thorax (Fig. 3; Table S2).

Analysis of DEG profiles showed temporal variations in transcriptional responses to heat stress across tissues. Notably, the proportion of DEGs relative to total expressed genes was highest at 168 h, indicating enhanced transcriptional regulation at this stage. Furthermore, tissue-specific response patterns were observed: ovaries exhibited higher DEG proportions than heads at 24 h and 168 h, while the opposite trend occurred at 96 h. Further functional characterization of these DEGs is required to determine their specific roles in heat stress response. This indicates that different tissues have varying capacities to respond to high temperature stress.

Functional analyses of DEGs in bees after high temperature stress

GO enrichment analysis was conducted on the DEGs, revealing several significant terms, including amino acid transmembrane transport, nucleoside transmembrane transporter activity, ammonium transmembrane transporter activity, secondary active sulfate transmembrane transporter activity, tricarboxylic acid cycle, cellular amino acid metabolic process, fatty acid biosynthesis, unsaturated fatty acid biosynthesis, aromatic amino acid family metabolism, signal transduction, Notch signaling pathway, DNA binding, fatty acid beta-oxidation, and stress response (Table 1). Most of these GO terms were enriched in the comparisons of CK-X_168_vs_HT-X_168, CK-T_168_vs_HT-T_168, and CK-L_168_vs_HT-L_168, with DNA binding and transmembrane transporter activity observed in almost all comparison groups. This suggests that these two GO terms play a crucial role at all time points under heat stress, while other terms are mainly relevant at the 168-hour time point. These findings indicate that DEGs involved in metabolic, signaling, and transport pathways are significantly altered after heat stress, potentially contributing to reduced reproduction and growth capacity in bees.

KEGG enrichment analysis revealed pathways such as biosynthesis of unsaturated fatty acids, MAPK signaling pathway, mTOR signaling pathway, Notch signaling pathway, protein processing in the endoplasmic reticulum, TGF-beta signaling pathway, Wnt signaling pathway, and ubiquitin-mediated proteolysis. The MAPK signaling pathway and protein processing in the endoplasmic reticulum were enriched across all comparison groups, highlighting their central role under heat stress. Other pathways were more prominent at the 168-hour time point. These results suggest that changes in protein processing, metabolic, and signaling pathways may underlie the reduced reproduction and growth capacity of bees after heat stress (Table 2).

Kmeans cluster

To better characterize gene expression patterns under heat stress across different treatments, we normalized the expression levels of 24,731 genes from 42 samples using K-means clustering analysis (Fig. 3), which grouped the genes into 15 clusters. Among these, genes in clusters 1 (4,582 genes), 2 (1,206 genes), and 11 (593 genes) exhibited increased expression levels under heat stress in most groups, representing 25.80% of the total genes (Fig. 4). Therefore, we hypothesize that genes in clusters 1, 2, and 11 may be strongly upregulated in response to heat stress in bees.

Given the limited GO and KEGG annotations for cluster 2, we focused on genes in clusters 1 and 11. GO enrichment analysis revealed significant terms related to metal ion binding, transcription regulation, DNA-templated protein phosphorylation, sequence-specific DNA binding, integral membrane components, transmembrane transport, and oxidation-reduction processes (Fig. 5). KEGG enrichment analysis identified pathways related to chromosomal proteins, protein kinases, transcription factors, the ubiquitin system, protein phosphatases, MAPK signaling, protein processing in the endoplasmic reticulum, cytochrome P450, and transporters (Fig. 4). These results suggest that genes exhibiting similar expression patterns under heat stress may play key roles in heat stress tolerance and the reduced reproductive capacity of bees.

DEGs identified in bees after high temperature stress

High-temperature stress alters metabolic processes, signaling, and transport pathways, which may be crucial for heat tolerance and reproductive capacity (Fig. 6). Many transporters were differentially expressed in bees under heat stress. Specifically, six ABC transporter genes showed differential expression, with most being downregulated, except one that was upregulated in ovaries at 24 h and another in the head after 168 h. Transporters such as amino acid transporters, glucose transporters,

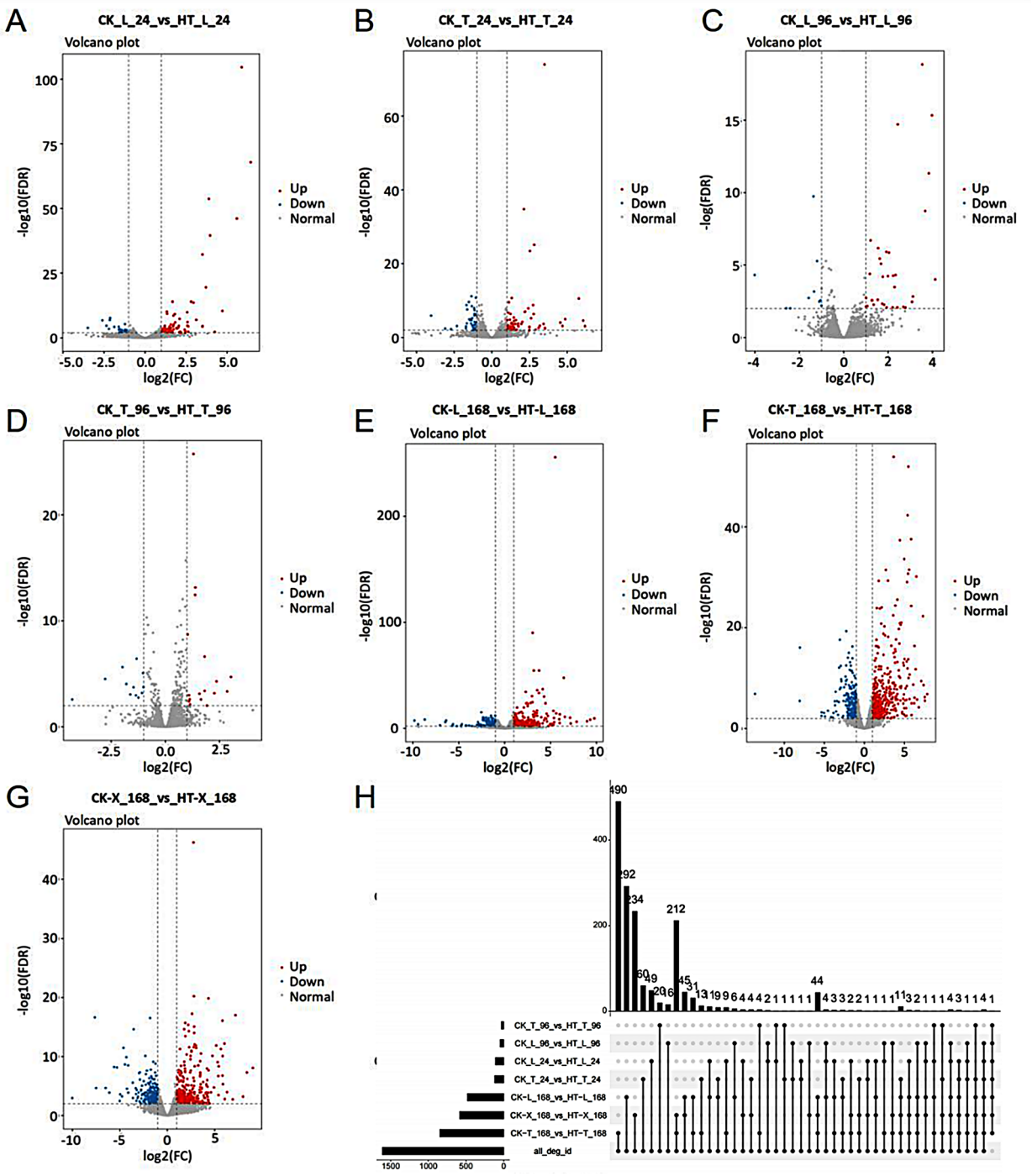

Fig. 3 DEGs of all samples. (**A**) DEGs identified in CK_L_24_vs_HT_L_24. (**B**) CK_T_24_vs_HT_T_24. (**C**) CK_L_96_vs_HT_L_96. (**D**) CK_T_96_vs_HT_T_96. (**E**) CK-L_168_vs_HT-L_168. (**F**) CK-T_168_vs_HT-T_168. (**G**) CK-X_168_vs_HT-X_168. (**H**) Venn diagram of all the DEGs

sugar transporters, and trehalose transporters also exhibited differential expression, predominantly downregulated under heat stress.

We also analyzed genes involved in sugar, amino acid, and fatty acid metabolism. Alpha, alpha-trehalase was downregulated in the head after 24 h of heat stress. Six glucose dehydrogenase genes were differentially expressed, with four upregulated and two downregulated after 168 h. One glyceraldehyde-3-phosphate dehydrogenase-like gene was upregulated in the head after 168 h. Additionally, two malate dehydrogenase genes were upregulated, with one in the head at 24 h and the other in the ovary after 168 h. An NADP-dependent malic enzyme was downregulated in the thorax after 168 h. Five

Table 1 Statistical analysis of GO enrichment analysis

Description	CK_T_24_vs_HT_T_24	CK_L_24_vs_HT_L_24	CK_T_96_vs_HT_T_96	CK_L_96_vs_HT_L_96	CK-X_168_vs_HT-X_168	CK-T_168_vs_HT-T_168	CK-L_168_vs_HT-L_168
Amino acid transmembrane transport	Acer016128	NA	NA	NA	NA	NA	Acer008661
DNA binding	XLOC_000965;Acer013508	Acer027966;Acer012865;Acer014457	NA	NA	Acer015199;Acer020777;Acer014826;Acer019038;Acer001833;Acer019073;Acer012008;Acer006133;Acer027477	Acer017944;Acer026162;XLOC_024245;Acer008764;Acer020777;Acer011175;Acer001833;Acer012071;Acer001832;Acer014840;Acer010138;Acer014457;Acer007555;Acer019073;Acer012008;Acer022666;Acer011416;Acer015017;Acer019180;XLOC_000965;Acer006047	XLOC_024245;Acer014826;Acer001833;Acer025072;Acer014822
Nucleoside transmembrane transporter activity	NA	NA	NA	NA	NA	NA	Acer016028
Tricarboxylic acid cycle	Acer024572	NA	NA	NA	Acer000387	NA	NA
Cellular amino acid metabolic process	NA	NA	NA	NA	Acer007144	NA	Acer007144
Fatty acid biosynthetic process	NA	NA	NA	NA	Acer006279	Acer006022	Acer007304
Fatty acid beta-oxidation	NA	NA	NA	NA	NA	Acer004541	NA
Unsaturated fatty acid biosynthetic process	NA	NA	NA	NA	NA	NA	Acer006466
Response to stress	NA	NA	NA	NA	Acer020955;Acer011141;Acer021075;Acer019907	Acer020955;Acer011141;Acer021075;Acer019907	Acer011141;Acer019907

Table 1 (continued)

Description	CK_T_24_vs_HT_T_24	CK_L_24_vs_HT_L_24	CK_T_96_vs_HT_T_96	CK_L_96_vs_HT_L_96	CK-X_168_vs_HT-X_168	CK-T_168_vs_HT-T_168	CK-L_168_vs_HT-L_168
Signal transduction	NA	Acer010925	Acer027596	NA	Acer006409;XLOC_017039;Acer004483;Acer006666;XLOC_005733;Acer022218;Acer021967;Acer010925	Acer003228;Acer004483;Acer027028;Acer012627;Acer021967	Acer027630;Acer026706;Acer010925
Notch signaling pathway	NA	NA	NA	NA	Acer006133	Acer024756	NA
Secondary active sulfate transmembrane transporter activity	NA	NA	NA	NA	Acer015698;Acer018206	Acer015698;Acer018024	NA
Ammonium transmembrane transporter activity	NA	NA	NA	NA	Acer016017	Acer016017	Acer016017;Acer009886
Aromatic amino acid family metabolic process	NA	NA	NA	NA	Acer019615	Acer019615	Acer019615;Acer019653
Amino acid transmembrane transporter activity	Acer016128	NA	NA	NA	NA	NA	Acer008661
Trehalose transmembrane transporter activity	Acer013526	NA	NA	NA	NA	NA	NA
Wnt signaling pathway	NA	NA	NA	NA	NA	Acer011104;Acer023231	NA
Amino acid binding	NA	NA	NA	NA	NA	NA	Acer014383;Acer019653
Transmembrane transporter activity	NA	Acer006226	Acer013090	NA	Acer020735;Acer009193	Acer020735	Acer015556

Table 1 (continued)

Description	CK_T_24_vs_HT_T_24	CK_L_24_vs_HT_L_24	CK_T_96_vs_HT_T_96	CK_L_96_vs_HT_L_96	CK-X_168_vs_HT-X_168	CK-T_168_vs_HT-T_168	CK-L_168_vs_HT-L_168
Cellular response to stress	NA	NA	NA	NA	NA	Acer027494	NA
Intracellular signal transduction	NA	NA	NA	NA	Acer024869;Acer005450;Acer003403;Acer019389	Acer009713;Acer005450	Acer024869;Acer010590;Acer005450;Acer003403;Acer009993
Sequence-specific DNA binding	NA	Acer015022;Acer002946;XLOC_010487;Acer011830	Acer011830	Acer015022	Acer020088;Acer014854;Acer015385;Acer019678;Acer002946;Acer026028;Acer009971;Acer007712;Acer021785;Acer009289	Acer020088;Acer021003;Acer007936;Acer011707;Acer015385;Acer007741;Acer022015;Acer002575;XLOC_000541;Acer015022;Acer013310;Acer009971;Acer015724;Acer027876;Acer025551	Acer020088;Acer014854;Acer001665;XLOC_000541;Acer009971;Acer012005
Metal ion transmembrane transporter activity	NA	NA	NA	NA	Acer000724;Acer012543;Acer001029	NA	NA

amino acid-related genes, including aspartate carbamoyltransferase, glutamine synthetase, tryptophan 2,3-dioxygenase, tryptophan 5-monooxygenase, and tyrosine 3-monooxygenase, were differentially expressed, with aspartate carbamoyltransferase, tryptophan 2,3-dioxygenase, and tryptophan 5-monooxygenase upregulated, while glutamine synthetase and tyrosine 3-monooxygenase were downregulated after 168 h.

Inositol-related genes, including inositol monophosphatase 2 isoform X1, inositol polyphosphate 5-phosphatase INPP5A, and myo-inositol-1(or 4)-monophosphatase, were downregulated after 168 h of heat stress. Fatty acid 2-hydroxylase isoform X2, isocitrate dehydrogenase (NAD+), and stearoyl-CoA desaturase were also differentially expressed, with most being downregulated.

Transcription factors were also affected, with one bZIP transcription factor, one C2H2-type zinc finger, one helix-loop-helix DNA-binding domain, and four homeobox protein genes showing differential expression, mostly upregulated, except for one homeobox gene. Seven zinc finger proteins were differentially expressed, most of which were upregulated.

Significant alterations in oxidative stress and heat shock response pathways were also observed under thermal stress. Antioxidant enzyme activity was notably affected, with one catalase (CAT) and one peroxidase (POD) downregulated, while another POD isoform was upregulated—suggesting a compensatory mechanism to mitigate oxidative damage. Cytochrome P450 (CYP) genes, critical for detoxification and redox homeostasis, exhibited pronounced differential expression: thirteen CYPs were dysregulated, predominantly downregulated in head tissues (CK_T_24 vs. HT_T_24; CK_T_168 vs. HT_T_168), though a subset was upregulated in thoracic and ovary tissues at 168 h (CK_X_168 vs. HT_X_168; CK_L_168 vs. HT_L_168), implying tissue-specific regulatory dynamics. The heat shock response was robustly activated, with ten heat shock proteins (HSPs) and two DnaJ cochaperones upregulated systemically. Notably, HSP induction was most pronounced in thoracic and ovary tissues at 168 h (CK_X_168 vs. HT_X_168; CK_L_168 vs. HT_L_168), with minor upregulation in ovaries at 24/96 h (CK_L_24 vs. HT_L_24; CK_L_96 vs. HT_L_96), aligning with prolonged stress adaptation. Additionally, fifteen serine/threonine kinases were upregulated. Intriguingly, E3 ubiquitin ligases (ten differentially expressed, evenly split between up/downregulation) and one antimicrobial peptide (transiently upregulated in heads at 24 h but suppressed by 168 h) also found to be differentially expressed under stress.

These expression changes may play a crucial role in heat stress tolerance and the reduced reproductive capacity of bees.

Table 2 Statistical analysis of KEGG enrichment analysis

Kegg_pathway	CK_T_24_vs_HT_T_24	CK_L_24_vs_HT_L_24	CK_T_96_vs_HT_T_96	CK_L_96_vs_HT_L_96	CK-X_168_vs_HT-X_168	CK-T_168_vs_HT-T_168	CK-L_168_vs_HT-L_168
Biosynthesis of unsaturated fatty acids	NA	NA	NA	NA	Acer006279;Acer011582	Acer004541	Acer006466
MAPK signaling pathway	Acer018381	Acer002712;Acer017219;XLOC_010487	NA	Acer017219	Acer006624;Acer011993;Acer026484;XLOC_020770	Acer006658;Acer018093;Acer020086;Acer021652;Acer025074;Acer025598;Acer026484	Acer003894;Acer011993;Acer017219;Acer018381
MAPK signaling pathway - fly	NA	XLOC_010487	NA	NA	Acer025391;XLOC_020770	Acer005103;Acer007632;Acer009872;Acer011985;Acer013342;Acer020558;Acer025087	Acer003894;Acer025087
mTOR signaling pathway	NA	NA	NA	NA	Acer000515	Acer000515;Acer001117;Acer002719;Acer011104;Acer023231	Acer007503
Notch signaling pathway	NA	NA	NA	NA	Acer004360;Acer006324	Acer013953;Acer023393;Acer024756;Acer026401	NA
Protein processing in endoplasmic reticulum	Acer011189;Acer025812;Acer027110;Acer027374;XLOC_016603	Acer017219;Acer025812;Acer026130;Acer027110;Acer027374;XLOC_016603	NA	Acer017219;Acer025812;Acer027374;XLOC_016603	Acer002381;Acer006692;Acer011141;Acer011993;Acer012969;Acer019907;Acer020758;Acer020871;Acer021075;Acer023184;Acer023267;Acer023614;Acer025532;Acer025808;Acer025812;Acer026259;Acer027110;Acer027374;XLOC_016603	Acer002381;Acer011141;Acer011189;Acer019907;Acer020871;Acer021075;Acer023184;Acer023267;Acer023614;Acer025532;Acer025812;Acer026259;Acer026450;Acer026823;Acer027110;Acer027374;XLOC_016603	Acer010241;Acer011141;Acer011189;Acer019933;Acer017001;Acer017219;Acer019907;Acer026130;Acer027110;Acer027374;XLOC_016603
TGF-beta signaling pathway	Acer018381	NA	NA	NA	Acer006873;Acer013512	Acer004301;Acer006873;Acer014627;Acer023393;Acer026401	Acer011437;Acer018381
Ubiquitin mediated proteolysis	NA	NA	NA	NA	NA	Acer000256;Acer004848	NA
Wnt signaling pathway	Acer018381	NA	NA	NA	Acer006624;Acer024230;Acer026484;XLOC_020770	Acer002719;Acer011104;Acer023231;Acer023393;Acer024230;Acer025074;Acer026401;Acer026484	Acer018381

PPI analysis

To further investigate the interaction of these DEGs, a PPI network was constructed using the STRING database. In the CK-L_168_vs_HT-L_168 comparison, the PPI network consisted of 89 nodes and 102 edges, with five genes - heat shock protein 90, heat shock protein 83, heat shock protein Hsp70Ab-like, stress-induced phosphoprotein 1, and heat shock 70 kDa protein 1/2/6/8—identified as hub genes. In the CK-T_168_vs_HT-T_168 comparison, the PPI network included 243 nodes and 507 edges, with six genes—heat shock protein 110 kDa, heat shock protein 90, heat shock protein cognate 3 precursor, heat shock protein 83, heat shock 70 kDa protein 1/2/6/8, and heat shock protein 90 kDa beta—selected as hub genes. In the CK-X_168_vs_HT-X_168 comparison, the PPI network contained 137 nodes and 264 edges, with PTK7 (protein tyrosine kinase 7) identified as a hub gene, suggesting its potential key role in heat stress tolerance in bees (Fig. 7).

Verification of DEGs using qRT-PCR

Ten genes (belongs to heat shock proteins) were selected from the DEGs identified in this study for qRT-PCR analysis. The relative mRNA expression levels, as determined by qRT-PCR, closely related with those obtained from the RNA-seq analysis (Fig. 8).

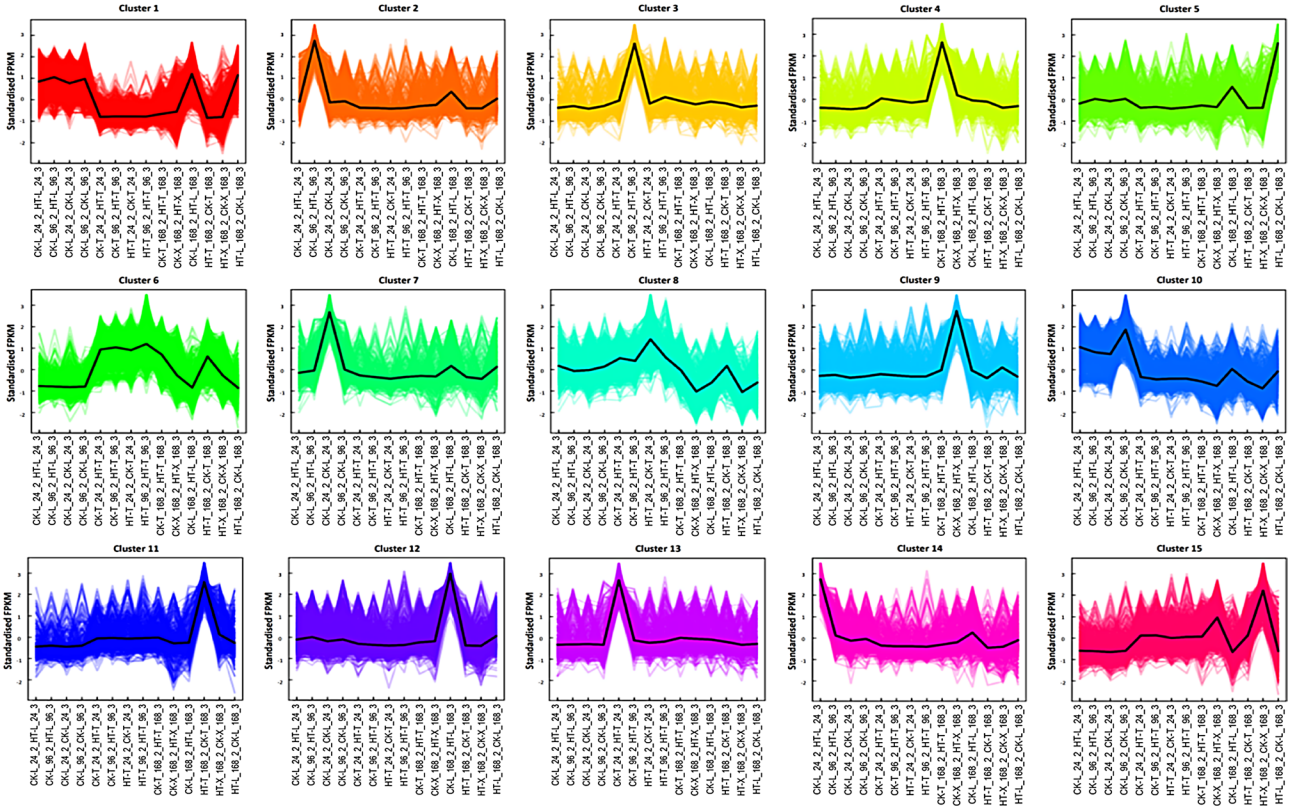

Fig. 4 Genes across 42 samples by K-means clustering analysis

Discussion

Apis cerana is widely utilized for pollination in controlled agricultural environments, where high temperatures pose significant challenges to colony health and productivity. Investigating the molecular mechanisms underlying heat stress responses in *A.* cerana queens is essential for developing strategies to enhance their thermal resilience, ensuring sustainable colony development and pollination efficiency under changing climate conditions. This study explored molecular mechanisms of heat adaptation in *A. cerana* queens through transcriptomic analysis. We identified tissue-specific (head, thorax and ovary) and time-dependent differentially expressed genes (DEGs) involved in metabolic processes, stress responses, and signaling pathways. Key genes related to antioxidant activity, heat shock proteins, and nutrient metabolism were differentially expressed, providing insights into the molecular mechanisms of heat stress adaptation. Our findings offer a comprehensive transcriptomic profile of *A. cerana* under heat stress, laying a foundation for future strategies to enhance heat tolerance in queen bees and improve colony resilience.

In this study, 168-hour time point is more crucial for the heat stress response than 24-hour time point and 96-hour time point, with a stronger molecular response compared to the 24-hour and 96-hour time points. DEGs associated with metabolic processes, signaling pathways, and transport were significantly altered after heat stress, potentially contributing to the reduced reproductive and growth capacity of bees. Additionally, genes related to antioxidant activity, nutrient metabolism, heat shock proteins, zinc finger proteins, and serine/threonine-protein kinases were differentially expressed, which may serve as candidate genes for explaining the mechanisms of reduced reproduction, growth, and stress resistance in bees under heat stress. Our findings provide a comprehensive expression profile of heat-resistance genes in *A. cerana*, offering valuable insights for future efforts to improve heat stress tolerance in queen bees.

High temperatures also trigger the accumulation of reactive oxygen species (ROS) in the queen's body, leading to oxidative stress. The antioxidant enzymes in insects can effectively eliminate active oxygen in the body and maintain normal physiological activities [42]. The queen bee, as the most reproductively capable individual in the colony, typically exhibits unique physiological and molecular responses to cope with heat stress, thereby protecting its reproductive function and maintaining colony survival [43]. Antioxidant responses are important physiological responses of insects to heat stress [43]. In our study, antioxidant oxidase has also been focused, some Cytochrome P450 was differentially expressed

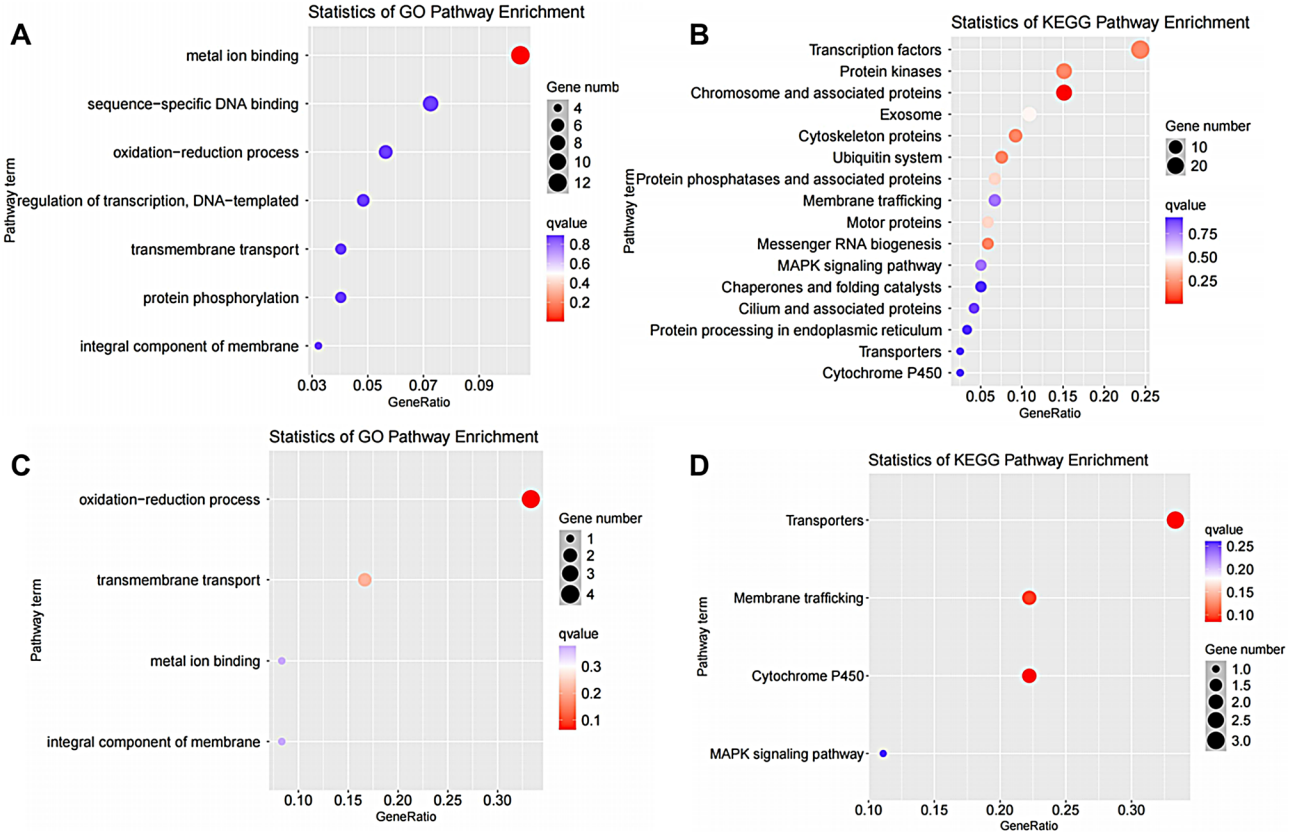

Fig. 5 KEGG and GO analysis of genes in cluster 1 and cluster 11. (**A**) GO analysis of DEGs in cluste 1. (**B**) KEGG analysis of DEGs in cluste 1. (**C**) GO analysis of DEGs in cluste 11. (**D**) KEGG analysis of DEGs in cluste 11

under heat stress tolerance, almost all these genes are down regulated. suggesting that heat stress can affect oxidative stress in bees.

Research has indicated that Zinc finger proteins (ZFPs) and serine/threonine protein kinases (STKs) are involved in the stress resistance mechanisms of organisms responding to various biotic and abiotic factors [44]. Recent studies have highlighted the significant role of several ZFP genes in the post-transcriptional regulatory processes when responding to heat stress [45]. Additional studies have shown that various ZFP genes, STKs, and antimicrobial peptide genes are critical components in the regulatory mechanisms activated by heat stress [45–48]. In our study, 7 zinc finger protein was differentially expressed under heat stress tolerance, almost all these genes are up regulated except one gene was down-regulated bees under heat stress tolenrance. 15 serine/threonine-protein kinase was differentially expressed under heat stress tolerance, almost all these genes are up regulated. This result show that ZFPs and STKs can be considered to be candidate genes for subsequent studies on the thermostable mechanism of bee.

Moreover, heat stress can trigger a reprogramming of the queen' s energy metabolism, leading to the activation of heat stress-related genes, such as heat shock proteins (HSPs), antioxidant genes, and stress signaling pathway genes (e.g., MAPK signaling pathway) [22]. Nutrient metabolism plays a crucial role in heat resistance in bees, as our findings confirm: under high-temperature stress, bee nutrient metabolism is significantly enhanced [49–52]. In our study, GO or KEGG analysis revealed the many DEGs invoved in nutrient metabolic pathways (including carbohydrate, amino acid and fatty acid). Heat stress can trigger a range of metabolic responses in insects, including an increase in energy expenditure [53]. and changed synthesis of metabolites, this could reflected the changed in expression of genes alpha, alpha-trehalase, Glucose dehydrogenase, malate dehydrogenase, glutamine synthetase, tryptophan 2,3-dioxygenase, fatty acid 2-hydroxylase isoform and etc., this may partially explain the reduced reproduction and growth ability of bees after high temperature stress. Free amino acids play a key role in enhancing cell water retention and stability [54]. The elevated levels of free amino acids in the hemolymph appear to be a significant physiological response of insects to heat stress [55]. Our results revealed DEGs associated with tyrosine, and tryptophan metabolism may partially explain response of bees after high temperature stress. Existing studies have revealed that the vesicular inhibitory amino acid transporter gene (AcVIAAT)

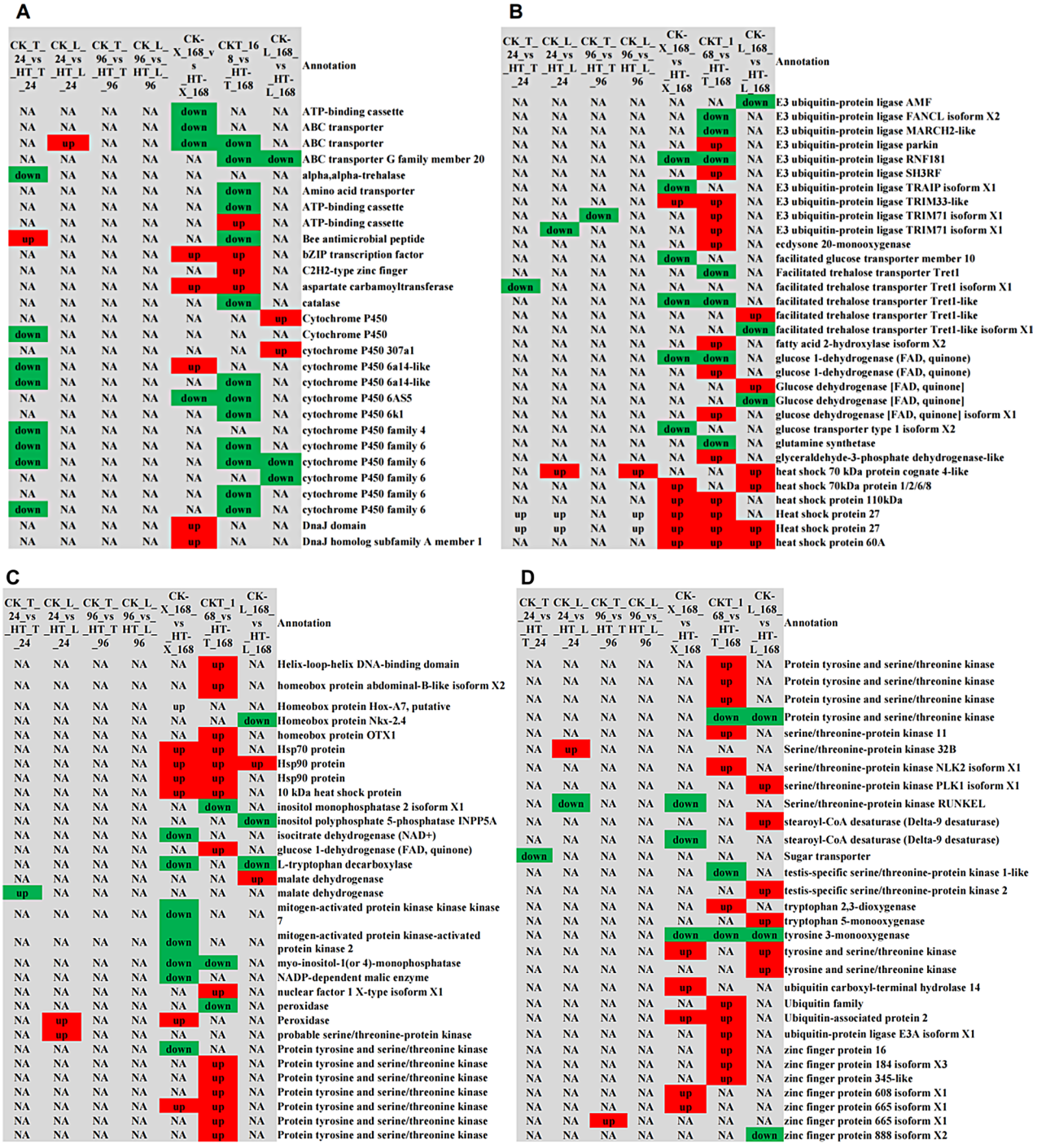

Fig. 6 DEGs with target annotation in different treatment groups. **A-D**. The division into **A-D** is for the aesthetic appearance of the layout, and **A-D** are the same table

in *Apis cerana* can enhance thermoregulatory capacity under heat stress by modulating oxidative stress-related genes. Administration of GABA to *A. cerana* significantly improved bee survival rates and upregulated oxidative stress-related gene expression [56].

Among the numerous thermal-resistance genes, the heat shock proteins (HSPs) gene family plays a crucial role, temperature stress can stimulate insects to produce heat shock proteins, enhancing their tolerance to elevated temperatures and shielding the body from thermal damage [57–61]. Previous research has demonstrated that the expression of the HSP protein family significantly increases following high-temperature exposure in various insect species [62–66]. These proteins mitigate

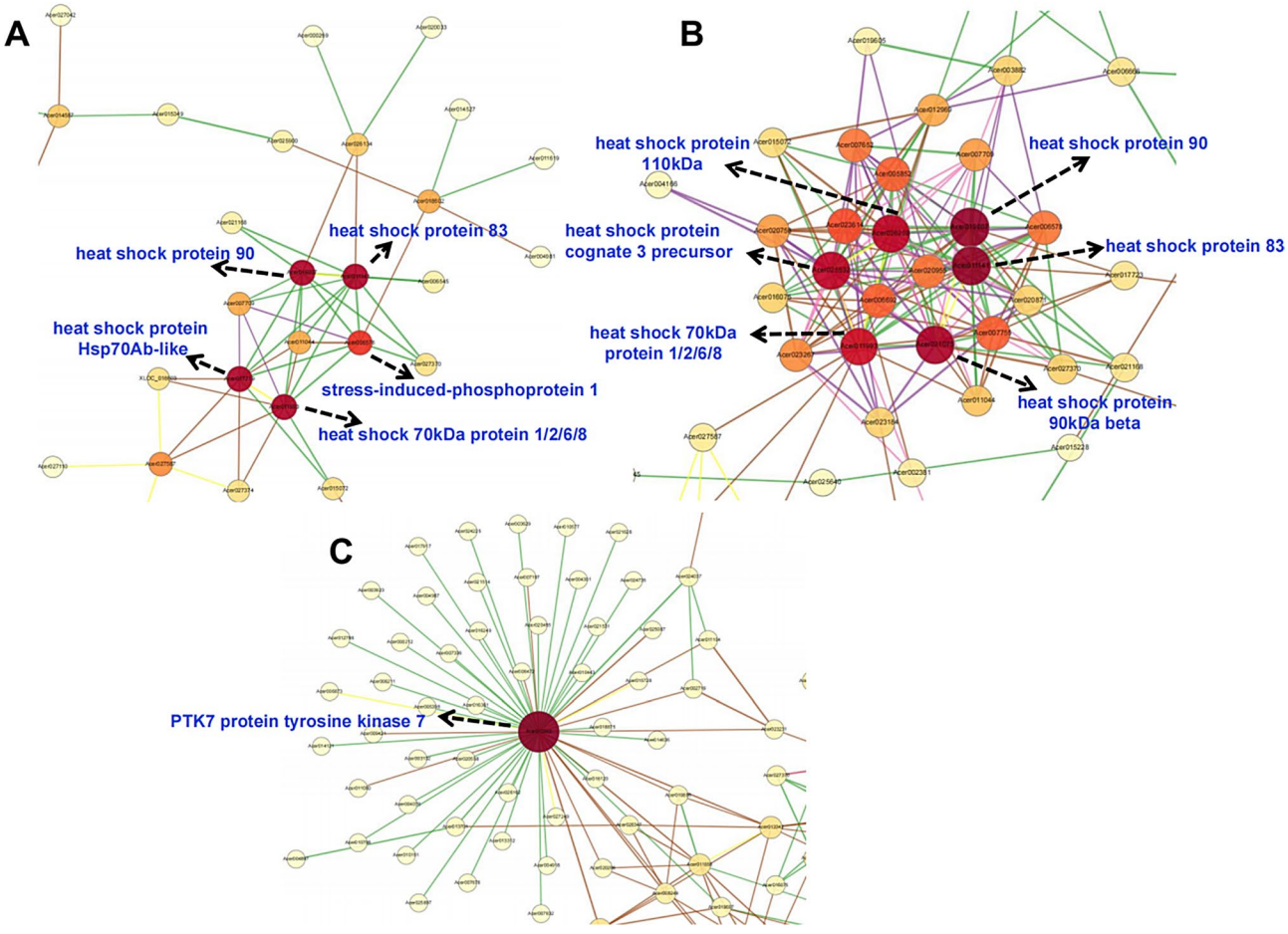

Fig. 7 PPI of DEGs. (**A**) PPI of DEGs identified in CK-L_168_vs_HT-L_168; (**B**) PPI of DEGs identified in CK-T_168_vs_HT-T_168; (**C**) PPI of DEGs identified in CK-X_168_vs_HT-X_168

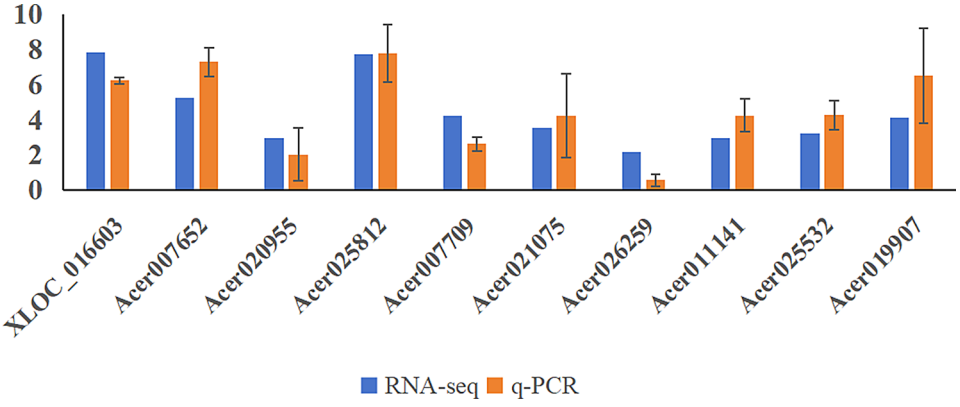

Fig. 8 Fold changes of the genes given by high-throughput sequencing and qRT-PCR. The Y axis indicates the fold change and the x axis indicates the gene name

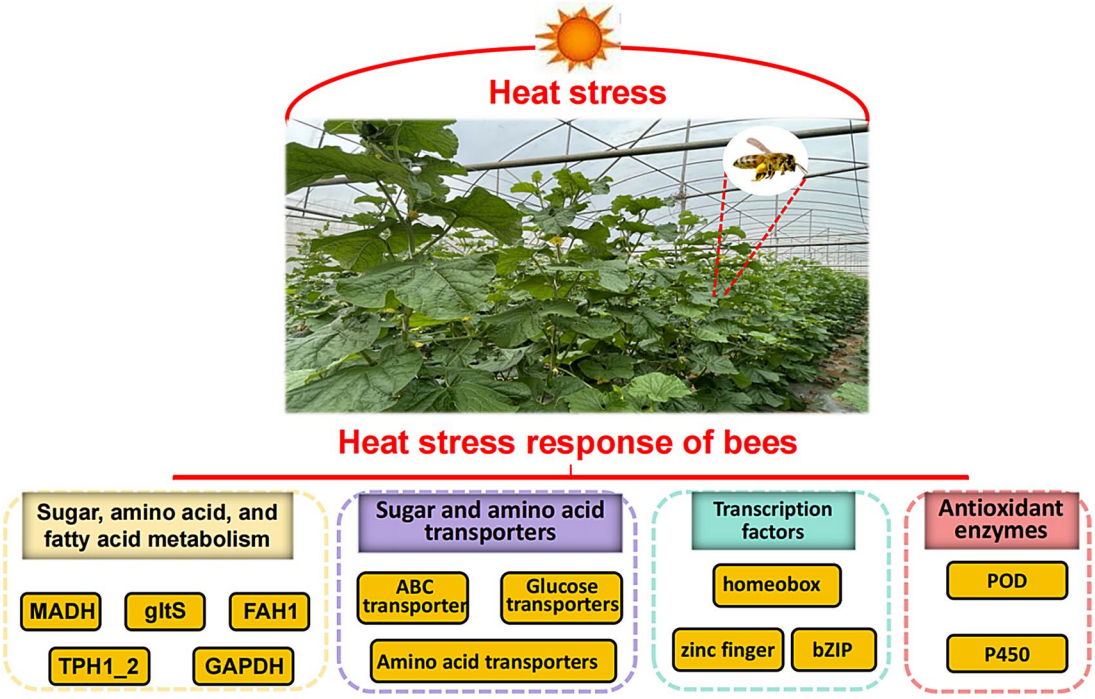

Fig. 9 The model of genetic basis underlying heat stress response of bees

damage to thermosensitive proteins at high temperatures and help maintain the integrity of cellular proteins [67]. Acting as molecular chaperones, they assist in repairing damaged proteins and preserving protein stability, thus safeguarding cells from heat-induced harm [26, 32–34]. We found nine DEGs associated with HSPs (heat shock 70, heat shock 27, heat shock 60 A) in different tissues at different time point after high temperature stress in bees under heat stress tolerance were almost all upregulated, indicating that Hsp family played important roles in the resistance of honeybees to heat shock. Overall, our results indicate that high temperature can induce the upregulation of various HSPs in honeybees.

Conclusions

Collectively, this study explored the transcriptome changes of *A. cerana* queens under heat stress. Initially, transcriptome analysis revealed a lot of DEGs in heads, thoraxes, and ovaries of queens at 24 h, 96 h and 168 h. And these DEGs were associated with metabolic processes, signaling, and transport pathways were significantly altered under heat stress, potentially contributing to the reduced reproductive and growth capacity of bees. Additionally, genes related to antioxidant activity, nutrient metabolism, heat shock proteins, zinc finger proteins, and serine/threonine-protein kinases were differentially expressed across treatments. Finally, a putative model was thus proposed for genetic underpinnings of heat stress responses in *A. cerana* queens (Fig. 9). Overall, the present study unveils the molecular basis underlying heat stress responses in *A. cerana* queen, and provides potential gene targets for enhancing their thermal resilience.

Abbreviations

DEGs	Differentially Expressed Genes
A. cerana	Apis cerana
CAT	Catalase
ROS	Reactive Oxygen Species
DDTase	DDT Dechlorinase
GST	Glutathione S-Transferase
CES	Carboxylesterase
CYP450	Cytochrome P450
AChE	Acetylcholinesterase
sHsp	Small Heat Shock Protein
Hsp60	Heat Shock Protein 60
Hsp70	Heat Shock Protein 70
Hsp90	Heat Shock Protein 90
HT	high-temperature treatment
RH	Relative Humidity
CK	Control
GO	Gene Ontology
PPI	Protein-Protein Interaction
SNP	Single Nucleotide Polymorphism
KEGG	Kyoto Encyclopedia of Genes and Genomes
AS events	Alternative Splicing events
CT	Threshold Cycle
NR	Non-redundant protein sequences
KOG	Kukaryotic Orthologous Groups
COG	Clusters of Orthologous Groups
MAPK	Mitogen-Activated Protein Kinase
mTOR	Mechanistic Target of Rapamycin
TGF-β	Transforming Growth Factor-beta
NADP-dependent malic enzyme	Nicotinamide Adenine Dinucleotide Phosphate-dependent Malic Enzyme
bZIP	basic Leucine Zipper
PTK7	Protein Tyrosine Kinase 7
ZFPs	Zinc Finger Proteins

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11714-7.

Supplementary Material 1

Supplementary Material 2

Acknowledgements
Not applicable.

Author contributions
Y-M L, J-X H designed the study and revised the manuscript. A-M L, C-X P, with the assistance from X-L C, F-C Z, B H, X-H H, conducted the experiment. A-M L carried out the bioinformatics analysis and drafted the manuscript. All authors contributed to this work and approved the submitted version.

Funding
This work was supported by the Natural Science Foundation of Guangxi (2024GXNSFAA010181), the Fundamental Research Fund of Guangxi Academy of Agriculture Sciences (2021YT050; GUINONGKE 2024YP048; 2025ZX19).

Data availability
The datasets generated and/or analysed during the current study are available from the Genome Sequence Archive in the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (https://bigd.big.ac.cn/gsa/browse/CRA021057).

Declarations

Ethics approval and consent to participate
All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 26 February 2025 / Accepted: 15 May 2025
Published online: 20 May 2025

References

1. Goulson D, Nicholls E, Botias C, Rotheray EL. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science. 2015;347(6229):1255957.
2. Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. Importance of pollinators in changing landscapes for world crops. Proc Biol Sci. 2007;274(1608):303–13.
3. Silva FDS, Carvalheiro LG, Aguirre-Gutiérrez J, Lucotte M, Guidoni-Martins K, Mertens F. Virtual pollination trade uncovers global dependence on biodiversity of developing countries. Sci Adv 2021;7(11).
4. Abou-Shaara HF, Owayss AA, Ibrahim YY, Basuny NK. A review of impacts of temperature and relative humidity on various activities of honey bees. Insect Soc. 2017;64(4):455–63.
5. Halsch CA, Shapiro AM, Fordyce JA, Nice CC, Thorne JH, Waetjen DP, Forister ML. Insects and recent climate change. Proc Natl Acad Sci U S A. 2021;118(2):1–9.
6. Martinet B, Zambra E, Przybyla K, Lecocq T, Anselmo A, Nonclercq D, Rasmont P, Michez D, Hennebert E, Pum Lee K. Mating under climate change: impact of simulated heatwaves on the reproduction of model pollinators. Funct Ecol. 2021;35(3):739–52.
7. Nguyen TM, Bressac C, Chevrier C. Heat stress affects male reproduction in a parasitoid Wasp. J Insect Physiol. 2013;59(3):248–54.
8. Southwick EE, Heldmaier G. Temperature control in honey bee colonies. Bioscience. 1987;37(6):395–9.
9. Vanderplanck M, Martinet B, Carvalheiro LG, Rasmont P, Barraud A, Renaudeau C, Michez D. Ensuring access to high-quality resources reduces the impacts of heat stress on bees. Sci Rep. 2019;9(1):12596.
10. Zvereva EL, Hunter MD, Zverev V, Kozlov MV. Factors affecting population dynamics of leaf beetles in a Subarctic region: the interplay between climate warming and pollution decline. Sci Total Environ 2016;566–7:1277–1288.
11. Parmesan C. Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst. 2006;37(0):637–69.
12. Walters J, Zavalnitskaya J, Isaacs R, Szendrei Z. Heat of the moment: extreme heat poses a risk to bee-plant interactions and crop yields. Curr Opin Insect Sci. 2022;52:100927.
13. Xu X, Du X, Zhou S, Zhou B, Lai K, Wang Q, Li H, Zhu C, Xu H, Zhang X, Cao M, Zhu X et al. Impacts of high temperature during early capped brood on pupal development and the size of appendages in adult workers *Apis Cerana*. Front Ecol Evol 2023;11.
14. McAfee A, Chapman A, Higo H, Underwood R, Milone J, Foster LJ, Guarna MM, Tarpy DR, Pettis JS. Vulnerability of honey bee queens to heat-induced loss of fertility. Nat Sustain. 2020;3(5):367–76.
15. Zhao H, Mashilingi SK, Liu Y, An J. Factors influencing the reproductive ability of male bees: current knowledge and further directions. Insects. 2021;12(6):529.
16. Xu X, Du X, Zhou S, Zhou B, Lai K, Wang Q, Li H, Zhu C, Xu H, Zhang X et al. Impacts of high temperature during early capped brood on pupal development and the size of appendages in adult workers *Apis cerana*. Front Ecol Evol 2023;11.
17. Medina RG, Paxton RJ, De Luna E, Fleites-Ayil FA, Medina ML, Quezada-Euan J. Developmental stability, age at onset of foraging and longevity of Africanized honey bees (*Apis mellifera* L.) under heat stress (Hymenoptera: Apidae). J Therm Biol. 2018;74:214–25.
18. Heidinger IMM, Meixner MD, Berg S, Büchler R. Observation of the mating behavior of honey bee (*Apis mellifera* L.) queens using radio-frequency identification (RFID): factors influencing the duration and frequency of nuptial flights. Insects. 2014;5(3):513–27.
19. Mayack C, Cook SE, Nino BD, Rivera L, Nino EL, Seshadri A. Poor air quality is linked to stress in honeybees and can be compounded by the presence of disease. Insects 2023, 14(8).
20. Lin Z, Zheng M, Li Z, Ji T. Editorial: biotic and abiotic stresses on honeybee physiology and colony health. Front Physiol. 2023;14:1260547.
21. Giacobino A, Molineri AI, Pacini A, Fondevila N, Pietronave H, Rodriguez G, Palacio A, Bulacio CN, Orellano E, Salto CE, et al. Varroa destructor and viruses association in honey bee colonies under different Climatic conditions. Environ Microbiol Rep. 2016;8(3):407–12.
22. Bordier C, Suchail S, Pioz M, Devaud JM, Collet C, Charreton M, Le Conte Y, Alaux C. Stress response in honeybees is associated with changes in task-related physiology and energetic metabolism. J Insect Physiol. 2017;98:47–54.
23. Flores JM, Gil-Lebrero S, Gámiz V, Rodríguez MI, Ortiz MA, Quiles FJ. Effect of the climate change on honey bee colonies in a temperate mediterranean zone assessed through remote hive weight monitoring system in conjunction with exhaustive colonies assessment. Sci Total Environ. 2019;653:1111–9.
24. Naug D. Nutritional stress due to habitat loss May explain recent honeybee colony collapses. Biol Conserv. 2009;142(10):2369–72.
25. Hsieh Y, Hsu C. Oxidative stress and anti-oxidant enzyme activities in the trophocytes and fat cells of queen honeybees (*Apis mellifera*). Rejuvenation Res. 2013;16(4):295–303.
26. Li X, Ma W, Jiang Y. Expression patterns of heat shock protein genes and antioxidase genes in *Apis cerana cerana* (Hymenoptera: Apidae) under heat stress. J Entomol Sci. 2023;58(1):95–103.
27. Zhai N, Jia H, Ma M, Chao Y, Guo X, Li H. Characteristics of AccSTIP1 in *Apis cerana cerana* and its role during oxidative stress responses. Cell Stress Chaperon. 2018;23(6):1165–76.
28. Li X, Ma W, Shen J, Long D, Feng Y, Su W, Xu K, Du Y, Jiang Y. Tolerance and response of two honeybee species *Apis cerana* and *Apis mellifera* to high temperature and relative humidity. PLoS ONE. 2019;14(6):e217921.
29. Morales M, Planello R, Martinez-Paz P, Herrero O, Cortes E, Martinez-Guitarte JL, Morcillo G. Characterization of Hsp70 gene in *Chironomus riparius*: expression in response to endocrine disrupting pollutants as a marker of ecotoxicological stress. Comp Biochem Physiol C Toxicol Pharmacol. 2011;153(1):150–8.
30. Zhang Y, Liu Y, Zhang J, Guo Y, Ma E. Molecular cloning and mRNA expression of heat shock protein genes and their response to cadmium stress in the grasshopper *Oxya chinensis*. PLoS ONE. 2015;10(7):e131244.

31. Ahmad A, Alghamdi, Yehya Z, Alattal. Expression levels of heat-shock proteins in *Apis mellifera jemenetica* and *Apis mellifera carnica* foragers in the desert climate of Saudi Arabia. Insects 2023;14(5):432–432.
32. Li G, Zhao H, Guo H, Wang Y, Cui X, Li H, Xu B, Guo X. Analyses of the function of DnaJ family proteins reveal an underlying regulatory mechanism of heat tolerance in honeybee. Sci Total Environ. 2020;716:137036.
33. Li G, Zhang S, Wang H, Liang L, Liu Z, Wang Y, Xu B, Zhao H. Differential expression characterisation of the heat shock proteins DnaJB6, DnaJshv, DnaJB13, and DnaJB14 in *Apis cerana cerana* under various stress conditions. Front Ecol Evol 2022;10.
34. Alqarni AS, Ali H, Iqbal J, Owayss AA, Smith BH. Expression of heat shock proteins in adult honey bee (*Apis mellifera* L.) workers under hot-arid subtropical ecosystems. Saudi J Biol Sci. 2019;26(7):1372–6.
35. Langmead B, Salzberg SL. Fast gapped-read alignment with bowtie 2. Nat Methods. 2012;9(4):357–9.
36. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L. Differential gene and transcript expression analysis of RNA-seq experiments with tophat and cufflinks. Nat Protoc. 2012;7(3):562–78.
37. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010;28(5):511–5.
38. Wang L, Feng Z, Wang X, Wang X, Zhang X. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics. 2010;26(1):136–8.
39. Young MD, Wakefield MJ, Smyth GK, Oshlack A. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol. 2010;11(2):R14.
40. Mao X, Cai T, John GO, Wei L. Automated genome annotation and pathway identification using the KEGG orthology (KO) as a controlled vocabulary. Bioinformatics. 2005;21(19):3787–93.
41. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, et al. The genome analysis toolkit: a mapreduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20(9):1297–303.
42. Wu H, Liu J, Zhang R, Zhang J, Guo Y, Ma E. Biochemical effects of acute Phoxim administration on antioxidant system and acetylcholinesterase in *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae). Pestic Biochem Physiol. 2011;100(1):23–6.
43. McAfee A, Tarpy DR, Foster LJ. Queen honey bees exhibit variable resilience to temperature stress. PLoS ONE. 2021;16(8):e255381.
44. Giri J, Vij S, Dansana PK, Tyagi AK. Rice A20/AN1 zinc-finger containing stress-associated proteins (SAP1/11) and a receptor-like cytoplasmic kinase (OsRLCK253) interact via A20 zinc-finger and confer abiotic stress tolerance in Transgenic *Arabidopsis* plants. New Phytol. 2011;191(3):721–32.
45. Droll D, Minia I, Fadda A, Singh A, Stewart M, Queiroz R, Clayton C. Post-transcriptional regulation of the trypanosome heat shock response by a zinc finger protein. Plos Pathog. 2013;9(4):e1003286.
46. Ma W, Li X, Shen J, Du Y, Xu K, Jiang Y. Transcriptomic analysis reveals *Apis mellifera* adaptations to high temperature and high humidity. Ecotoxicol Environ Saf. 2019;184:109599.
47. Li X, Ma W, Jiang Y. Heat stress affects the expression of antimicrobial peptide genes in adult honeybee (*Apis Cerana* and *Apis mellifera*). Int J Trop Insect Sci. 2022;42(3):2465–71.
48. Li X, Ma W, Du Y, Xu K, Jiang Y. Differential expression of endocrine regulatory genes in *Apis cerana* and *Apis mellifera* (Hymenoptera: Apidae) at high temperature. J Entomol Sci. 2022;57(3):411–24.
49. Salvucci ME, Stecher DS, Henneberry TJ. Heat shock proteins in whiteflies, an insect that accumulates sorbitol in response to heat stress. J Therm Biol. 2000;25(5):363–71.
50. Managbanag JR, Torzilli AP. An analysis of Trehalose, glycerol, and mannitol accumulation during heat and salt stress in a salt marsh isolate of *Aureobasidium pullulans*. Mycologia. 2002;94(3):384–91.
51. Thomson LJ, Macfadyen S, Hoffmann AA. Predicting the effects of climate change on natural enemies of agricultural pests. Biol Control. 2010;52(3):296–306.
52. Siqueira TCS, Brito DV, Carvalho Zilse GA. Research Article heat shock genes in the stingless bee *Melipona interrupta* (Hymenoptera, Meliponini). Genet Mol Res 2018;17(3).
53. Koo J, Son T, Kim S, Lee K. Differential responses of *Apis mellifera* heat shock protein genes to heat shock, flower-thinning formulations, and Imidacloprid. J Asia-Pac Entomol. 2015;18(3):583–9.
54. Malmendal A, Overgaard J, Bundy JG, Sorensen JG, Nielsen NC, Loeschcke V, Holmstrup M. Metabolomic profiling of heat stress: hardening and recovery of homeostasis in *Drosophila*. Am J Physiol Regul Integr Comp Physiol. 2006;291(1):R205–12.
55. Schou MF, Kristensen TN, Pedersen A, Karlsson BG, Loeschcke V, Malmendal A. Metabolic and functional characterization of effects of developmental temperature in *Drosophila melanogaster*. Am J Physiol Regul Integr Comp Physiol. 2017;312(2):R211–22.
56. Li Y, Song H, Xie L, Tang X, Yan J, Yao Y, Peng X, Cui J, Zhou Z, Xu J et al. Surviving high temperatures: the crucial role of vesicular inhibitory amino acid transporter in Asian honeybee, *Apis Cerana*[J]. Int J Biol Macromol 2024;279(Pt3):135276.
57. Sun Y, Zhao J, Sheng Y, Xiao YF, Zhang YJ, Bai LX, Tan Y, Xiao LB, Xu GC. Identification of heat shock cognate protein 70 gene (Alhsc70) of *Apolygus lucorum* and its expression in response to different temperature and pesticide stresses. Insect Sci. 2016;23(1):37–49.
58. Jerbi-Elayed M, Lebdi-Grissa K, Foray V, Muratori F, Hance T. Using multiple traits to estimate the effects of heat shock on the fitness of *Aphidius colemani*. Entomol Exp Appl. 2015;155(1):18–27.
59. King AM, MacRae TH. Insect heat shock proteins during stress and diapause. Annu Rev Entomol. 2015;60(1):59–75.
60. Bhattacharyya M, Chakraborty SK, Acharya SK. Proboscis extension reflex in *Apis florea* (Hymenoptera: Apidae) in response to temperature. J Entomol Sci. 2019;54(3):238–49.
61. Shih SR, Bach DM, Rondeau NC, Sam J, Lovinger NL, Lopatkin AJ, Snow JW. Honey bee sHSP are responsive to diverse proteostatic stresses and potentially promising biomarkers of honey bee stress. Sci Rep. 2021;11(1):22087.
62. Dahlgaard J, Loeschcke V, Michalak P, Justesen J. Induced thermotolerance and associated expression of the heat-shock protein Hsp70 in adult *Drosophila melanogaster*. Funct Ecol. 1998;12(5):786–93.
63. Huang LH, Kang L. Cloning and interspecific altered expression of heat shock protein genes in two leafminer species in response to thermal stress. Insect Mol Biol. 2007;16(4):491–500.
64. Huey RB, Kearney MR, Krockenberger A, Holtum JA, Jess M, Williams SE. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos Trans R Soc Lond B Biol Sci. 2012;367(1596):1665–79.
65. Cheng L, Du Y, Hu J, Jiao D, Li J, Zhou Z, Xu Q, Li F. Proteomic analysis of ubiquitinated proteins from deltamethrin-resistant and susceptible strains of the Diamondback moth. *Plutella Xylostella* L. Arch Insect Biochem. 2015;90(2):70–88.
66. Wu C, Wang C, Li D, Liu Y, Sheng Q, Lv Z, Yu W, Nie Z. BmHSP20.8 is localized in the mitochondria and has a molecular chaperone function in vitro. J Insect Sci. 2015;15(1):99.
67. Bakthisaran R, Tangirala R, Rao C. Small heat shock proteins: role in cellular functions and pathology. Biochim Biophys Acta. 2015;1854(4):291–319.

Publisher's note