Study of thermotolerant mechanism of *Stropharia rugosoannulata* under high temperature stress based on the transcriptome sequencing

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**ABSTRACT**

*Stropharia rugosoannulata* is a popular edible mushroom in the world. High temperature seriously affects its yield and quality. In this study, transcriptome sequencing was performed on the mycelia of heat-resistant strains and heat-sensitive strains heat-treated at 38 °C for 0 h and 24 h. The changes of catalase (CAT) activity, superoxide dismutase (SOD) activity and trehalose content in the mycelia under high temperature stress were also measured and analyzed. We find that the differential genes are mainly enriched in the pathways of glycerophospholipid metabolism, starch and sucrose metabolism, protein processing in the endoplasmic reticulum, etc. The expression levels of genes encoding trehalose-6-phosphate phosphatase (TPP), CAT, SOD, etc. are quite different. And these genes' variation range in the thermotolerant strain are higher than that in heat-sensitive strain. The CAT activity and trehalose content of the two strains increase first and then decrease, and the SOD activity increase slowly. The CAT, SOD activity and trehalose content of the thermotolerant strain are higher than those of the heat-sensitive strain. This study will provide a basis for further research on important signal pathways and gene function identification of *S. rugosoannulata* related to high temperature stress.

**Keywords:** catalase, superoxide dismutase, thermotolerant gene, trehalose

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1. Introduction

*Stropharia rugosoannulata* is one of the edible fungi recommended by the Food and Agriculture Organization of the United Nations (FAO) to developing countries (Hawksworth et al., 1996; Gong et al., 2018). It is an important edible and medicinal fungus with excellent taste, beautiful color and rich nutrition (Huang, 1998). In the Chinese edible mushroom market, the price and sales volume of *S. rugosoannulata* in winter and spring are better than in other seasons. Therefore, in northern China, people tend to cultivate *S. rugosoannulata* in Aug in order to harvest fresh mushrooms to sell in early winter, so as to obtain better income. However, the mycelia of this fungus grow weakly and is vulnerable to infection by bacteria or other fungi at 32 °C or higher, which often occurs in the cultivation strategy of autumn sowing and winter harvest. In order to overcome the high temperature stress problem faced by the cultivation of *S. rugosoannulata* in Aug, it took us 6 y to select a thermotolerant strain (shannonqjilu No.3, patent number 201910546457.5) through monosporic hybridization, high-temperature domestication, protoplast fusion and other breeding techniques.

When the organism is under high temperature, its physiological and biochemical changes will occur, and its gene expression and transcription accumulation will change at the molecular level (Iba, 2002). For example, the catalase (CAT, EC: 1.11.1.6), peroxidase, superoxide dismutase (SOD, EC: 1.15.1.1) and glutathione reductase content of *Pleurotus eryngii* change under high temperature stress (Meng et al., 2015). And the regulation of trehalose metabolism in *P. ostreatus* and *P. pulmonarius* also changes (Liu, 2013). At gene expression level, studies have shown that under high temperature stress, the relative expression levels of *hyd1* gene of *Lentinula edodes* is much higher in heat-resistant strain than in heat-sensitive strain (Xin et al., 2016). After 48 h of high temperature stress at 40 °C, the heat shock protein genes *hsp60*, *grp78*, *hsp90* and *hsp104* in the mycelia of *P. ostreatus* were up-regulated by 28-fold, 6-fold, 10-fold and 15-fold (Zhang, 2016). In *Saccharomyces cerevisiae*, the expression of *NTH1* and *NTH2* genes encoding neutral trehalose under 40 °C temperature stress is higher than under 30 °C (Nwaka et al., 1995). However, there is still little research on the changes of physiology, biochemistry and transcription level of *S. rugosoannulata* under high temperature stress. Research in this area will provide theoretical guidance for the breeding of high temperature resistant strain of *S. rugosoannulata*.

Transcriptome sequencing analysis is an important means of studying cell phenotype and function, and plays an important role in revealing the relationship between gene expression and specific biological processes (Wang et al., 2009). This technology has be-
come an important method to study the biological response mechanism to adversity. Xu (2014) sequenced the transcriptomes of different heat-resistant grape samples, and mastered the overall expression patterns of differential genes related to high temperature stress. Han et al. (2015) used transcriptome sequencing technology to analyze the changes in gene expression of *Haloxylon* under adversity stress and the main ways of regulation, laying the foundation for revealing the mechanism of drought tolerance and heat resistance for *Haloxylon*.

Herein, we will combine transcriptome sequencing analysis and physiological index determination to reveal the changes of physiology and gene expression in the mycelia of *S. rugosoannulata* under heat stress, and explore the gene enrichment pathways of thermotolerant strain and heat-sensitive strain under high temperature. We analyze the changes in the content of heat-resistant functional genes and some gene-encoded substances, with a view to providing a basis for further research on the heat resistance mechanism and related functional genes of *S. rugosoannulata*.

2. Materials and methods

2.1 Strain information

Two strains, L3 and SM, with different heat resistance were selected for this study. L3 is from the strain of Shannongqiugai No. 3 which is bred and selected from 28 strains of *S. rugosoannulata* at home and abroad by using methods, such as protoplast fusion, protoplast regeneration, single-spore hybrid and multiple stability and cultivation verification. L3 is a thermotolerant strain with the adaptable temperature range for mycelial growth 5–42 °C. This strain is deposited in China General Microbial Culture Collection Centre (CGMCC No.: 17676) and has applied for a patent for invention (NO.: 201910546457.5). And the intellectual property of this strain belongs to us. SM is from the strain of Mine-da 128 (national product recognition NO.: 200805) which is provided by Fujian Sanming Fungal Research Institute. And SM is a heat-sensitive strain with the adaptable temperature range for mycelial growth 5–34 °C. These two strains are currently commonly used in production and cultivation due to their stable and high yield.

2.2 Mycelia treatment, RNA extraction and transcriptome sequencing

The L3 and SM strains of the same generation were selected for colony propagation. For each strain, six culture medium cakes (5 mm diam) were obtained from the outer edge of the colony. Each of the cakes was transferred to the center of a new petri dish (9 cm diam) containing PDA medium. The petri dishes and PDA medium were all sterile, and cakes transfer were carried out at a clean environment to ensure that there was no contamination. Then these new petri dishes were placed in a 26 °C incubator and kept in the dark for 10 d. After that, three petri dishes were selected randomly and moved to a 38 °C incubator for 24 h high temperature stress treatment (treatment group), and the others stayed in the 26 °C incubator (control group). We scraped the mycelia of each colony in the 26 °C and 38 °C incubators, and took the same weight (2 g) of mycelia to store in the –80 °C refrigerator until RNA extraction. As shown in Table 1: The code of the control group (heat treatment at 38 °C for 0 h) and the treatment group (heat treatment at 38 °C for 24 h) of L3 strain is L and HL. And the code of the control group (heat treatment at 38 °C for 0 h) and the treatment group (heat treatment at 38 °C for 24 h) of SM strain is S and HS. Each group had three biological replicates. A total of 12 mycelial samples were obtained from the two strains for RNA extraction and transcriptome sequencing.

Total RNA was extracted using fungal RNA extraction kit (Sangon Co., Ltd, Shanghai, China) according to the instruction manual. The concentration of total RNA was initially detected by 1.0% agarose gel electrophoresis. Further, the Nanodrop ND-2000 (Boya-invation Co., Ltd, Beijing, China), Ultra Trace Nucleic Acid Protein Analyzer (Nuohai Life Science Co., Ltd, Shanghai, China) and Agilent 2100 (Agilent Technology Co., Ltd., China) were used to test the concentration, integrity and purity of RNA. The qualified RNA was sent to Beijing Novogene Co., Ltd. (Beijing, China) for sequencing with the platform of Illumina. Since there is no reference genome in this experiment, the screening, filtering, quality evaluation of the raw data (Clear Reads) and the splice of the transcripts were all carried out by software of Trinity. The transcript sequences were obtained after splice, and the longest transcript sequences in each gene were used as Unigene for subsequent analysis. The reads produced in this study had been deposited in the National Center for Biotechnology Information (NCBI) SRA database with accession number: PRJNA660560.

2.3 Functional annotation of genes and analysis of differentially expressed genes

Seven major databases were used to annotate the gene function of unigenes. The databases, annotation software and corresponding parameters were as follows: nr database (annotation software: DIAMOND v0.8.22; parameter: e-value = 1e-5, --more-sensitive), KOG/COG database (annotation software: DIAMOND v0.8.22; parameter: e-value = 1e-5, --more-sensitive), Swiss-prot database (annotation software: DIAMOND v0.8.22; parameter: e-value = 1e-5, --more-sensitive), nt database (annotation software: NCBI BLAST v2.2.28+; parameter: e-value = 1e-5), KEGG database (annotation software: KAAS r140224; parameter: e-value = 1e-10), Pfam database (annotation software: hmmscan HMMER 3; parameter: e-value = 0.01), GO database (annotation software: Blast2GO bg2gpipe_v2.5; parameter: e-value = 1.0E-6). The read count data obtained in the analysis of gene expression levels was normalized, and the genes that express significant differences between samples were screened by using DESeq2 (1.16.1) R package with the threshold of adjusted P-Value (padj) < 0.05 and |log2FoldChange| > 1. Four groups comparison, i.e. HL-L, HS-S, L-S and HL-HS, were analyzed. The first two groups comparison (HL-L and HS-S) were carried out to find the differentially expressed genes between hyphae with and without heat treatment of the same strain. The other two groups comparison (L-S and HL-HS) were carried out to find the differentially expressed genes between heat-resistant strains and heat-sensitive strains. Then, GO (software: GOSeq, version:

| Table 1. Experimental design. |
|--------------------------------|
| **Strain code** | **Control group** | **Treatment group** |
| L3 | Heat treatment at 38 °C for 0 h | Heat treatment at 38 °C for 24 h |
| SM | L | HS |
| L3 | S | HS |

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2.4 RT-qPCR verification

Some unigenes were selected for RT-qPCR verification, and the reverse transcription kit HiScrip® III RT SuperMix for qPCR (Vazyme Biotech Co., Ltd., Nanjing, China) was used to synthesize the first-strand cDNA of total RNA from different RNA samples of *S. rugosoannulata*. The gene β-actin was used as the internal reference gene. The primer sequence of the internal reference gene and the selected differentially expressed genes (Table 2) were designed by using Premier 5.0. And the primers were synthesized in BGI Tech Solutions (Beijing Liuhe) Co., Ltd. (Beijing, China). The RT-qPCR reaction was performed using the ChamQTM Universal SYBR® qPCR Master Mix kit (Vazyme Biotech Co., Ltd.) according to the instruction. And the relative quantitative $2^{-\Delta\Delta C_t}$ method was used to analyze the gene expression level (Livak, & Schmittgen, 2001). And the T-test was used to determine the significance of relative expressions level (heat shock 24 h-heat shock 0 h) of each gene.

2.5 Measurement of physiological indexes

The preparation of the colony is the same as 2.2. For each strain, 21 newly inoculated petri dishes were placed upside down in a 26 °C incubator and kept in the dark for 6 d. Three petri dishes were randomly selected to determine the activities of catalase and superoxide dismutase and the content of trehalose in the mycelia. The remaining 18 petri dishes were moved to a 38 °C incubator, and three petri dishes were randomly selected at 6 h, 12 h, 24 h, 36 h, 48 h and 60 h to determine the catalase and superoxide dismutase activity and trehalose content of the mycelia. Catalase activity was measured using CAT-2-Y kit (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China), and superoxide dismutase activity was measured using SOD-2-Y kit (Suzhou Keming Bio Technology Co., Ltd.). The determination of trehalose content was performed using high-performance liquid chromatography (HPLC) according to Kong et al. (2012). The Duncan’s test in the software of SPSS (Statistics 22.0) was used to determine the significant differences.

3. Results

3.1 Functional annotation of genes

A total of 15,654 unigenes were successfully annotated. The number of unigenes successfully annotated in each database was shown in Table 3. Among them, 10081 unigenes were successfully annotated in the nr database, accounting for 64.39% of the entire sequences, which provided an important reference value for the functional analysis of differential genes. 9208 unigenes were successfully annotated in GO, which were divided into three categories: biological processes, cellular components, and molecular functions, with a total of 55 functional groups (Fig. 1). Among them, the three functional groups of cell process, metabolic process and catalytic activity contained the largest number of unigenes (5719, 5479 and 4551). Besides, 4075 unigenes were divided into 32 metabolic pathway groups in KEGG (Fig. 2). The number of genes in signal transduction, carbohydrate metabolism and energy metabolism pathways were relatively higher than that in other metabolic pathways, which indicated that the signal transduction, carbohydrate metabolism and energy metabolism played an important role in the response to heat stress of *S. rugosoannulata*.

3.2 Analysis of differentially expressed genes between heat-sensitive and heat-resistant strains

As shown in Table 4, the number of differential expression genes (DEG) for the comparison HL-L, HS-S, HL-HS and L-S were 3660, 6941, 2972 and 1555, respectively. The number of DEG in the comparison HS-S was the highest, with 3115 up-regulated genes and 3100 down-regulated genes. The number of DEG in the comparison HL-L was 3660, 3660, 3660 and 3660, respectively. The number of DEG in the comparison HL-HS and L-S was 3660, 3660, 3660 and 3660, respectively. The number of DEG in the comparison HS-S was 3115 up-regulated genes and 3100 down-regulated genes. The number of DEG in the comparison HL-L was 3660, 3660, 3660 and 3660, respectively. The number of DEG in the comparison HL-HS and L-S was 3660, 3660, 3660 and 3660, respectively. The number of DEG in the comparison HS-S was 3115 up-regulated genes and 3100 down-regulated genes.
Table 3. The results of unigene annotation supported by hits from the corresponding public databases.

| Database                                  | Number of Unigenes | Percentage |
|--------------------------------------------|--------------------|------------|
| Annotated in NR                            | 10,081             | 64.39      |
| Annotated in NT                            | 6,129              | 39.15      |
| Annotated in KO                            | 4,075              | 26.03      |
| Annotated in SwissProt                     | 8,248              | 52.68      |
| Annotated in PFAM                          | 9,208              | 58.82      |
| Annotated in GO                            | 9,208              | 58.82      |
| Annotated in KOG                           | 5,066              | 32.36      |
| Annotated in all Databases                 | 2,187              | 13.97      |
| Annotated in at least one Database         | 12,862             | 82.16      |
| Total Unigenes                             | 15,654             | 100.00     |

Fig. 1. GO classification of transcriptome (A: Biological process. B: Cellular component. C: Molecular Function).

Fig. 2. KEGG annotation of unigene (A: Cellular Processes. B: Environmental Information Processing. C: Genetic Information Processing. D: Metabolism. E: Organismal Systems).
and 3826 down-regulated genes, indicating that heat-sensitive strain responded more intensely to high temperature stress at the transcriptome level compared to heat-resistant strain. Under the same normal temperature and heat treatment culture conditions, the gene expression of heat-resistant strain was different from that of heat-sensitive strain. The degree of difference under heat treatment culture conditions was greater than that under normal temperature culture conditions. Under the heat stress, differential expression genes of the four treatment combinations enriched mainly in the 20 KEGG metabolic pathways (Fig. 3). The up-regulated differential genes were mainly enriched in protein processes in the endoplasmic reticulum, glycerophospholipid metabolism, RNA degradation, peroxisomes, glycolysis/gluconeogenesis, and mitogen-activated protein kinase (MAPK) signaling pathways. The

| Comparisons | Differential expression gene | Up-regulated gene | Down-regulated gene |
|-------------|-------------------------------|-------------------|---------------------|
| HL-L        | 3,660                         | 1,650             | 2,010               |
| HS-S        | 6,941                         | 3,115             | 3,826               |
| HL-HS       | 2,972                         | 1,394             | 1,578               |
| L-S         | 1,555                         | 590               | 965                 |

Note: HL: Heat-resistant strain heat shock 24 h; L: Heat-resistant strain heat shock 0 h; HS: Heat-sensitive strain heat shock 24 h; S: Heat-sensitive strain heat shock 0 h.

Fig. 3. Top 20 enriched KEGG pathways of differential expressed genes. A: HL-L; B: HS-S; C: HL-HS; D: L-S. HL: Heat-resistant strain heat shock 24 h; L: Heat-resistant strain heat shock 0 h; HS: Heat-sensitive strain heat shock 24 h; S: Heat-sensitive strain heat shock 0 h.
down-regulated genes were mainly enriched in starch and sucrose metabolism, glycerophospholipid metabolism, arginine and proline metabolism, proteasome, and galactose metabolism pathways.

Twelve unigenes with large mobilization under high temperature stress in each group comparison (HL-L, HS-S, HL-HS and L-S) were selected for comprehensively analysis (Table 5). Among them, the gene with the largest mobilization range was the gene encoding the heat shock protein HSP20. The log2FC value in the comparison HL-L reached 15.88, and the log2FC value in the comparison HS-S reached 10.48. The biggest difference in the expression level was the genes encoding the heat shock proteins HSP70 and CAT. The expression level of the HSP70 gene in the comparison HL-L was up-regulated with a log2FC value of 2.49, but there was no change of the expression level of this gene in the comparison HS-S. In the comparison HL-L, the CAT gene expression level was up-regulated with a log2FC value of 1.31, while down-regulated in the comparison HS-S with a log2FC value of 1.20. In the comparison HL-HS, the expression levels of genes encoding CAT, endoplasmic reticulum mannosidase I (ERManI, EC: 3.2.1.113), HSP20, HSP40, HSP70, HSP90, HspBP1, GroEL, ERMan1, pyruvate decarboxylase (PDC, EC: 4.1.1.1), CAT and SOD were up-regulated, while that encoding sphingomyelin phosphodiesterase 1 (SMPD1, EC: 3.1.4.12) and trehalose-6-phosphate phosphatase (TPP, EC: 3.1.3.12) were down-regulated under heat stress.

In the heat-sensitive strain, the expression levels of genes encoding HSP20, HSP40, HSP70, HSP90, HspBP1, GroEL, ERMan1, PDC, and SOD were up-regulated, while that encoding CAT, SMPD1 and TPP were down-regulated. CAT is an important enzyme that removes reactive oxygen species. Our results showed that the CAT gene was up-regulated in heat-resistant strain and down-regulated in heat-sensitive strain, which indicated that the heat-sensitive strain had a weaker ability to scavenge active oxygen and relieve oxidative damage than heat-resistant strain.

### 3.3 RT-qPCR to verify transcriptome sequencing

The results of 12 unigenes were verified by RT-qPCR. The results showed that the expression changes of the differential genes were basically consistent with the transcriptome sequencing results (Fig. 4). In the heat-resistant strain, the expression levels of genes encoding HSP20, HSP40, HSP70, HSP90, HspBP1, GroEL, ERMan1, pyruvate decarboxylase (PDC, EC: 4.1.1.1), CAT and SOD were up-regulated, while that encoding sphingomyelin phosphodiesterase 1 (SMPD1, EC: 3.1.4.12) and trehalose-6-phosphate phosphatase (TPP, EC: 3.1.3.12) were down-regulated under heat stress. In the heat-sensitive strain, the expression levels of genes encoding HSP20, HSP40, HSP70, HSP90, HspBP1, GroEL, ERMan1, PDC, and SOD were up-regulated, while that encoding CAT, SMPD1 and TPP were down-regulated. CAT is an important enzyme that removes reactive oxygen species. Our results showed that the CAT gene was up-regulated in heat-resistant strain and down-regulated in heat-sensitive strain, which indicated that the heat-sensitive strain had a weaker ability to scavenge active oxygen and relieve oxidative damage than heat-resistant strain.

### 3.4 Physiological indexes of different thermotolerant strains of S. rugosoannulata under high temperature stress

#### 3.4.1 Determination results of catalase activity

Under high temperature stress, a large amount of active oxygen is produced in the mycelia, and CAT is an important enzyme that removes active oxygen. In this study, the CAT activity in the mycelia of heat-resistant strain and heat-sensitive strain showed a trend of first increasing and then decreasing with the increase of high temperature treatment time (Fig. 5). And the time for the CAT activity of the heat-resistant strain to reach its peak value (36 h) was

### Table 5. Differential gene expression and annotation of different thermotolerant trains of Stropharia rugosoannulata.

| Gene ID     | Gene function            | log2FC (padj) |
|-------------|--------------------------|---------------|
| HL-L (padj) | HS-S (padj) | HL-HS (padj) | L-S (padj) |
| Cluster-2809.135 | SMPD1 | -4.71 (0.001) | -2.11 (0.001) | -- | |
| Cluster-2809.6782 | CAT | 1.31 (0.001) | 0.03 (0.001) | 1.20 (0.001) | -- | |
| Cluster-2809.3736 | TPP | -2.26 (0.001) | -3.42 (0.001) | -- | -1.24 (0.001) | |
| Cluster-2809.3513 | ERMan1 | 6.04 (0.001) | 4.01 (0.001) | 1.26 (0.001) | -- | |
| Cluster-2809.4299 | HSP20 | 15.88 (0.001) | 10.48 (0.001) | 2.60 (0.001) | -- | |
| Cluster-2809.4586 | HSP40 | 3.45 (0.001) | 1.26 (0.001) | 2.22 (0.001) | 1.07 (0.001) | |
| Cluster-2809.6765 | HSP70 | 2.49 (0.001) | -- | 1.98 (0.001) | -- | |
| Cluster-2809.4875 | HspBP1 | 4.04 (0.001) | 1.59 (0.001) | 2.54 (0.001) | -- | |
| Cluster-2809.6835 | HSP90 | 3.32 (0.001) | 1.22 (0.001) | 2.14 (0.001) | -- | |
| Cluster-2809.3519 | Chaperonin GroEL | 5.37 (0.001) | 5.93 (0.001) | 2.40 (0.001) | -- | |
| Cluster-2809.4473 | SOD | 2.72 (0.001) | 1.65 (0.001) | 1.56 (0.001) | -- | |

Note: HL: L3 heat treatment at 38 °C for 24 h; L: L3 heat treatment at 38 °C for 0 h; HS: SM heat treatment at 38 °C for 24 h; S: SM heat treatment at 38 °C for 0 h. --: The value of log2FC is 0. padj: Corrected P-Value of multiple hypothesis test (FDR calculation method: BH; significant criteria of differential gene screening: padj <0.05).

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postponed to the time for the CAT activity of the heat-sensitive strain to reach the peak value (24 h). In each period of high temperature treatment, the CAT activity of heat-resistant strain was higher than that of heat-sensitive strain, and the difference in CAT activity of the two strains was the largest at 36 h. All these indicated that the CAT activity of the two strains of mycelia responded to high temperature stress. At the same time, compared to the heat-sensitive strain, the higher-level CAT activity and the delayed time of CAT activity peak of the heat-resistant strain implied that the heat-resistant strain had a stronger active oxygen scavenging ability so that it could effectively reduce the oxidative damage caused by the high temperature stress.

3.4.2 Measurement results of superoxide dismutase activity

SOD can remove superoxide anions in cells and convert them to hydrogen peroxide, which is at the core of the antioxidant enzyme system. In this study, the SOD activity of heat-sensitive strain (SM) and heat-resistant strain (L3) showed a slow growth trend with no significant change before 36 h under high temperature stress, and began to increase rapidly after 36 h under high temperature stress (Fig. 6). The SOD activity of heat-sensitive strains at 6–36 h under high temperature stress was lower than that at 0 h. The SOD activity of heat-resistant strain increased with the time of high temperature stress, which indicated that the SOD activity continued to increase under high temperature stress to remove active oxygen in cells. However, the SOD activity of the heat-sensitive strain was lower than that of the control at the initial stage of high temperature stress, and the
increase amplitude in the SOD activity of the heat-sensitive strain was lower than that of the heat-resistant strain, which indicated that the heat-resistant strain was more resistant to oxidative damage caused by high temperature stress.

3.4.3 Determination results of trehalose content

Trehalose, a stress protective substance, exists widely in organisms and affects signal transduction pathways. In this study, the trehalose content of heat-resistant strain (L3) and heat-sensitive strain (SM) showed a trend of increasing first and then decreasing under high temperature stress (Fig. 7). The trehalose content of heat-resistant strain was 32.9% higher than that of heat-sensitive strain at 0 h of high temperature stress. The trehalose of the heat-resistant strain reached a peak at 36 h of high temperature stress, and was 128% higher than the control. The trehalose of heat-sensitive strain reached its highest value at 6 h of high temperature stress, and was 50.6% higher than the control. Throughout the process of high temperature stress, the trehalose content of heat-resistant strain was higher than that of heat-sensitive strain. The results showed that the heat-resistant strain of *Stropharia rugosoannulata* accumulated the trehalose content during high-temperature stress to alleviate the damage to the mycelia caused by high temperature. While the heat-sensitive strain had weaker trehalose accumulation ability than the heat-resistant strain under high temperature stress.

4. Discussion

Transcriptome can study gene function and structure at a holistic level and reveal the molecular mechanism of biological processes. In this study, by comparing and analyzing the mycelial transcriptome data of heat-resistant strain and heat-sensitive strain of
S. rugosoannulata at 0 h and 24 h under high temperature stress, it was found that the differentially expressed genes mainly exist in heat shock protein family HSP90, HSP70, HSP40, HSP20, HspBP1, chaperone protein GroEL, catalase, superoxide dismutase, pyruvate decarboxylase, sphingomyelin phosphodiesterase 1, trehalose-6-phosphatase, endoplasmic reticulum mannosidase I, etc.

Heat shock proteins are a class of important proteins that resist high temperature and other stresses. They have the role of molecular chaperones to prevent protein misfolding and denaturation, and reduce the damage caused by stress (Parsell & Lindquist, 1993). The function of HSP90 is mainly to carry out post-mature processing and assembly of proteins, and at the same time interact with intracellular calmodulin, agonist protein, tubulin, kinase and other related receptor proteins (Matsumiya et al., 2009). Du (2008) found that under high temperature stress, rice heat shock protein HSP90 expression increased, reducing the damage caused by high temperature. HSP70 is the most important group of cell functional proteins in the heat shock protein family. When subjected to stress such as high temperature, the transcriptional expression of its genes increases, which makes the organism’s function recoverable when it is under certain pressure. The Yhsp70 gene has been cloned in corn, Eupatorium adenophorum and Eryngium adenophorum, and its expression increased under high temperature stress (Gong et al., 2010). HSP40 regulates ATPase activity so that HSP70 can complete its function. Zhou et al. (2012) found that under high temperature conditions, the expression levels of heat shock protein HSP40-related genes in Arabidopsis were up-regulated. The small heat shock protein HSP20 assists the refolding of the structure of denatured proteins in the organism, stabilizes the cytoskeleton, and improve resistance to heat stress, etc. (Åhrman & Emma, 2007). Auesukaree et al. (2012) found that under high temperature stress, the expression levels of genes hsp26, hsp82 and hsp104 encoding the heat shock proteins small HSP, HSP90 and HSP100 in Saccharomyces cerevisiae increased. In this study, the expression levels of genes encoding heat shock proteins HSP20, HSP40, HSP70, HSP90 related to heat-resistant strain and heat-sensitive strain were significantly up-regulated under high temperature stress, and the mobilization range of heat-resistant strain was greater than that of heat-sensitive strain. It shows that the heat shock protein HSP20 and other shock proteins work together to reduce the damage caused by high temperature, improve the heat resistance of the strain, and play an important role in the resistance of the mycelia to heat stress.

HspBP1 is a nucleotide exchange factor of HSP70. Shomura et al. (2005) found that HspBP1 can promote the dissociation of adenosine-diphosphate (ADP) and the binding of adenosine-triphosphate (ATP) on HSP70, and drive HSP70 to ATP binding state, thus drive a new round of protein folding. Bochkareva et al. (1996) found that under high temperature and other stress, the chaperone protein GroEL plays an important role in the correct folding and assembly of nascent proteins, the recovery process of denatured proteins, and the process of transmembrane transfer or protein insertion into the plasma membrane in Escherichia coli cells. Rudolph et al. (2010) found that increased expression of the chaperone protein GroEL reduced the damage caused by temperature stress in Escherichia coli. High temperature stress will cause cells to produce a large amount of reactive oxygen species. Nali et al. (1998) found that the increased activity of SOD and CAT can reduce oxidative damage caused by high temperature. Wang et al. (2017) found that increasing the expression of catalase gene can improve the resistance of Pleurotus ostreatus to heat stress. ERManI is involved in important control pathways of endoplasmic reticulum-related protein degradation. Liebminger et al. (2009) found that ERManI promotes the development of plant roots, affects the synthesis of cell wall organisms, and has a great effect on the stress resistance of plants. Pyruvate decarboxylase plays an important role in the fermentation metabolic pathway, participates in energy and material metabolism in plants, and responds to various biological and abiotic stresses such as hypoxia, low temperature, and high salt (Bolton et al., 2008). In this study, the gene expression levels associated with HspBP1, chaperone GroEL, SOD, CAT, ERManI, PDC were increased under high temperature stress, and the up-regulation amplitude of heat-resistant strain was higher than that of heat-sensitive strain, indicating that the up-regulated expression of these genes promotes the synthesis of their encoded substances and enhances the ability of the strain to resist high temperature stress.

Fig. 7. Changes in the concentrations of trehalose induced by heat stress in mycelia of different heat sensitive strains of Stropharia rugosoannulata. Different lowercase letters indicate significant differences (P-Value <0.05, according to Duncan’s test).
stress through mutual cooperation. At the same time, the results of SOD and CAT activities in mycelia under high temperature stress showed that with the time prolongation of high temperature stress, the activities of SOD and CAT in heat-resistant strain were higher than that in heat-sensitive strain, which was consistent with the results of gene expression studies, indicating that heat-resistant strain has a stronger ability to remove reactive oxygen species. This is consistent with the results of Liu (2013)’s study of *P. palmonarius*.

Trehalose is involved in the growth and development, stress resistance, and adaptation mechanisms to cope with extreme environments, alleviating oxidative damage caused by high temperatures, and reducing the degree of plasma membrane peroxidation of many kinds of organisms (Luo et al., 2008). The TPS-TPP pathway is trehalose synthesis pathway which the most detailed and reported currently in many kinds of organisms, such as *Selaginella lepidophylla* (Zentella et al., 1999), *Arabidopsis thaliana* (Schluempmann et al., 2003), etc. In this pathway, trehalose-6-phosphate synthase (TPS) catalyzes uracil diphosphate glucose (UDPG) and glucose-6-phosphate (G-6-P), and then synthesis trehalose-6-phosphoric acid (T6P). Finally, trehalose-6-phosphate phosphatase (TPP) catalyzes the production of trehalose (Cabië & Leloir, 1958). Neves and François (1992) found that when cell culture temperature of *S. cerevisiae* was increased from 28 °C to 40 °C, TPS and TPP could regulate the rapid accumulation of trehalose. Generally speaking, down-regulation (or up-regulation) of TPS gene expression means that trehalose content will decrease (or increase). However, the synthesis pathway of trehalose is not necessarily the same between different organisms. TreS pathway, TreY-TreZ pathway, TreP pathway, TreF pathway, etc. can also synthesize trehalose (Iturríaga et al., 2009). Different species, even different individuals of the same species, or the same individual under different conditions may have different trehalose synthesis pathways. For instance, in *Bradyrhizobium japonicum*, the TPS-TPP is the main pathway for trehalose synthesis under 60 mmol/L NaCl stress, while the TreS is the main pathway for trehalose synthesis under drought stress (Sugawara et al., 2010). In this study, the gene encoding TPP in the heat-sensitive strain SM was down-regulated and the content of synthetic trehalose decreased, which may be ascribed to that the TPS-TPP pathway is the main way to synthesize trehalose in the mycelia of heat-sensitive strain of *S. rugosoannulata*. Interestingly, we found that under heat stress, the TPP gene of L3 strain was down-regulated, but its trehalose content in mycelia increased. This means that under heat stress, compared with the trehalose synthesis pathway of the heat-sensitive strain SM, the trehalose synthesis pathway of the heat-resistant strain L3 has its particularity, which requires further study.

Sphingolipid is a component of the plasma membrane of eukaryotic cells. It affects the regulating transmembrane signal transduction by affecting protein kinases, and plays an important role in the process of resisting thermal stimulation, penetration and low pH stimulation of organisms (Ogawa et al., 1998). SMPD1 is an important enzyme in sphingomyelin and ceramide metabolic pathways, and plays an important role in signal transmission (Lin et al., 2018). Characterization and gene expression profiles of thermotolerant Saccharomyces cerevisiae isolates from Thai fruits. *Journal of Bioscience and Bioengineering*, 114(2), 144–149. https://doi.org/10.1016/j.jbiosc.2012.03.012.

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*Disclosure*

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of the People’s Republic of China.

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