High-throughput profiling of diapause regulated genes from *Trichogramma dendrolimi*, an important egg parasitoid

Xue Zhang
Jilin Agricultural University
Wenmei Du
Jilin Agricultural Sciences
Junjie Zhang
Jilin Agricultural University
Zhen Zou
Chinese Acedemy of Sciences
Changchun Ruan (ruanchangchun@126.com)
Jilin Agricultural University

Research article

Keywords: Trichogramma dendrolimi, transcriptome, RNA-Seq, diapause, diapause-related genes

Posted Date: September 3rd, 2020

DOI: https://doi.org/10.21203/rs.2.24668/v3

Version of Record: A version of this preprint was published at BMC Genomics on December 4th, 2020.
See the published version at https://doi.org/10.1186/s12864-020-07285-4.

Abstract

Background: The parasitoid wasp Trichogramma dendrolimi can successfully enter diapause at the prepupal stage. Thus, diapause is an efficient preservation method during the mass production of T. dendrolimi . Previous studies on diapause have focused largely on ecological characteristics, so the molecular mechanism of diapause in T. dendrolimi is mostly unknown. In this study, we compared transcriptomes of diapause and non-diapause T. dendrolimi to identify key genes and pathways involved in the development of diapause.

Results: Transcriptome sequencing was performed using different samples, including diapause prepupae, pupae after diapause, normal prepupae, and pupae. Initially, it yielded a total of 87,022 unigenes with an average length of 1,604 bp. By removing redundant sequences and those without significant BLAST hits, a non-redundant dataset was generated, containing 7,593 sequences with an average length of 3,351 bp. Among them, 5,702 genes were differentially expressed. The result of Gene Ontology (GO) enrichment analysis revealed that regulation of transcription, DNA-templated, oxidation-reduction process, and signal transduction were significantly affected. Ten genes were selected for validation using a quantitative real-time PCR (qPCR). The changes showed the same trend between the qPCR and RNA-Seq results. Based on our data, several genes were identified to be involved in diapause, including ribosomal proteins, zinc finger proteins, homeobox proteins, forkhead box proteins, UDP-glucuronosyltransferase , Glutathione-S-transferase , p53 , DNA damage-regulated gene 1 (pdrg1), and genes related to lipid metabolism were also included.

Conclusions: In this study, we generated a great amount of transcriptome data from T. dendrolimi , providing a resource for gene function research. The diapause-related genes that we identified establish a valuable basis for future studies on the molecular mechanisms of diapause, not only for T. dendrolimi , but for other species as well.

Background

Trichogramma dendrolimi Matsumura, an egg parasitoid of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), is one of the most successful biological control agents for several important Lepidopteran insect pests, such as *Chilo suppressalis* [1], *Cnaphalocrocis medinalis* [2], and *Ostrinia furnacalis* [3]. Currently, the application of *T. dendrolimi* is primarily inundative and augmentative release into fields to control insect pests, so large numbers of *T. dendrolimi* are needed for biological control programs. Thus, the preservation of parasitoids for timely supply is essential for the continuous production of parasitoids throughout the year [4]. At present, cold storage of parasitized host eggs is the most commonly used method for parasitoid preservation [5]. Although *Trichogramma* can be kept for a long period at low temperatures, the performance, such as emergence rate and longevity, decreases as storage time increases. For example, the survival rate of *T. dendrolimi* has been shown to significantly decrease after three weeks of storage at low temperatures [6], and similar phenomena have been found

among other *Trichogramma* species [7, 8]. Therefore, it is important to improve the quality of preservation. Diapause can be an effective tool to solve this problem.

Diapause is one of the essential processes used to help insects overcome extreme circumstances [9]. Insects can utilize this ability to resist adverse environmental conditions. When the environmental conditions are not suitable for insects, diapause may be initiated to reduce energy expenses and metabolic activities, and to enhance stress resistance and extend lifespan [10-12]. Previous studies have comprehensively reviewed diapause from many aspects, ranging from insects to mammals, and shown that diapause is a complicated process [13-15]. To date, many studies have focused on ecological characteristics to optimize conditions for diapause induction or termination to improve biological control programs[16-20]. Several diapause-associated genes, such as *dilp1*, forkhead box protein O (*foxo*), and *akt*, have been identified in other insect species[21-26]. Few studies, however, have been carried out to decipher molecular mechanism of diapause in *Trichogramma* spp. Moreover, many of the phenomena that arose from diapause in *T. dendrolimi* have not yet been explained. For example, adults of *T. dendrolimi* experienced diapause development could produce more eggs [27]. Why and how they improve their ability to lay eggs? Therefore, it is necessary to understand the diapause mechanism. Transcriptome sequencing has become a favorable tool for gene expression research. In fact, many studies have used RNA-sequencing (RNA-Seq) to solve a wide variety of problems. Some studies focused on insect resistance to insecticide [28-31], some on insect adaptability to extreme environments[32, 33], and others on selected genes, like chemosensory genes [34]. But there have been few studies on insect diapause using RNA-Seq. Hao et al. (2019) identified the candidate genes (*rai1* and *foxo*) related to the FOXO pathway in the egg diapause regulation of *Locusta migratoria* [35].

In this study, we are the first to report the gene expression profiles of *T. dendrolimi* in diapause and non-diapause states. The objective of this study was to use RNA-Seq to characterize diapause-related genes in this species. The results of this study are expected to provide a reference for deciphering the diapause mechanism in *T. dendrolimi* and guiding the use of *T. dendrolimi* in biological control programs.

Results And Discussion

Diapause induction, termination, sequencing, and gene identification

After the completion of induction process, the parasitized host eggs were dissected to verify whether *T. dendrolimi* entered diapause successfully or not. Diapausing parasitoids should stay at the prepupal stage, noted as Dpre. If diapause induction failed, they would die or continue to develop into pupae or adults. Following the diapause termination process, the parasitized host eggs were transferred to normal development conditions (26°C ± 1°C, 60% ± 5% RH, 16: 8 L: D). If diapause was disrupted, the parasitoids would convert from prepupa to pupa within several days, noted as Dp. Conversely, if diapause was undisrupted, the *T. dendrolimi* would remain in the prepupa stage. In this study, 99% of the parasitoids entered diapause successfully, and about 95% broke out of the diapause state after the 70 days

termination treatment. And the prepupae and pupae of *T. dendrolimi* developed under normal conditions were also obtained as references noted as NDpre and NDp, respectively.

RNA samples obtained from distinct stages of this species were prepared and sequenced using the Illumina Hiseq2000 sequencing platform. Four cDNA libraries were constructed from the samples described above (Dpre, Dp, NDpre, and NDp). After filtering raw reads (reads containing adaptor, reads containing N larger than 10%, and low-quality reads (Qphred < 20) were removed), clean reads were retained (Table 1). The clean data were assembled by Trinity and Corset with 87,022 unigenes, and an average length of 1,604 nt and an N50 of 3,148. Of the unigenes, 35,231 (40.5%) were longer than 1000 bp (Table 2).

In order to gain information of gene function, transcripts were annotated using BLASTX searches against the non-redundant (NR) sequence database; 39,969 (45.92%) of them displayed homology to known proteins ($E < 1e^{-5}$; Fig. 1A). Nearly 25,000 annotated unigenes, over 65% of the annotated unigenes, were homologous to *T. pretiosum*, probably because the genome of *T. pretiosum* was the only available one in *Trichogramma*. Fewer unigenes were homologous to *Nasonia vitripennis* (1,217, 3.1%), *Apis florea* (26, 0.03%), *A. dorsata* (12, 0.01%), or *A. cerana* (15, 0.01%) (Fig. 1B). Less than 40 unigenes were matched to those from *Microplitis demolitor*. Among all annotated unigenes, 73.0% unigenes had significant homology with an E-value of $<10^{-30}$ (Fig. 1C), and 52.3% had a similarity higher than 80.0% (Fig. 1D). After filtering and removing redundant sequences, we kept those with significant BLAST hits and constructed a non-redundant dataset containing 7,593 sequences with an average length of 3,351 nt. Based on the annotation, such as gene length, ID, and speculative function, the diapause-related genes and potential genes involved in diapause were sorted out for further analysis.

Identification of DEGs and functional classification

Ten genes were selected for validation with qPCR, and glyceraldehyde phosphate dehydrogenase (*GAPDH*) was selected as the reference gene after measuring its stable expression level for diapause and non-diapause. The tendencies of these genes' expression profilings were similar according to RNA-Seq and qPCR (Fig. 2). Among these ten selected genes, all except *trehalase* (*tre*) were up-regulated during diapause.

To get more acquainted with diapause-specific transcriptional changes in *T. dendrolimi* induced by low temperature, we performed pairwise comparisons between different libraries to identify the DEGs. The results showed that there were 5,702 DEGs were identified among four groups. Among these DEGs, there were 3,182 DEGs changed in Dpre compared to NDpre. While, DESeq2 identified 3,251 and 3,442 DEGs exclusively changed in Dp vs NDp and Dpre vs Dp, respectively. In addition, DEGs changed in NDpre vs NDp were 1,511. This group of DEGs may be the genes relative to normal development, namely from prepupa to pupa, not to diapause development. According to the venn diagram, there were 463 genes

changed throughout the diapause development process, while in normal development process, the expression of these genes did not change (Fig. 3).

To figure out the potential function of identified DEGs, GO enrichment was performed. From the results, in all combination except for Dpre vs Dp, more genes were up-regulated. But, when compared Dpre to Dp, there was little difference in the number of up- and down-regulated genes. Besides, more DEGs were assigned to the same category among different groups. Regulation of transcription, DNA-templated, oxidation-reduction process, and signal transduction were the top three in all these four groups. Interestingly, the number of DEGs involved in ribosome biogenesis was much higher during diapause development than normal development (Fig. 4). Moreover, the subsequent analyses are based directly upon these results.

Comparative analysis of genes involved in diapause

Diapause is a dynamic process accompanied by a series of physiological transitions. Several studies have focused on the general gene expression pattern of insect diapause without clear elucidation of the diapause mechanism. This is due to the complexity of the diapause process as well as the variations among insect species.

According to the results of GO enrichment, firstly, we focused on the genes enriched in the oxidation-reduction process, regulation of transcription, DNA-templated and signal transduction. In addition, ribosome biogenesis was something that we were looking at.

A total of 342 genes were identified in oxidation-reduction process, 16 of which belong to cytochrome P450s (CYP450s). CYP450s are hemoproteins involved in several physiological processes, such as biosynthesis of hormones and degradation of xenobiotics [36]. There are four clans in P450 supergene family, namely CYP2, CYP3, CYP4, and Mitochondrion (Mito) [37]. In *T. dendrolimi* transcriptome, 22 CYP450s were identified, and 16 were differentially expressed. These 16 genes belonged to 4 clans. In diapause stages (Dpre), there were ten genes were up-regulated. While, in the pupae after diapause (Dp), five genes highly expressed. And only one gene (CYP9E2) was extremely up-regulated in normal pupae (NDp) (Fig. 5). From these results, we can see that the number of up-regulated genes during diapause in this species was significantly higher than that in other stages. Previous studies showed that CYP450 of *Schistosoma mansoni* was essential for worm survival and egg development [38]. Besides, CYP4G1 was proved to be related to cuticular hydrocarbon biosynthesis in *Drosophila* [39]. These genes were up-regulated in diapause individuals, suggesting that when *T. dendrolimi* entered diapause, the environmental conditions were not suitable for survival. The condition was worse than that under normal condition. In this process, many harmful substances may be produced. Thus, a possible function for these genes is to balance out harmful substances, maintaining cellular homeostasis. Besides, this phenomenon still occurred in pupae after diapause (Dp) compared to that under normal condition (NDp). Given to this result, we could infer that although diapause helped insect survive adverse environments,

the process itself also had an effect on insect growth. Diapause development is a tradeoff that insects make in order to survive.

According to the results, there were 36 transcription factors differentially expressed during diapause development. Based on these results, it was speculated that three kinds of transcription factors might be associated with diapause in *T. dendrolimi*.

Firstly, zinc finger protein. There were three genes encoded zinc finger protein, zinc finger protein 271, zinc finger 184, and zinc finger 544, were identified in the transcriptome. They were all up-regulated that the expressions of these three genes of Dpre were higher than that of Dp. Zinc finger protein gene 271 had an SFP domain. Genes containing this domain were considered to be a putative transcriptional repressor during G2/M (second gap period to mitotic period) transition. One of the most noticeable characteristics of diapause is the blockage of ontogeny, and this blockage always occurs with the cell cycle cessation [40, 41]. In *N. vitripennis*, the S phase of the cell cycle disappeared in the beginning stage of diapause due to the cells being arrested in the G0/G1 (stop cell division to first gap) and G2 phases[42]. Similarly, in drosophilid fly, *Chymomyza costata*, the cell cycle of CNS cells was arrested in the G0/G1 (86.6%) and G2 (12.8%) division phases during diapause [43]. The *wee1* gene, encoding for a kind of inhibitory kinase, was up-regulated during diapause in this species[43]. We obtained similar results in *T. dendrolimi* (Fig. 6). Therefore, the wee1 gene might be a molecular marker of diapause. Besides, zinc finger protein 184 contained a GDT1 domain, which is a putative Ca^{2+}/H^{+} antiporter. Ca^{2+}/H^{+} antiporter, which maintain the homeostasis, has been studied a lot in plants, but there are few studies in insects.

Second, homeobox domain proteins. In *T. dendrolimi* transcriptome, 11 homeobox-containing genes were differentially expressed during diapause stage except for *pit1*, which was significantly up-regulated in the individuals which terminated diapase. Among these genes, homeobox protein homothorax (*hth*) showed the greatest change in expression, followed by homeotic protein distal-less (*dll*) and homeobox protein six1 (*six1*). In *Tribolium castaneum*, during embryogenesis, *hth* plays a broad role in the segmentation process and is required for specification of body wall identities in the thorax [44]. In other species, like *D. melanogaster*, *hth* has different functions in different tissues. *Hth* located in head leads to opposite effects on eye and antennal development as a negative regulator of eye development, and it acts with extradenticle (*exd*) to delimit the eye field and prevent inappropriate eye development [45]. Transcriptome factor *dll* plays a role in larval and adult appendage development [46]. The gene expression of another homeobox protein six1 was similar to *hth*, expression of which increased after entering diapause stage. And this gene is postulated to be involved in the regulation of cell proliferation, apoptosis, and embryonic development. In mammals, *six1* is essential for early neurogenesis in the development of olfactory epithelium [47]. They were crucial genes regulating the myogenesis and extremely up-regulated during diapause in *T. dendrolimi*. But the aberrant expression of these genes may cause the cessation of growth. So, we inferred that these genes up-regulated at diapause stage blocked the normal cell cycle in diapause *T. dendrolimi.*

Third, forkhead box proteins. In previous studies, FOXOs have been identified as promising candidates for the molecular control of embryonic diapause in some species, like *Culex pipiens* [22, 48]. In *T. dendrolimi*, three forkhead box proteins, *foxo*, forkead box protein E3 (*foxe3*), and forkhead box protein D3 (*foxd3*) were identified. These genes were up-regulated both in the prepupae and pupae individual in diapause conditions. So, we inferred that they may play a role in diapause development.

In signal transduction term, a variety of enzymes were speculated to play an important role in diapause. Protein phosphatase 2A (PP2A) was one that caught our attention. It is a key serine-threonine protein phosphatase, which regulates several cellular processes, including metabolism, transcription, cell cycle, autophagy, and signal transduction [49, 50]. Cell cycle withdrawal, from G1 to S stage, is negative related to the activity of PP2A [51, 52]. In *T. dendrolimi* transcriptome, the expression of PP2A was down-regulated during diapause stage. This result was consistent with that obtained in the cotton bollworm, *Helicoverpa armigera.* Low PP2A expression in diapause-destined individuals contributed the accumulation of *p*-Akt, and *p*-Akt leads to *H. armigera* into diapause [24, 53]. And based on the previous studies, PP2A plays a multi-faceted role in the regulation of a couple of pathways, such as mTOR and wnt signaling pathway, which were related to cell cycle [49, 54]. So, we supposed that PP2A may have a certain function in *T. dendrolimi* diapause.

Except for these three biological processes, ribosome biogenesis also has an vital role in the control of cell growth and division in eukaryotes and is worth paying attention to [55]. In the present study, ribosome biogenesis involves 31 DEGs, of which 29 genes were up-regulated during diapause in the prepupae stage. Only two genes, 40S ribosomal protein S11 (*rpS11*) and 28S ribosomal protein S5 (*rpS5*). And all the 60S ribosomal proteins were up-regulated (Fig. 7). It has been reported that the rate of ribosome synthesis during diapause was lower than that of non-diapause eggs in *Bombyx mori*, so the up-regulation of ribosomal proteins played an important role in blocking diapause [56, 57]. In mosquitos, *C. pipiens*, the expression of ribosomal protein S3a (*rpS3a*) was dramatically reduced for a short time in diapause stage. After the injection of *rpS3a* dsRNA into non-diapaused females, the development of follicle was arrested, similarly to in the diapause state [58]. Conversely, in *T. dendrolimi*, diapause prepupae had a higher expression of ribosomal proteins compared to non-diapause wasps. It is noticeable that *B. mori* and *C. pipiens* enter diapause as adults, whereas *T. dendrolimi* enter diapause as prepupae. Although diapause decreased metabolism level, they may still need more energy to maintain the diapause condition. It was the first time to report that the ribosomal protein was related to diapause in *T. dendrolimi*. But the specific function of these genes involving in diapause still need to be verified.

In addition, some genes, even if they were not involved in the biological process with a considerable number of genes enriched, were still important in diapause development of *T. dendrolimi*. Taking the following genes for examples.*p53* induces growth arrest or apoptosis and has a negative regulation on cell division by controlling an array of genes in this process [59]. In addition, *p53* and DNA damage-regulated gene 1 (*pdrg1*) are involved in multiple cellular processes, such as apoptosis, DNA damage repair, cell cycle. In *Artemia sinica*, PDRG1 takes an essential role in diapause termination and regulation

of cell cycle during early embryonic development [60]. By blocking the expression of *pdrg1* in human colon cancer cells, cell growth reduced significantly. Transcriptome based analysis indicated that the expression of *p53* significantly increased during diapause, and the contrary result was observed for pdrg1 in *T. dendrolimi*. Moreover, this result also revealed that apoptosis activity was enhanced although the diapause individual remained in a dormancy state. It is speculated that, the wasps had to face the adverse condition, when more harmful substances could be accumulated during diapause. So, it was necessary to boost apoptosis activity for wasp to survive.

UDP-glucuronosyltransferase (*UDPGT*) is of central importance in the union and next elimination of toxic xenobiotics and endogenous compounds. *Glutathione-S-transferase* (*GST*) belongs to a multifunctional protein family mainly located in the cytoplasm. Both are related to cellular detoxification. The transcriptional expression of *GST* and *UDPGT* were up-regulated during *T. dendrolimi* prepupal diapause. This was different from other species, such as solitary bee *Tetrapedia diversipes* and *Tetranychus urticae* in which transcripts are down-regulated during diapause [62]. In other species, the downregulation of *GST* and *UDPGT* might be correlated with non-feeding conditions, so the amount of exogenous substances decreases accordingly. However, *T. dendrolimi* is a kind of endoparasitoid wasp that spends its whole life cycle within the host egg, it means that starvation is a rare situation until the prepupae stage. Therefore, we speculated that the reason for the observed up-regulation of these two genes in diapause *T. dendrolimi* might be due to the increased resistance under unfavorable environmental conditions.

Lipid metabolism is essential for energy homeostasis. It has been shown that some diapausing insects use lipids as predominant energy stores[63, 64]. During diapause, almost all selected lipid metabolism related genes were up-regulated, coinciding with the mobilization of TAG reserves (Fig. 8). These genes have important influence in the formation of triacylglycerol, which is the main caloric reserve during diapause. In addition, several genes' expressions significantly increased after diapause termination. This might be due to post-diapause development. In our previous study, diapause *T. dendrolimi* had higher numbers of parasitized hosts than non-diapause *T. dendrolimi* [27]. The increasing lipid storage could provide more energy for maintaining activities.

Conclusions

Diapause is not only an important physiological process in insects but has also shown great potential as an effective method for the preservation of natural enemy commodities. Taking advantage of the characteristics of diapause, the developmental cycle can elongate to increase the application efficiency of *T. dendrolimi*. This is important to the mass production of *Trichogramma* on commercial and industrialized scales. Although diapause has been successfully used as a technical means, the molecular mechanism of diapause remains largely unknown.

In this study, we compared gene expression profiles among different diapause stages of *T. dendrolimi*. Our results were either consistent with previous studies or provided additional information to understand the mechanism of diapause. Other novel genes, such as *p53* and *pdrg1* might be relevant to diapause or

apoptosis processes. Further studies are needed to elucidate the functions of candidate genes during diapause. Hence, we should not ignore the role of independent genes.

Our results not only provide crucial information to generate the genetic diapause toolkit but also establish a basis for improving the practical application of *T. dendrolimi* in biological control programs. However, as diapause is a complex process influenced by many factors, future studies are required to build a dynamic network to elucidate the adaptability between insects and environment.

Methods

Insect culture

T. dendrolimi adult wasps were gained from a corn field in Yitong, Jilin Province, China (125°11'E, 43°3'N) in 2015. The species was identified through the morphological characteristics of male genital capsules. A wasp population was reared on the eggs of *Antheraea pernyi* (fresh eggs dissected from the ovaries of female *A. pernyi* were supplied in glass tubes to newly emerged *T. dendrolimi* for oviposition), maintaining under the condition of 26°C ± 1°C and 60% ± 5% relative humidity (RH) with a 16: 8-h light: dark (L: D) photoperiod.

Diapause induction and termination

Approximately 1,000 eggs of *A. pernyi* were parasitized for 2 h by approximately 2,500 *T. dendrolimi* adults. Then, the parasitized host eggs were separated into two groups for different treatments (Table 3). *T. dendrolimi* entered diapause at the prepupa stage. Diapause prepupae and pupae after diapause were denoted as Dpre and Dp, respectively. Non-diapause prepupae and non-diapause pupae were denoted as NDpre and NDp. The methods of diapause induction and termination in this experiment were selected based on those of Zhang et al. (2017) [18]. Specifically, diapause was induced by keeping *T. dendrolimi* at 12 °C for 30 days, and diapause was terminated by keeping *T. dendrolimi* at 3 °C for 70 days (Fig. S1; Table 3).

RNA isolation and qualification and library preparation

Total RNA was extracted from four sample sets described above using TRIzol Reagent (Sigma Aldrich, St. Louis, MO). Three replicates of four parasitized eggs were evaluated for each experiment.

A total of 1.5 μg RNA per sample was used as input material for library preparation. Libraries used for sequencing were generated using the NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations, and index codes were added to attribute sequences in each sample. The prepared libraries were then sequenced with Illumina HiSeq (Novogene, China). The RNA-seq

raw reads were deposited as project number PRJNA597631 in the Sequence Read Archive of the National Center for Biotechnology Information (NCBI). Three replicates were sequenced for each sample.

De novo transcriptome assembly and functional annotation

Firstly, raw data (raw reads) were processed using in-house Perl scripts. Clean data, namely clean reads, were got after removing adaptor, ploy-N, and low-quality reads. In the meantime, the Q20, Q30, GC-content, and sequence duplication level were measured. *De novo* transcriptome assembly was performed with Trinity 2.4.0 software [72] with min_kmer_cov set to 25 and all other parameters at the default values [65]. All assembled unigenes were aligned using DIAMOND v0.8.22, NCBI blast 2.2.28+, HMMER 3.0, and KAAS, and with the protein and nucleotide sequences in five public databases (NR, NT, Pfam, KEGG, and Swiss-Prot) with a threshold of e < 0.00001. Functional annotation of all the unigenes was carried out using the Blast2GO v2.5 [66], and databases Gene Ontology (GO) and Clusters of Orthologs Groups for Eukaryotic Complete Genomes (KOG) were used.

Identification of differential expression genes (DEGs) and functional classification

Transcript expression levels were estimated by RSEM (RNA-Seq by Expectation-Maximization) [67]. Clean data were mapped back to the assembled transcriptome, and the read count for each gene was obtained from the mapping results. Then the read count was transferred into FPKM (fragments per kilobase million), which was expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced. DEGs between four conditions (Dpre vs. NDpre; Dpre vs. Dp; NDpre vs. NDp; Dp vs NDp) were identified using the DESeq R package. The resulting *P*-values were adjusted using the Benjamini and Hochberg's method to control the false discovery rate (FDR). Transcripts with an adjusted *P*-value less than 0.05 and fold change greater than two found by DESeq were considered as differentially expressed. Venn diagram (http://bioinformatics.psb.ugent.be/beg/) was used to compare these four DEG lists.

Gene Ontology (GO) enrichment analysis of DEGs was implemented by the GOseq R package [68]. For GO enrichment analysis, corrected *P*-values less than 0.05 were considered as significantly enriched in DEGs. Then, the top ten biological processes involved in these four lists were also analyzed. According to the results of GO enrichment analysis, candidate genes, which related to different stage during diapause development in *T. dendrolimi*, were selected and analyzed.

Validation experiment by quantitative real-time PCR (qPCR) analysis

Quantitative real-time PCR (qPCR) was performed on a qTOWER^{3}G system (Analytikjena, Germany) using SYBR green PCR Master Mix (Tiangen, China). The thermal cycling conditions were as follows: one cycle

of 95°C for 5 min, 40 cycles of 95°C for 5 s, and 60°C for 15 s, following the melt curve program. The *GAPDH* of *T. dendrolimi* was selected as the internal standard to normalize cDNA templates. Primers with product sizes of 100-200 bp were designed with Primer Premier 6 using the default settings. The selected candidate genes and corresponding primers are listed in Table 4. Three biological replicates were performed for each treatment with four technical replicates for each primer pair, and the relative transcript expression level among the different treatments were measured by the $2^{-\Delta\Delta Ct}$ method.

List Of Abbreviations

RNA-Seq: RNA sequencing

GO: Gene Ontology

Dpre: diapause prepupae

Dp: pupae after diapause

NDpre: non-diapause prepupae

NDp: non-diapause pupae

NR: Non-redundant

DEGs: differentially expressed genes

GAPDH: glyceraldehyde phosphate dehydrogenase

tre: trehalase

CYP450s: cytochrome P450s

Mito: mitochondrion

CNS: central nervous system

hth: homeobox protein homothorax

dll: homeotic protein distal-less

exd: extradenticle

foxo:forkhead box protein O

foxe3: forkhead box protein E3

foxd3: forkhead box protein D3

PP2A: protein phosphatase 2A

rpS11: ribosomal protein S11

rpS5: ribosomal protein S5

rpS3a: ribosomal protein S3a

pdrg1: DNA damage-regulated gene 1

UDPGT: UDP-glucuronosyltransferase

GST: Glutathione-S-transferase

TAG: triacylglycerol

KOG: Clusters of orthologs groups for eukaryotic complete genomes

FPKM: fragments per kilobase million

RSEM: RNA-Seq by expectation-Maximization

FDR: false discovery rate

qPCR: quantitative real-time PCR

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Because a lot of data related to the ongoing research. So, the data have been submitted to the NCBI while the data will release until the paper published.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the National Key R&D Program of China (Grant No. 2017YFD0200400, 2019YFC1200504). The funder of No.2017YFD0200400 was JJZ and he designed the research and preformed part of the research. The funder of No.2019YFC1200504 was ZZ and he designed the research, analyzed the data and wrote the manuscript.

Authors' contributions

JJZ, ZZ, and CCR designed the research; XZ, JJZ and WMD preformed the research; ZZ and XZ analyzed the data; and XZ and ZZ wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Not applicable

References

1. Yuan XH, Song LW, Zhang JJ, Zang LS, Zhu L, Ruan CC, Sun GZ: Performance of four Chinese *Trichogramma* species as biocontrol agents of the rice striped stem borer, *Chilo suppressalis*, under various temperature and humidity regimes. *J Pest Sci* 2012, 85(4):497-504.
2. Tian JC, Wang ZC, Wang GR, Zhong LQ, Zheng XS, Xu HX, Zang LS, Lu ZX: The effects of temperature and host age on the fecundity of four *Trichogramma* species, egg parasitoids of the *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). *J Econ Entomol* 2017, 110(3):949-953.
3. Huang J, Hua HQ, Wang LY, Zhang F, Li YX: Number of attacks by *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) affects the successful parasitism of *Ostrinia furnacalis* (Lepidoptera: Crambidae) eggs. *Bull Entomol Res* 2017, 107(6):812-819.
4. Zhang JJ, Ruan CC, Zang LS, Shao XW, Shi SS: Technological improvements for mass production of *Trichogramma* and current status of their applications for biological control on agricultural pests in China. *Chinese Journal of Biological Control* 2015, 31(5):638-646.
5. Pitcher SA, Hoffman MP, Gardner J, Wright MG, Kuhar TP: Cold storage of *Trichogramma ostriniae* reared on Sitotroga cerealella eggs. *BioControl* 2002, 47:525-535.
6. Ma CS, Chen YW: Effects of constant temperature, exposure period, and age on diapause induction in *Trichogramma dendrolimi*. *Biological Control* 2006, 36(3):267-273.
7. Rundle B, Thomson L, Hoffmann A: Effects of cold storage on field and laboratory performance of *Trichogramma carverae* (Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. carverae*, *T. nr. brassicae*, and *T. funiculatum*) to cold. *J Econ Entomol* 2004, 97(2):213-221.
8. Chen KW, Liu LZ, Fang CM, Huang SS: Effect of cold storage on mass-rearing population quality of *Trichogrammatoidea bactrae*. *Journal of South China Agricultural University* 2004, 25(2):56-59.
9. Cira TM, Koch RL, Burkness EC, Hutchison WD, Venette RC: Effects of diapause on *Halyomorpha halys* (Hemiptera: Pentatomidae) cold tolerance. *Environmental entomology* 2018, 47(4):997-1004.

10. Zhang Q, Denlinger DL: Molecular characterization of heat shock protein 90, 70 and 70 cognate cDNAs and their expression patterns during thermal stress and pupal diapause in the corn earworm. *J Insect Physiol* 2010, 56(2):138-150.
11. Lin X, Xu W: Hexokinase is a key regulator of energy metabolism and ROS activity in insect lifespan extension. *Aging* 2016, 8(2):245-258.
12. Zhang XS, Wang T, Lin XW, Denlinger DL, Xu WH: Reactive oxygen species extend insect life span using components of the insulin-signaling pathway. *PNAS* 2017, 114(37):E7832-E7840.
13. Fenelon JC, Renfree MB: The history of the discovery of embryonic diapause in mammals. *Biology of reproduction* 2018, 99(1):242-251.
14. Denlinger DL, Armbruster PA: Mosquito diapause. *Annu Rev Entomol* 2014, 59:73-93.
15. Hand SC, Denlinger DL, Podrabsky JE, Roy R: Mechanisms of animal diapause: recent developments from nematodes, crustaceans, insects, and fish. *American journal of physiology Regulatory, integrative and comparative physiology* 2016, 310(11):1193-1211.
16. Ahmadi F, Moharramipour S, Mikani A: The effect of temperature and photoperiod on diapause induction in pupae of *Scrobipalpa ocellatella* (Lepidoptera: Gelechiidae). *Environmental entomology* 2018, 47(5):1314-1322.
17. Ryan SF, Valella P, Thivierge G, Aardema ML, Scriber JM: The role of latitudinal, genetic and temperature variation in the induction of diapause of *Papilio glaucus* (Lepidoptera: Papilionidae). *Insect Sci* 2018, 25(2):328-336.
18. Zhang JJ, Desneux N, Benelli G, Zang LS, Du WM, Ruan CC: Geographic variation of diapause induction rates in *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) in China. *J Econ Entomol* 2017, 110(2):386-391.
19. Takano Y, Ullah MS, Gotoh T: Effect of temperature on diapause termination and post-diapause development in *Eotetranychus smithi* (Acari: Tetranychidae). *Experimental & applied acarology* 2017, 73(3-4):353-363.
20. Hiroyoshi S, Reddy GVP, Mitsuhashi J: Effects of photoperiod, temperature and aging on adult diapause termination and post-diapause development in female Asian comma butterflies, *Polygonia caureum* Linnaeus (Lepidoptera: Nymphalidae). *Journal of comparative physiology A, Neuroethology, sensory, neural, and behavioral physiology* 2018, 204(9-10):849-858.
21. Chen W, Xu WH: Wnt/beta-catenin signaling regulates *Helicoverpa armigera* pupal development by up-regulating c-Myc and AP-4. *Insect Biochem Mol Biol* 2014, 53:44-53.
22. Sim C, Kang DS, Kim S, Bai X, Denlinger DL: Identification of FOXO targets that generate diverse features of the diapause phenotype in the mosquito *Culex pipiens*. *PNAS* 2015, 112(12):3811-3816.
23. Lin XW, Tang L, Yang J, Xu WH: HIF-1 regulates insect lifespan extension by inhibiting c-Myc-TFAM signaling and mitochondrial biogenesis. *Biochim Biophys Acta* 2016, 1863(11):2594-2603.
24. Li HY, Wang T, Yang YP, Geng SL, Xu WH: TGF-β signaling regulates p-Akt levels via PP2A during diapause entry in the cotton bollworm, *Helicoverpa armigera*. *Insect Biochem Mol Biol* 2017, 87:165-173.

25. Post S, Liao S, Yamamoto R, Veenstra JA, Nassel DR, Tatar M: Drosophila insulin-like peptide dilp1 increases lifespan and glucagon-like Akh expression epistatic to dilp2. *Aging Cell* 2018, 18(1):e12863.
26. Song Z, Yang YP, Xu WH: PTEN expression responds to transcription factor POU and regulates p-AKT levels during diapause initiation in the cotton bollworm, *Helicoverpa armigera*. *Insect Biochem Mol Biol* 2018, 100:48-58.
27. Zhang JJ, Zhang X, Zang LS, Du WM, Hou YY, Ruan CC, Desneux N: Advantages of diapause in *Trichogramma dendrolimi* mass production on eggs of the Chinese silkworm, *Antheraea pernyi*. *Pest Manag Sci* 2018, 74(4):959-965.
28. Antony B, Johny J, Abdelazim MM, Jakse J, Al-Saleh MA, Pain A: Global transcriptome profiling and functional analysis reveal that tissue-specific constitutive overexpression of cytochrome P450s confers tolerance to imidacloprid in palm weevils in date palm fields. *BMC Genomics* 2019, 20(1):1-23.
29. Xu N, Sun XH, Liu ZH, Xu Y, Sun Y, Zhou D, Shen B, Zhu CL: Identification and classification of differentially expressed genes in pyrethroid-resistant *Culex pipiens* pallens. *Mol Genet Genomics* 2019, 294(4):861-873.
30. Peterson B, Sanko TJ, Bezuidenhout CC, van den Berg J: Transcriptome and differentially expressed genes of *Busseola fusca* (Lepidoptera: Noctuidae) larvae challenged with Cry1Ab toxin. *Gene* 2019, 710:387-398.
31. Li Z, Yu T, Chen Y, Heerman M, He J, Huang J, Nie H, Su S: Brain transcriptome of honey bees (*Apis mellifera*) exhibiting impaired olfactory learning induced by a sublethal dose of imidacloprid. *Pestic Biochem Physiol* 2019, 156:36-43.
32. Govaere L, Morin MD, Frigault JJ, Boquel S, Cohen A, Lamarre SG, Morin PJ: Transcriptome and proteome analyses to investigate the molecular underpinnings of cold response in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Cryobiology* 2019, 88:54-63.
33. Xiong Y, Liu XQ, Xiao PA, Tang GH, Liu SH, Lou BH, Wang JJ, Jiang HB: Comparative transcriptome analysis reveals differentially expressed genes in the Asian citrus psyllid (*Diaphorina citri*) upon heat shock. *Comp Biochem Physiol Part D Genomics Proteomics* 2019, 30:256-261.
34. Wu Z, Kang C, Qu M, Chen J, Chen M, Bin S, Lin J: Candidates for chemosensory genes identified in the Chinese citrus fly, *Bactrocera minax*, through a transcriptomic analysis. *BMC Genomics* 2019, 20(1):646-660.
35. Hao K, Jarwar AR, Ullah H, Tu X, Nong X, Zhang Z: Transcriptome Sequencing Reveals Potential Mechanisms of the Maternal Effect on Egg Diapause Induction of Locusta migratoria. *Int J Mol Sci* 2019, 20(8):1974-1993.
36. Hannemann F, Bichet A, Ewen KM, Bernhardt R: Cytochrome P450 systems--biological variations of electron transport chains. *Biochim Biophys Acta* 2007, 1770(3):330-344.
37. Yin CL, Ye XH, Chen MY, Mei Y, M XH, Li F: Evolution Analysis of Cytochrome P450 Gene Family in Parasitoid Wasps. *Chinese Journal of Biological Control* 2019, 35(3):335-342.

38. Ziniel PD, Karumudi B, Barnard AH, Fisher EM, Thatcher GR, Podust LM, Williams DL: The *Schistosoma mansoni* cytochrome P450 (CYP3050A1) is essential for worm survival and egg development. *PLoS Negl Trop Dis* 2015, 9(12):e0004279.
39. Qiu Y, Tittiger C, Thomas C, Goff G, Young S, Wajnberg E, Fricaux T, Taquet N, Blomquist G, Feyereisen R: An insect-specific P450 oxidative decarbonylase for cuticular hydrocarbon biosynthesis. *PNAS* 2012, 109(14858-14863).
40. Chen DF, Lin C, Wang HL, Zhang L, Dai L, Jia SN, Zhou R, Li R, Yang JS, Yang F *et al*: An La-related protein controls cell cycle arrest by nuclear retrograde transport of tRNAs during diapause formation in *Artemia*. *BMC Biol* 2016, 14(16):1-13.
41. Chen L, Barnett RE, Horstmann M, Bamberger V, Heberle L, Krebs N, Colbourne JK, Gomez R, Weiss LC: Mitotic activity patterns and cytoskeletal changes throughout the progression of diapause developmental program in *Daphnia*. *BMC Cell Biol* 2018, 19(1):30-43.
42. Shimizu Y, Mukai A, Goto SG: Cell cycle arrest in the jewel wasp *Nasonia vitripennis* in larval diapause. *J Insect Physiol* 2018, 106(Pt 2):147-152.
43. Kostal V, Simunkova P, Kobelkova A, Shimada K: Cell cycle arrest as a hallmark of insect diapause: changes in gene transcription during diapause induction in the drosophilid fly, *Chymomyza costata*. *Insect Biochem Mol Biol* 2009, 39(12):875-883.
44. Smith FW, Jockusch EL: Hox genes require homothorax and extradenticle for body wall identity specification but not for appendage identity specification during metamorphosis of *Tribolium castaneum*. *Developmental Biology* 2014, 395(1):182-197.
45. Pai CY, Kuo TS, Jaw TJ, Kurant E, Sun YH: The Homothorax homeoprotein activates the nuclear localization of another homeoprotein, Extradenticle, and suppresses eye development in *Drosophila*. *Genes & Development* 1998, 12(3):435-446.
46. Vachon G, Cohen B, Pfeifle C, McGuffin ME, Botas J, Cohen SM: Homeotic genes of the Bithorax complex repress limb development in the abdomen of the *Drosophila* embryo through the target gene Distal-less. *Cell* 1992, 71(3):437-450.
47. Ikeda K, Ookawara S, Sato S, Ando ZI, Kageyama R, Kawakami K: Six1 is essential for early neurogenesis in the development of olfactory epithelium. *Developmental Biology* 2007, 311(1):1-68.
48. Sim C, Denlinger DL: Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. *PNAS* 2008, 105(18):6777-6781.
49. Wlodarchak N, Xing Y: PP2A as a master regulator of the cell cycle. *Critical reviews in biochemistry and molecular biology* 2016, 51(3):162-184.
50. Bánréti Á, Lukácsovich T, Csikós G, Erdélyi M, Sass M: PP2A regulates autophagy in two alternative ways in *Drosophila*. *Autophagy* 2012, 8(4):623-636.
51. Altiok S, Xu M, Spiegelman BM: PPARgamma induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. *Genes Dev* 1997, 11(15):1987-1998.
52. Kolupaeva V, Daempfling L, Basilico C: The B55α regulatory subunit of protein phosphatase 2A mediates fibroblast growth factor-induced p107 dephosphorylation and growth arrest in

chondrocytes. *Mol Cell Biol* 2013, 33(15):2865-2878.

53. Tian K, Xu WH: High expression of PP2A-Aα is associated with diapause induction during the photoperiod-sensitive stage of the cotton bollworm, *Helicoverpa armigera*. *J Insect Physiol* 2013, 59(6):588-594.
54. Tang Y, Berlind J, Mavila N: Inhibition of CREB binding protein-beta-catenin signaling down regulates CD133 expression and activates PP2A-PTEN signaling in tumor initiating liver cancer cells. *Cell communication and signaling : CCS* 2018, 16(1):9-21.
55. Chaillou T, Kirby TJ, McCarthy JJ: Ribosome biogenesis: emerging evidence for a central role in the regulation of skeletal muscle mass. *J Cell Physiol* 2014, 229(11):1584-1594.
56. Kurata S, Koga K, Sakaguchi B: Nueleolar size in parallel with ribosomal RNA synthesis at diapause termination in the eggs of *Bombyx mori Chromosoma* 1978, 68:313-317.
57. Gong J, Tian S, Zhou X, Yang H, Zhu Y, Hou Y: Transcriptional response of silkworm (*Bombyx mori*) eggs to O2 or HCl treatment. *Int J Mol Sci* 2016, 17(12):1838-1857.
58. Kim M, Sim C, Denlinger DL: RNA interference directed against ribosomal protein S3a suggests a link between this gene and arrested ovarian development during adult diapause in *Culex pipiens*. *Insect Mol Biol* 2010, 19(1):27-33.
59. Levine A: p53, the Cellular Gatekeeper for Growth and Division. *Cell* 1997, 88:323-331.
60. Zhang W, Yao F, Zhang H, Li N, Zou X, Sui L, Hou L: The potential roles of the apoptosis-related protein PDRG1 in diapause embryo restarting of *Artemia sinica*. *Int J Mol Sci* 2018, 19(1):126-144.
61. Perez C, Perez-Zuniga FJ, Garrido F, Reytor E, Portillo F, Pajares MA: The Oncogene PDRG1 Is an Interaction Target of Methionine Adenosyltransferases. *PLoS One* 2016, 11(8):e0161672.
62. Santos PKF, de Souza Araujo N, Francoso E, Zuntini AR, Arias MC: Diapause in a tropical oil-collecting bee: molecular basis unveiled by RNA-Seq. *BMC Genomics* 2018, 19(1):305-318.
63. Reynolds JA, Poelchau MF, Rahman Z, Armbruster PA, Denlinger DL: Transcript profiling reveals mechanisms for lipid conservation during diapause in the mosquito, *Aedes albopictus*. *J Insect Physiol* 2012, 58(7):966-973.
64. Batz ZA, Armbruster PA: Diapause-associated changes in the lipid and metabolite profiles of the Asian tiger mosquito, *Aedes albopictus*. *J Exp Biol* 2018, 221(Pt 24):jeb189480.
65. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q *et al*: Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol* 2011, 29(7):644-652.
66. Gotz S, Garcia-Gomez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talon M, Dopazo J, Conesa A: High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Res* 2008, 36(10):3420-3435.
67. Li B, Dewey C: RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 2011, 12:16.

68. Young MD, Wakefield MJ, Smyth GK, Oshlack A: Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biology* 2010, 11.

Tables

Table 1. Summary of Illumina transcriptome assembly for *T. dendrolimi*.

Sample name	Raw reads	Clean reads	Clean bases	Error rate (%)	Q20 (%)	Q30 (%)	GC content (%)
Dpre-A	49071974	47695622	7.15G	0.03	96.52	91.43	41.30
Dpre-B	49810990	47210830	7.08G	0.01	97.26	93.17	43.77
Dpre-C	47347676	44732274	6.71G	0.01	97.29	93.25	43.84
Dp-A	49517974	48189484	7.23G	0.02	95.98	90.34	41.11
Dp-B	51270660	48977252	7.35G	0.02	96.97	92.69	40.74
Dp-C	45943884	43816628	6.57G	0.02	96.84	92.38	42.41
NDpre-A	45701770	44515808	6.68G	0.01	96.28	90.97	40.07
NDpre-B	50860900	48522830	7.28G	0.01	97.37	93.48	41.57
NDpre-C	47589266	45544062	6.83G	0.01	97.36	93.45	38.73
NDp-A	56029606	54463466	8.17G	0.01	96.88	92.35	38.69
NDp-B	46605166	44410060	6.66G	0.01	97.19	93.11	40.63
NDp-C	62287702	59900798	8.99G	0.01	97.24	93.26	40.71

Note: A, B, and C represent the three biological replicates of each sample.

Table 2. General features of the *de novo* assembled transcriptome by Trinity.

	Transcripts	Unigenes
200-500 bp	131,509	22,869
500-1 kbp	42,525	28,922
1 k-2 kbp	18,308	18,119
>2 kbp	17,115	17,112
Total	209,457	87,022
Min length	201	201
Mean length	865	1,604
Median length	389	814
Max length	29,327	29,327
N50	1,732	3,148
N90	308	647
Total nucleotides	181,250,941	139,571,319

Table 3. Different treatments for *T. dendrolimi* used in this study.

State	Treatment	Sampling Period	Sample Set
Non-diapause	26 ± 1°C, 60 ± 5% RH, 16:8 L:D	Prepupal stage	NDpre
	26 ± 1°C, 60 ± 5% RH, 16:8 L:D	Pupal stage	NDp
Diapause	12 ± 1°C, 60 ± 5% RH, 0:24 L:D, 30 d	Prepupal stage	Dpre
	12 ± 1°C, 60 ± 5% RH, 0:24 L:D, 30 d 3 ± 1°C, 60 ± 5% RH, 0:24 L:D, 70 d 26 ± 1°C, 60 ± 5% RH, 16:8 L:D, until pupal stage	Pupal stage	Dp

[a] Groups 1 and 2 represent control and treatment, respectively.

Table 4. Candidate genes and primers used for qPCR analysis.

	Gene name	Primer sequence	Amplicon size (bp)
	Heat shock protein 68 (*HSP68*)	F: TTCTGCCGATGAGACGCTTGG R: TGCCTTCACCGACACCGAGA	104
	Autophagy-related protein 2 (*ATG2*)	F: TCTGGAGCAGTAGGTGGAGTGT R: GCAGCCTCATGTCTGGCATCT	137
	Autophagy-related protein 9 (*ATG9*)	F: TCGGCATCGTCAACTTCGTCCT R: GCAGATAGAGGCGGCTGTAGGT	144
	Autophagy-related protein 13 (*ATG13*)	F: CGCAGCAGCAACAACAACAACC R: TGGTAGTGGTCGCCGATCTCTG	111
	cyclin D	F: GCCACGAGCTGATCGAGGAGAT R: GCTGTTGCTGTTGCTGCTGTTG	102
	Trehalose transporter (*Tret*)	F: CGAGGCGAACATCCAGAAGGT R: GGCAGCATCAGCATCGTCAC	111
	Trehalase (*Tre*)	F: GCCGACATCACAACCGAAGACA R: TCGTTCCAGAGCACCTCGTCAA	173
	Fatty acid synthase (*FAS*)	F: CGACGAGAAGCAGTTCAAGGC R: CGGACGAGAAGCAGACGAAGT	116
	lipid storage droplets surface-binding protein 2 (*LSD2*)	F: GGCCGTGTCGAGGATCAACTAC R: ACGAGCATCTCCAGGACGAAGG	162
	stearoyl-CoA desaturase 5 (*SCD5*)	F: TGGAGTCGCTGGAGGAATACAC R: ACGGGATCGGCATCGGTTTC	174
rol	Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)	F: CCAGCCACCTACGACGAGATCA R: ACCACGAGATGAGCTTGACGAA	187

Figures

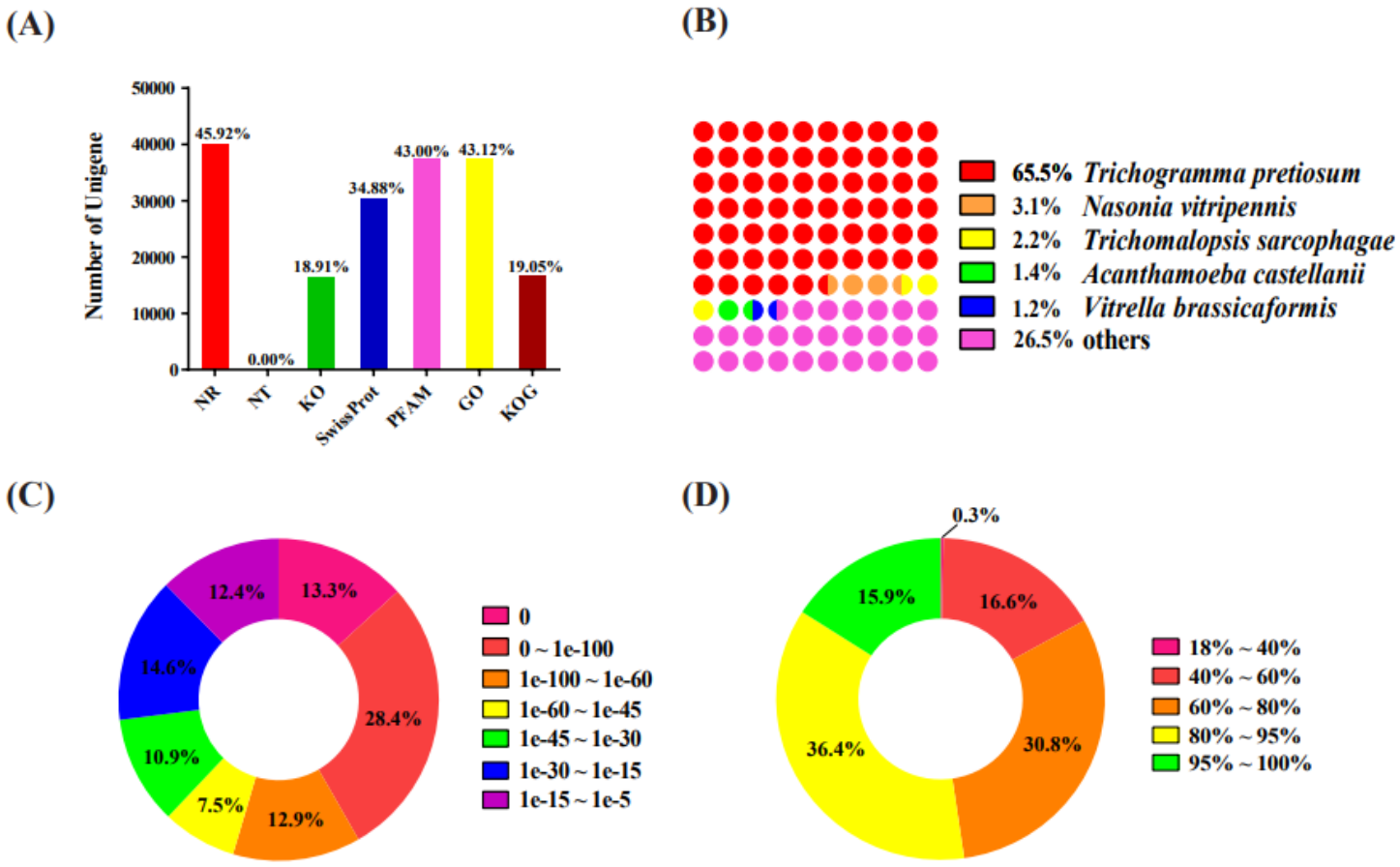

Figure 1

Sequence annotation and homology search against NR database for T. dendrolimi unigenes. (A) Annotation results in seven major databases. (B) Distribution of species of top BLAST hit. (C) Distribution of E-value of top BLAST hit with a cut-off E-value of 1e-5. (D) Distribution of similarity of top BLAST hit.

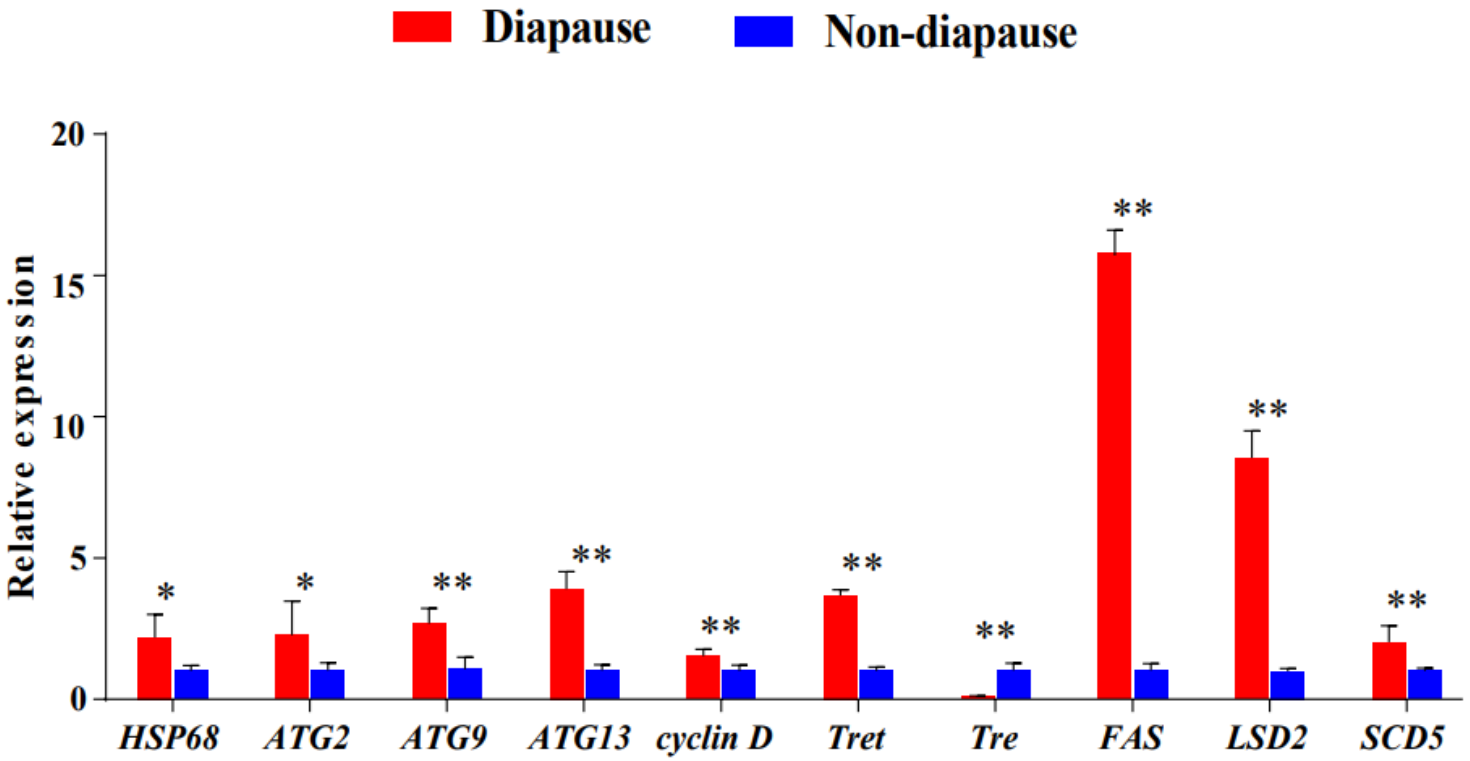

Figure 2

The relative expressions of 10 selected genes by qPCR analysis. Nine genes were up-regulated, and one gene was down-regulated during the diapause stage. The changing trends of all ten genes between diapause and non-diapause were identical between qPCR and RNA-Seq. The red bar represents the diapause, while the blue bar represents the non-diapause. The relative mRNA levels are represented as the mean ± S.D. *, $p < 0.05$; **, $p < 0.01$.

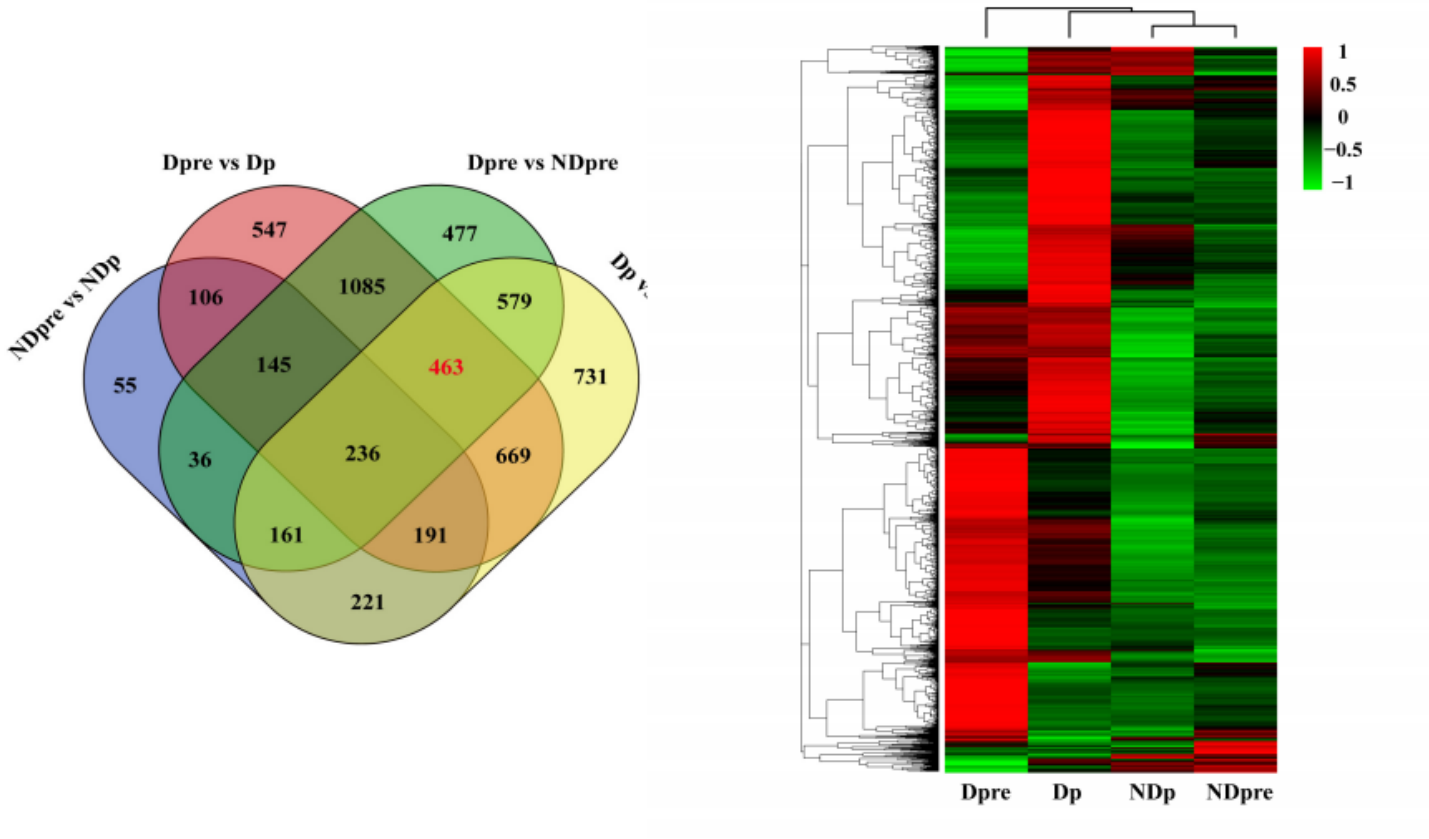

Figure 3

Venn graph and heatmap of DEGs across the four treatments. In heatmap graph, red indicates relatively high expression, green indicates relatively low expression, and black represents moderate expression.

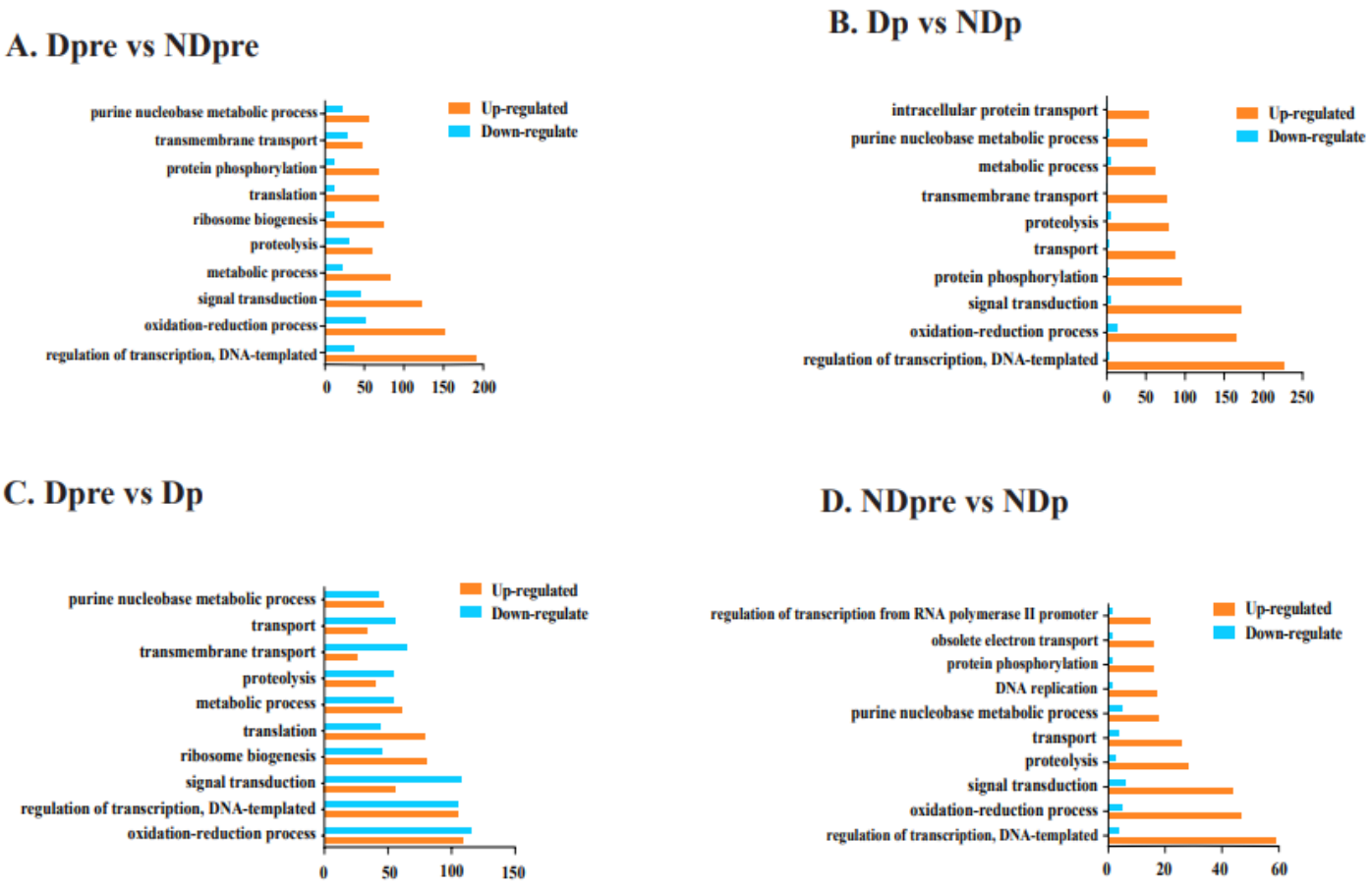

Figure 4

GO enrichment analysis. According to our data, ten GO items were selected according to the gene number of four comparisons. The value of the horizontal ordinate represented the number of DEGs in each GO item. Up- or down-regulated genes were coded by different colors.

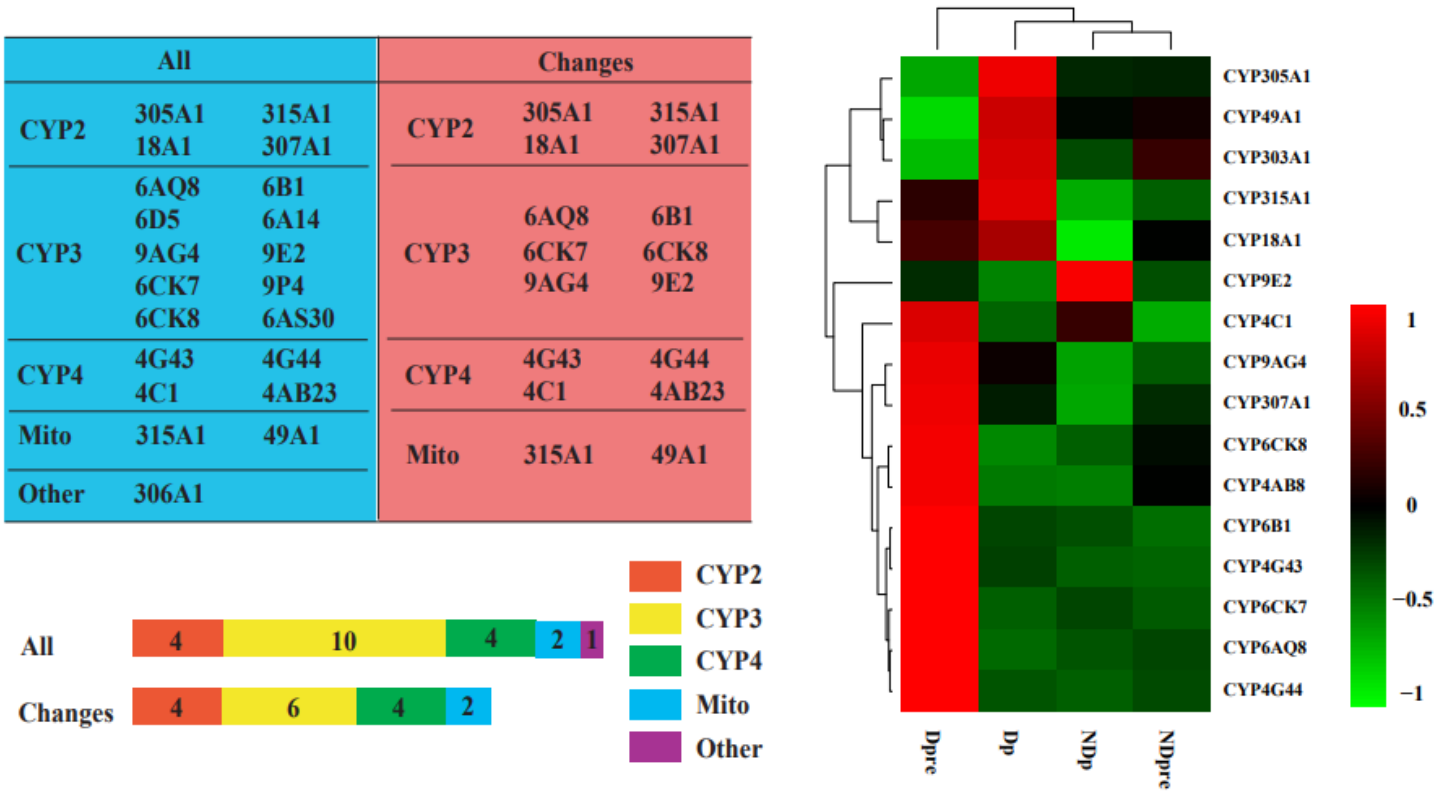

Figure 5

Cytochrome P450s (CYPs) genes selected in T. dendrolimi transcriptome. The left table compared the number of CYPs between two groups. ALL means all the unigenes of T. dendrolimi transcriptome, and Changes means the differentially expressed genes. The right heatmap graph showed the expression of differentially expressed CYPs genes.

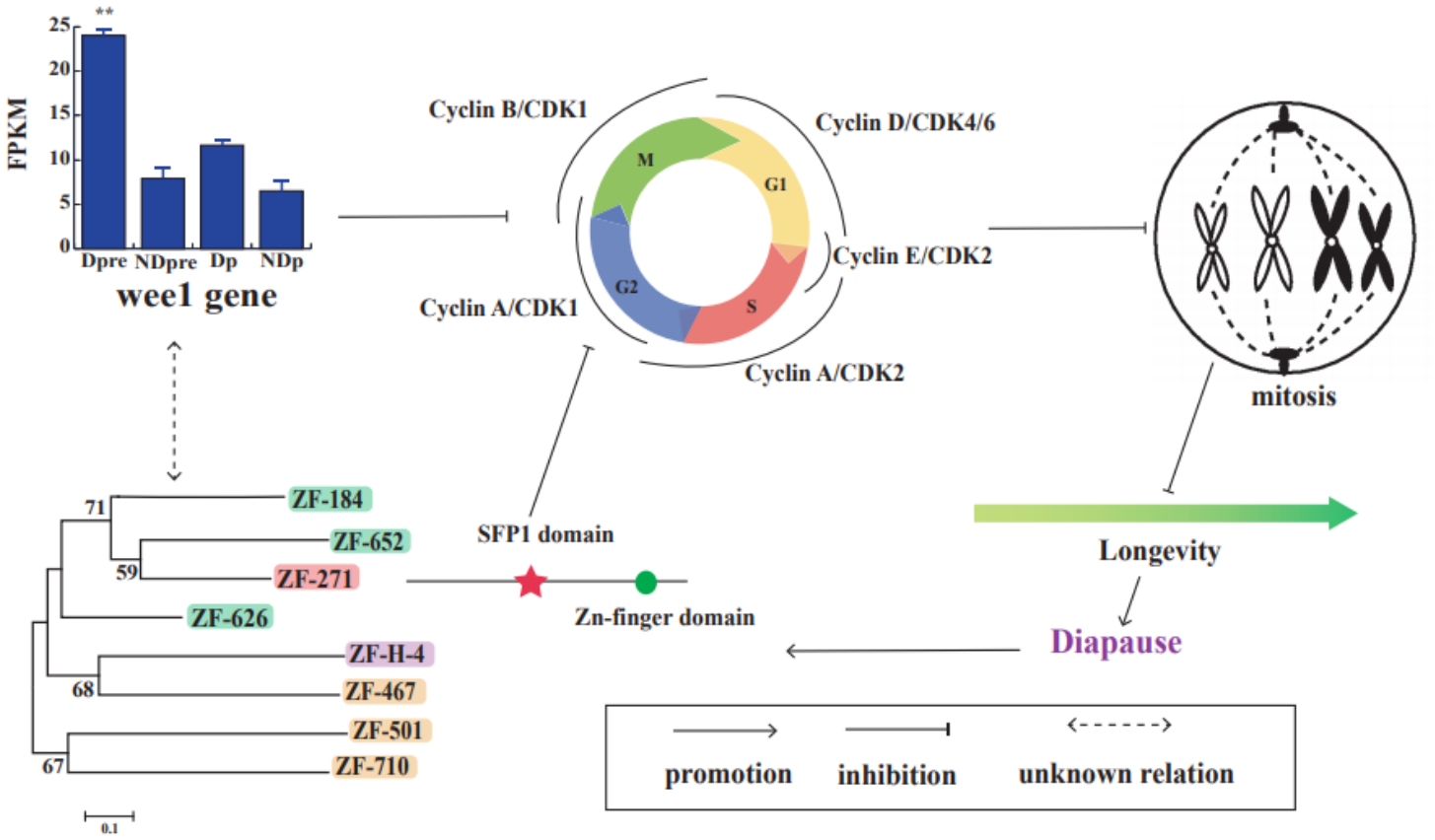

Figure 6

Speculated pattern diagram of zinc finger protein genes in cell cycle. Eight zinc finger protein genes were screened in our data. ZF-271, indicated with a read background color, showed an interesting SFP1 domain, which is a putative transcriptional repressor regulating G2/M transition. The expression of wee1 gene is also shown in the histogram. The wee1 gene has been studied in other species and has been shown to have the ability to make the cell stay at the G2 stage.

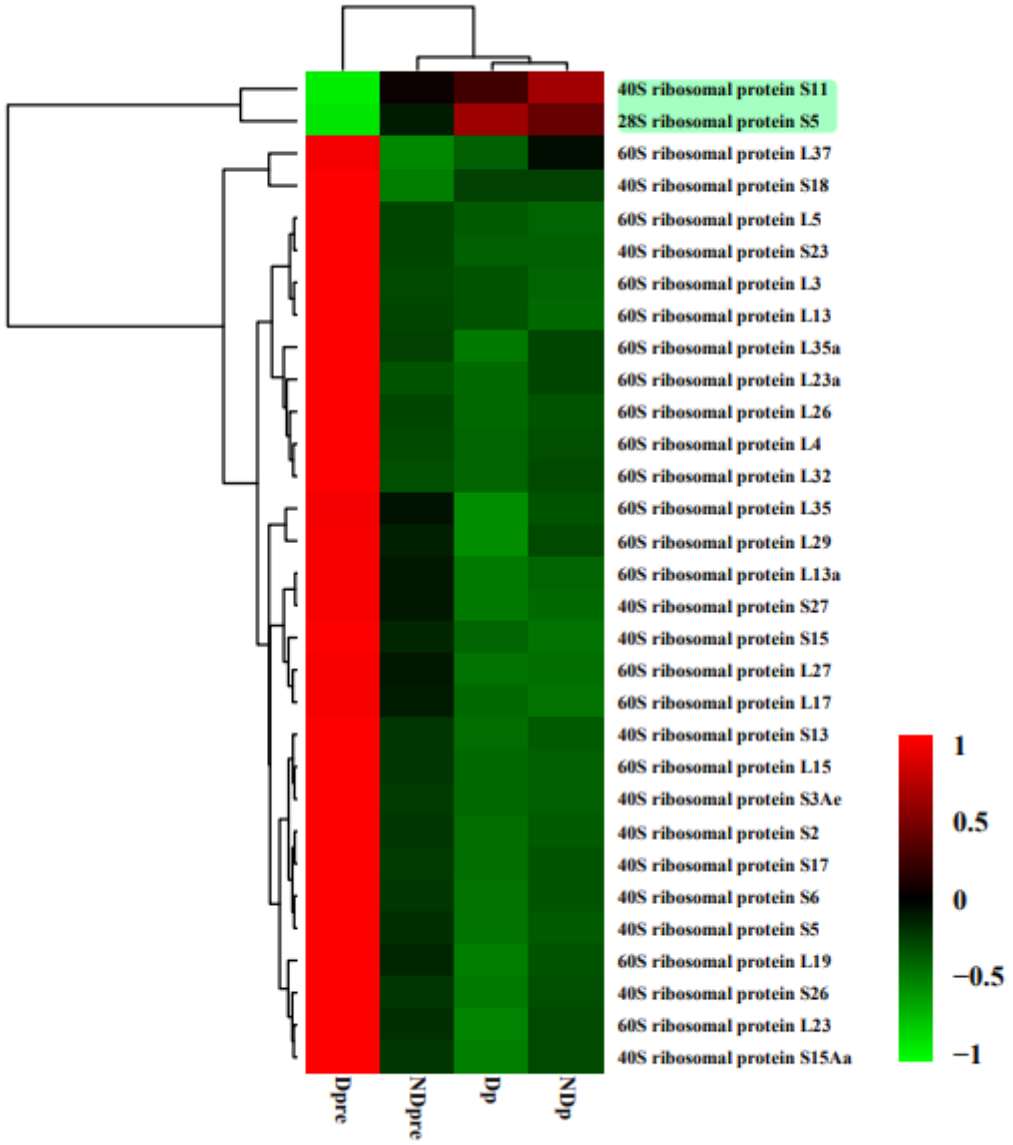

Figure 7

Hierarchical clustering analysis of ribosomal protein (RP) genes in T. dendrolimi at four different stages. FPKM values of 31 RP genes were used to construct the expression profiling. Hierarchical clustering of FPKM values was performed with the Pearson correlation-based metric and average linkage clustering method. The two genes with green background were down-regulated during diapause development process.

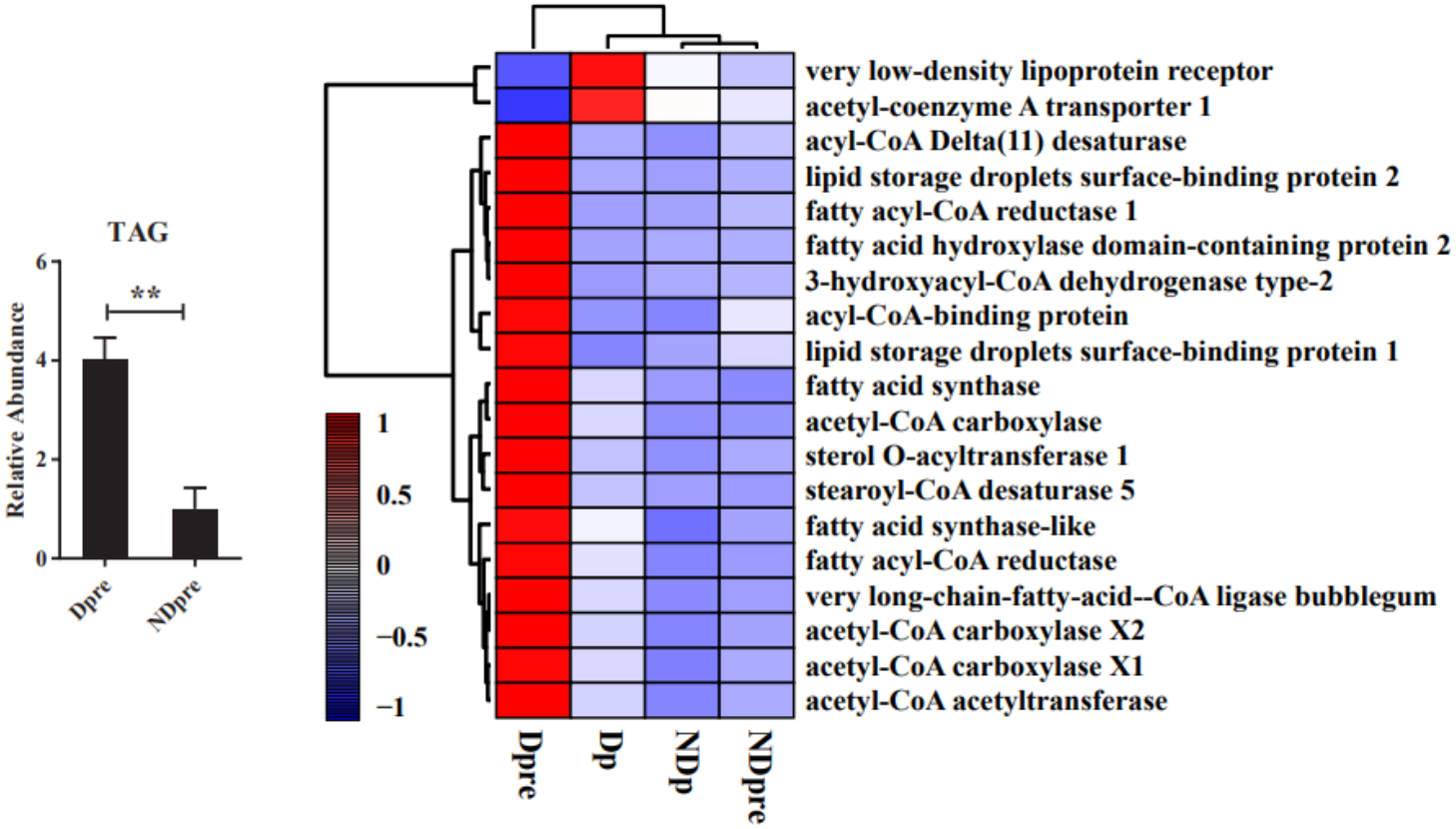

Figure 8

Lipid metabolism changes during diapause development. (A) TAG was measured in diapause and non-diapause T. dendrolimi. At least three independently collected samples for each treatment were analyzed. Dpre treatments were normalized to NDpre. The error bar represents the SEM; **p < 0.01. (B) Heatmap of expression patterns of lipid metabolism-related genes with fold change large than 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- S1new.pdf