Comparative transcriptomic analysis reveals molecular processes involved in pileus morphogenesis in Pleurotus eryngii under different light conditions

Fang Du, Yajie Zou, Qingxiu Hu, Huiying Zhang, Dou Ye

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Comparative transcriptomic analysis reveals molecular processes involved in pileus morphogenesis in *Pleurotus eryngii* under different light conditions

Fang Du[1], Yajie Zou[1], Qingxiu Hu[*], Huiying Zhang, Dou Ye

Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

*Corresponding author:

Qingxiu Hu

Chinese Academy of Agricultural Sciences, Zhongguancun south street 12, Haidian District, Beijing, 100081, China

Huqingxiu@caas.cn

86-10-82108681

[1] Fang Du and Yajie Zou contributed equally to this work

Abstract: Light plays an important role in pileus differentiation in *Pleurotus eryngii* cultivation, and pileus morphology is influenced by light quality. To understand the effects of light quality on pileus morphology at the transcriptional level, we performed a comparative transcriptomic analysis of pilei grown under blue and red light irradiation. We identified 3,959 differentially expressed genes (DEGs) between the blue and red light–treated pilei, which included 1,664 up-regulated and 2,295 down-regulated genes. These DEGs were significantly associated with light sensing, signal transduction, cell wall degradation and melanogenesis, suggesting that these processes are involved in pileus morphogenesis. Multiple DEGs related to respiratory functions were differentially expressed, suggesting that respiratory activity increased during pileus development regardless of light quality. These results provide a valuable view of the transcriptional changes and molecular processes involved in pileus morphogenesis under different light conditions and provide a foundation for yield improvement and quality control of *P. eryngii*.

Keywords: *Pleurotus eryngii*; Pileus morphogenesis; Light quality; Transcriptomics; Light sensing

1. Introduction

Pleurotus eryngii, commonly called the king oyster mushroom, is a commercially cultivated edible mushroom with high nutritional and medicinal value[1,2]. *P. eryngii* is rich in proteins, vitamins, amino acids, polysaccharides, lipids, and peptides[3]. Pharmacological studies have confirmed its medicinal properties, including antitumor, antiviral, and antioxidant activities[4-6]. Its desirable flavor, nutritional value, and biological functions have made *P. eryngii* a popular edible mushroom.

Light is a key environmental factor that influences fruiting body development in many basidiomycetes. Pileus differentiation and development require light stimulation, and different light qualities can produce different morphological features. Many studies have demonstrated that dark-cultivated fruiting bodies exhibit an abnormal shape. For example, dark growth results in tiny or absent pilei in *Coprinopsis cinerea*[7], pinhead fruiting bodies in *Flammulina velutipes*[8], or abnormal stipes and undeveloped pilei in *P. eryngii*[9,10]. Upon exposure to light, pilei quickly differentiate at the apical region of the fruiting body[8,11].

In *P. eryngii*, pileus size and shape are important commercial factors that are influenced by light quality. Fruiting body with longer stipe and closed pileus is considered as high quality product. *P. eryngii* fruiting bodies developed under a fluorescent lamp and blue light irradiation exhibited thicker and larger pilei but shorter stipes than those grown under LEDs[12]. We previously showed that *P. eryngii* fruiting bodies exposed to red light were of higher quality, with longer stipes and closed pilei, and blue-light treatment produced larger pilei with darker colors and open pilei[13]. These observations indicate that pileus morphogenesis under different light conditions involves variations in metabolic and physiological processes. An

understanding of these processes at the molecular level will not only be useful in *P. eryngii* cultivation but also reveal mechanisms underlying pileus differentiation. Previously molecular-level studies of the relationship between pileus induction and light were performed in *F. velutipes*[8], *C. cinerea*[7], and *P. eryngii*[9]. The functions of some pileus-specific genes, such as *psh* in *F. velutipes*, have been investigated. Unfortunately, the mechanisms driving light response in pileus morphogenesis remain unclear. Closed and open *P. eryngii* pilei (by blue and red light, respectively) are an informative model for investigating the molecular processes underlying pileus morphogenesis under different light quality treatments.

Light response processes are enacted by altering the expression of thousands of genes. The identification and functional analysis of genes differentially expressed under different light quality conditions will improve our understanding of the genetic and molecular mechanisms that drive light quality–regulated differences in pileus morphology. Illumina RNA sequencing (RNA-seq) has been successfully used to explore functional genes and molecular mechanisms associated with growth and development of edible mushrooms, including *Pleurotus tuoliensis*[14], *Lentinula edodes*[15], *Hypsizygus marmoreus*[16], and *Cordyceps militaris*[17]. In *P. eryngii*, transcriptomic analysis of blue light–induced differentiation of primordia into fruiting bodies revealed that carbohydrate-active enzyme (CAZyme) function was associated with fruiting body development[9]. To our knowledge, a comparative transcriptomic analysis of pileus morphogenesis under different light conditions has not been performed.

In this study, we used RNA-seq to perform transcriptomic analysis on *P. eryngii* pileus exposed to blue light or red light illumination. These transcriptomic informations could

facilitate our understanding of the genetic and molecular mechanisms of *P. eryngii* pileus development in response to different light wavelengths.

2 Materials and Methods

2.1 Cultivation of *P. eryngii*

P. eryngii (ACCC52611) was provided by the Agricultural Culture Collection of China. Mycelia were grown at 25ºC in polypropylene bags containing 455 g of cultivation substrate (23.2% sawdust, 23.2% bagasse, 18.4% corncob, 18.4% wheat bran, 8.4% soybean meal, 6.6% corn flour, 1% lime, and 1% gypsum) in the dark for 40 days, after which the substrate was fully colonized. The cultivation bags were randomly divided into two groups and were cultivated at 12-14ºC under (1) 12 h red light/dark (R group) and (2) 12 h blue light/dark (B group) conditions until fruiting body maturation. We randomly selected three fruiting bodies from each treatment group on day 16 after primordium formation and collected their pilei into three corresponding tubes, which were frozen at -80ºC until RNA extraction.

2.2 RNA extraction, cDNA library construction, and Illumina sequencing

Total RNA was extracted from the pilei of fruiting bodies obtained from the R and B groups using the mirVana miRNA Isolation Kit (Ambion) and evaluated for integrity and quality using an Agilent Technologies 2100 Bioanalyzer (Santa Clara, CA, USA). Samples with an RNA Integrity Number (RIN) $\geq$ 7 were subjected to subsequent analysis. Six cDNA libraries (2 treatments with 3 biological replicates per treatment) were constructed using the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's standard protocol. Libraries were sequenced on an Illumina HiSeq 2000 platform by Shanghai OE Biotech Co., Ltd. (Shanghai, China).

2.3 Transcriptome assembly and functional annotation

Raw data were processed using the NGS QC Toolkit[18]. Reads containing poly-Ns, duplicate sequences, and low-quality sequences were removed to obtain high-quality clean reads. These clean reads were then mapped to the *P. eryngii* genome derived from the JGI transcriptome reference database using hisat2[19]. Alignments to the *P. eryngii* genome were performed using Tophat v2.0.10 with default parameters. The FPKM value of each gene was calculated using Cufflinks, and read counts for each gene were obtained using htseq-count. Raw Illumina sequencing data were deposited in GenBank under the BioProject accession PRJNA551567.

Differentially expressed genes (DEGs) were filtered and identified using the DESeq functions estimateSizeFactors and nbinomTest. Significant gene expression differences were assessed using a threshold for the false discovery rate (p-value < 0.05) and fold change ($|\log_2$ fold change$| > 1$). Heatmaps were generated using HemI software (Version 1.0.3.3, Heatmap Illustrator, Huazhong University of Science and Technology, Wuhan, China).

Gene ontology (GO) enrichment analysis of DEGs was performed using the GOseq R package based on a Wallenius non-central hyper-geometric distribution, which can be adjusted for gene length bias in DEGs[20]. Functional analysis of DEGs was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG)[21].

2.4 Validation of RNA-seq by quantitative real-time PCR

RNA-seq results were verified using qRT-PCR. Total RNA used for qRT-PCR was extracted from the same batch of samples as were used for RNA-seq. Each RNA sample was treated with RNase-free DNase I (TaKaRa, Shiga, Japan) following the manufacturer's

protocol to remove any residual genomic DNA. DNase I–treated RNA was reverse transcribed using oligo (dT) primers and Prime-ScriptTM Reverse Transcriptase (TaKaRa, Shiga, Japan) according to the manufacturer's protocol. Total RNA (2 μg) was used to synthesize cDNA with the PrimeScript™ RT Reagent Kit (Perfect Real Time, TaKaRa, Japan). A *P. eryngii* housekeeping gene, glyceraldehyde-phosphate dehydrogenase (gapdh), was used as an internal control to normalize for varying amounts of input cDNA. Primers for qRT-PCR of 9 DEGs were designed in Primer 6.0 software and are shown in Table 1. Three biological replicates were analyzed per sample. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method[22].

2.5 Data analysis

All experiments were repeated at least three times, and the results are expressed as mean ± standard deviation (S.D.). Statistical evaluation was performed using ANOVA (SPSS 18.0), and P values < 0.05 was considered significant.

3 Results

3.1 Morphological features and quality of fruiting bodies under red and blue light conditions

Under low temperatures and red light (R) or blue light (B) conditions, *P. eryngii* mycelia transitioned from vegetative to reproductive growth, and the fruiting bodies matured 16 days after primordium formation. We found that fruiting bodies of R group were of higher quality, with longer stipes and closed pilei, and blue light stimulation promoted larger stipe diameter and deeper pileus color (Figure 1a). The average of the maximum and minimum diameter of pilei under R group was 52.1 ± 2.5 mm and 44.8 ± 3.4 mm, respectively, while B group pilei

diameter is larger, with the maximum diameter of 113.5 ± 4.13 mm and the minimum diameter of 99.8 ± 1.87 mm (Figure 1b). The total yield (281.1 ± 54.2 g) and biological efficiency (61.78 ± 19.13 %) under red light treatment is significantly higher than that (the total yield of 236.7 ± 30.1 g and biological efficiency of 52.68 ± 6.62 %) under red light stimulation (Table 2). These results indicate that different light signals exert different regulatory effects on the morphological features of *P. eryngii* fruiting bodies.

3.2 RNA sequencing and transcriptome assembly

To investigate the molecular basis of pileus differentiation in response to varying light signals, we performed RNA-seq analysis of pilei from R- and B-group fruiting bodies collected 16 days after primordium formation. A total of six cDNA libraries were prepared and subjected to Illumina deep sequencing.

After cleaning and filtering the raw reads, we observed an average of 49,554,576 clean reads per sample. All Q30 percentages for the sequences for each library were over 85%. For all libraries, approximately 88% of the reads mapped to the *P. eryngii* reference genome, and 87% of reads were unique matches (Table 3). Gene expression values were normalized as fragments per kilobase of exon per million fragments mapped (FPKM). Taken together, our sequencing data were of high quality and displayed good correlations among biological replicates and thus could be used for subsequent analyses.

3.3 Identification of differentially expressed genes

To identify differentially expressed genes (DEGs) between the B and R sample groups, comparative transcriptome analysis was performed. Compared with the B group, 3,959 DEGs were significantly and differentially expressed in response to red light, suggesting that these

genes were closely associated with pileus morphogenesis induced by different light qualities. Among these DEGs, 1,664 and 2,295 were up-regulated and down-regulated, respectively (Figure 2).

3.4 Functional annotation of differentially expressed genes

To better understand the functions of DEGs involved in pileus morphology, all DEGs were subjected to GO enrichment analysis. The enriched annotations were grouped into three functional categories: biological processes, cellular components, and molecular functions (Table S1). Figure 3a shows the top 30 GO terms enriched in each category (10 terms per functional category [$P < 0.05$]). Our analysis revealed significantly enriched biological process terms ($P < 0.05$) associated with growth and development, such as positive regulation of the cell cycle (GO:0045787) and ascospore formation (GO:0030437). Within the cellular components category, mitochondiral respiratory chain supercomplex (GO:0097249) was enriched in our DEGs. In addition, we found that 15 DEGs were associated with photoperiodism (GO:0009648), deoxyribodipyrimidine photo-lyase activity (GO:0003904), red and far-red light phototransduction (GO:0009585), and response to light stimulus (GO:0009416) terms (Figure 3b).

We used KEGG analysis to further classify the functions of annotated DEGs. The significant enrichment pathways of these DEGs are shown in Table S2. In addition to pathways related to energy metabolism, we also observed that some signaling pathways, including the Notch, AMPK, and Hippo signaling pathways, were enriched in our up-regulated genes in the B and R groups. These pathways are likely associated with the observed differences in pileus morphology in *P. eryngii* cultivated under blue and red light

(Figure 4).

3.5 Identification of DEGs related to pileus morphology under different light qualities

Pileus morphology is easily affected by variations in light quality. We first analyzed DEGs related to light response and transmission (Figure 5). As expected, DEGs enriched in functional terms related to blue light (cellular response to blue light; GO:0071483), such as the transcriptional regulator *CRZ1* (1407225), were up-regulated in B-group pilei, while DEGs involved in response to red light exposure (red, far-red light phototransduction; GO:0009585), such as the DNA damage-binding protein *DDB1*(789470), displayed increased expression in R-group pilei. Genes encoding deoxyribodipyrimidine photolyases (*phrA* and *phrB*) were significantly enriched under blue light stimulation. Expression of flavonol synthase/flavanone 3-hydroxylase FLS (1508022), which is related to light stimulation, was significantly up-regulated following red-light treatment. Quinone oxidoreductase (1509938), which is involved in response to high light intensity (GO:0009644), displayed reduced expression in R-group pilei, similar to casein kinase II (432546), which is associated with photoperiodism (GO:0009648).

We further analyzed the expression profiles of DEGs associated with other signaling pathways. This included transcription factors and protein kinases that may be related to photomorphogenesis. We observed that kinase-encoding genes, such as MOB kinase activator-like 1A (*MOB1A*, 1281117), and multiple serine/threonine-protein kinases (*Chek2*, 514294; *CBK1*, 1351763; *CBK1*, 1396431), exhibit significant up-regulation in expression under red-light treatment. The transcription factor gene *GTE3* (1396279) was up-regulated 156-fold under red light stimulation, and the genes encoding the transcriptional activator Myb

(1386426 and 1386496) and transcriptional regulatory proteins (1266696 and 1383034) displayed significantly increased expression in pilei stimulated by blue light. Furthermore, under red light, we observed 171-fold up-regulation of the nucleoporin gene (*NDC1*, 253478), which has been reported to be associated with environmental stress and plays a fundamental role in regulating transport between the cytoplasm and nucleus[23]. The gene encoding hydrophobin-1 (*HYD-1*, 1366453), which has been shown to play an important role in fruiting body formation[24], was also significantly up-regulated in R-group pilei. Genes encoding tyrosinases (470110, 1406860 and 1438411), which are rate-limiting enzymes in melanin synthesis[25], were significantly up-regulated in B-group pilei, which were darker in color than R-group pilei.

CAZymes, including glycosyl hydrolases (GHs), polysaccharide lyases (PLs), carbohydrate-binding modules (CBMs), carbohydrate esterases (CEs), glycosyl transferases (GTs), and auxiliary activities (AAs), are involved in degradation of plant cell wall polysaccharides[9] and related to the utilization of cultivation substrate. By searching our DEGs against the carbohydrate-active enzyme database (CAZy), we found that 18 DEGs, including 11 GHs, 4 CEs, 2 GTs, and 1 PL, can be identified as CAZymes. An acetyl xylan esterase (*CE*, 1450626), cellulase (*GH5*, 1568780), and beta-galactosidase (*GH1*, 1578439) were significantly up-regulated in pilei subjected to blue-light treatment, while alpha-xylosidase 1 (*GH31*, 1483237) and endoglucanase (*GH45*, 1594060) were up-regulated in R-group pilei.

Finally, we also noticed that multiple genes related to respiratory functions were differentially expressed under the two light conditions. These included respiratory supercomplex factor 2 homolog (*rcf2*; 1354663), NADH dehydrogenase [ubiquinone]

iron-sulfur protein (*Ndufs8*; 1385578) and coiled-coil domain-containing protein (*CCDC90B*; 1384237), demonstrating that respiratory activity increased during pileus development regardless of light quality.

3.6 Validation of DEGs by qRT-PCR

To validate our RNA-seq findings, the expression levels of selected DEGs, namely, *CRZ1* (1407225), *phrB* (1374073), *FLS* (1508022), *qor* (1509938), *MOB1A* (1281117), GTE3 (1396279), *NDC1* (253478), *HYD-1* (1366453), *rcf2* (1354663), and *CCDC90B* (1384237), were quantified by qRT-PCR (Additional file 8: Table S8). Overall, the results of our qRT-PCR were consistent with those obtained by RNA-seq (Figure 6).

4. Discussion

Our transcriptome analysis revealed 3,259 genes differentially expressed in *P. eryngii* pilei under different light conditions. We found that genes involved in sensing and transducing blue light signals were significantly up-regulated in blue light-treated pilei, while red light–responsive genes displayed high expression in red light groups of pilei. Moreover, we found that the transcription factor *GTE3* and serine/threonine-protein kinases related to signal transduction were significantly up-regulated following red-light treatment, and the transcriptional activator gene *Myb* and several genes encoding transcriptional regulatory proteins displayed increased expression under blue-light treatment, suggesting possible involvement of these genes or their protein products in regulation of pileus development under distinct light conditions.

Hydrophobins are involved in fruiting body formation in basidiomycete fungi[24, 26]. Sakamoto reported that the expression of the hydrophobin PSH was induced by light

stimulation and was specifically transcribed in *F. velutipes* pilei, and was thus designated as a pileus-specific protein[27]. We demonstrated that the expression of hydrophobin-1 was slightly greater in R-group pilei than B-group pilei, suggesting that this hydrophobin is related to pileus development.

P. eryngii yield and biological efficiency were significantly different under red light and blue light stimulation. We observed differential expression of CAZymes in *P. eryngii* pilei under two light conditions, such as cellulase (*GH5*), GPI mannosyltransferase 2 (*GT76*), peptidoglycan-N-acetylglucosamine deacetylase (*CE4*), and chitosanase (*GH5*). The differentially expression of these genes might indicate a difference in conversion efficiency of substrate into mushroom biomass and associated with the total yield and biological efficiency. Alpha-xylosidase 1 (*GH31*), which catalyzes the liberation of alpha-xylose from the non-reducing terminal glucose of xyloglucan oligosaccharides, was significantly up-regulated in R-group pilei. We deduced that high expression of this gene is only related to oligosaccharides degradation.

Under blue light, pileus appeared deeper colouration (melanin synthesis). It has been reported activation of tyrosinases is related to melanogenesis[28, 29]. Interestingly, all tyrosinase-encoding genes were significantly up-regulated in B-group pilei, which displayed increased coloration. This result indicates that blue light exposure leads to increased tyrosinase activity and increased pigment formation. Genes encoding phenylalanine ammonia-lyases (PALs) were also up-regulated in B-group pilei, suggesting that, along with tyrosinases, PALs are coordinately involved in pileus pigment formation.

Blue light (higher energy) exposure can lead to high-light stress and thus influence

growth and development. In our study, genes encoding deoxyribodipyrimidine photolyases, which participate in DNA replication and repair, were significantly upregulated in B-group pilei and likely function to counteract blue light stress. Blue light also induces oxidative stress, which inhibit growth[30]. Quinone oxidoreductase is capable of scavenging superoxide anions generated during oxidative stress and regenerating reduced forms of protective antioxidant compounds[31] and was upregulated in B-group pilei to resist oxidative damage caused by blue light exposure.

Respiration and the concentration of carbon dioxide during fruiting body formation have been investigated. Respiration activity increases following primordia formation and peaks in the later stages of fruiting body development[32]. In our study, genes related to respiration were differentially expressed regardless of light quality.

In summary, we have described the transcriptomes and DEGs in pilei cultivated under red and blue light. Further research of the molecular mechanisms and roles of the genes identified in our study will provide a better understanding of the effects of different light qualities on pileus differentiation.

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Supporting information description

Table S1: GO enrichment terms of differential expressed genes

Table S2: KEGG enrichment pathways of differential expressed genes.

Author contributions

FD, YZ, DY and HZ conceived, designed, and performed the experiments, analyzed the data, and wrote and revised the manuscript. QH conceived and designed the experiments.

Conflict of interest

The authors declare no competing financial interest.

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Figure captions

Figure 1 *P. eryngii* pilei morphological features under red light and blue light conditions. (a) pilei shape under different light conditions. (b) the average of maxinum diameter and minimum diameter of pilei from red light group and blue light group.

Figure 2 The number of up- and down-regulated differentially expressed genes under blue light and red light conditions. The total number of genes differentially expressed is indicated on the top.

Figure 3 GO functional classification of differentially expressed genes in pilei under blue light and red light stimulation. (a) The top 30 GO enrichment terms from R vs B. The red bars represent biological process; green bars represent cellular component; blue bars represent molecular function. (b) GO enrichment terms relating to light response under R vs B.

Figure 4 The top 20 KEGG enrichment pathways from R vs B. The left Y-axis shows the KEGG pathway. The X-axis shows the rich factor. A high *q*-value is represented by blue and a low q-value is represented by red ($q < 0.05$).

Figure 5 Genes involved in the regulation of pilei mophology in *P. eryngii* under red light and blue light conditions. The right side of the heatmap indicates the gene ID in *P. eryngii* and the homologous gene name. The gene expression values (FPKMs) were transformed to Z-score values.

Figure 6 qRT-PCR validation of the expression levels of 9 selected genes was determined. *Gapdh* expression was used as the internal control. Error bars indicates SE (n=3). CRZ1, transcriptional regulator; phrB, deoxyribodipyrimidine photolyases; FLS, flavonol

synthase/flavanone 3-hydroxylase; qor, quinone oxidoreductase; MOB1A, MOB kinase activator-like 1A; GTE3, transcription factor; NDC1, nucleoporin; rcf2, respiratory supercomplex factor 2 homolog; CCDC90B, coiled-coil domain-containing protein.

Table 1 Primers for qRT-PCR of 9 DEGs

Gene ID	Annotation	Primer
1407225	Transcriptional regulator (CRZ1)	F:CACGAACCTACGAACAGTA
		R:ACCTACGAAGAAGAGAATGG
1374073	Deoxyribodipyrimidine photolyases (phrB)	F: CATCACCGCAGTCAAGAA
		R: GATAGCAGCACCGTCATT
1508022	Flavonol synthase/flavanone 3-hydroxylase (FLS)	F: CAATCCATAGGCTCGTCAA
		R: CAATCCATAGGCTCGTCAA
1509938	Quinone oxidoreductase (qor)	F: ATACAAGAGCGAGGATACTG
		R: GTTGCCGTAATGGAATGC
1281117	MOB kinase activator-like 1A (MOB1A)	F: ATATCTACCGCCAACTCCT
		R: ATCGCCTCTGATGTCCTT
1396279	Transcription factor GTE3	F: GAACAACTCGCCAACAAG
		R: CATCATATCTTCTGCCATCG
253478	Nucleoporin (NDC1)	F: CTGGTCTCTGTGTTCTGTC
		R: ACTGTCTTGCTGATTGGTAA
1354663	Respiratory supercomplex factor 2 homolog (rcf2)	F: GCTGTCGGTGTATCATATTG
		R: CTTCTGCTGCTCCTTCTC
1384237	Coiled-coil domain-containing protein (CCDC90B)	F: ACTCTTCTCGGTGTCCTC
		R: CTCCTGCTCTATCCTCGTA

Table 2 Effect of light on yield and biological effciency of fruiting body

Treatment	Yield (g)	Biological efficiency (%)
R	236.7 ± 30.1 g[b]	52.68 ± 6.62 %[b]
B	281.1 ± 54.2 g[a]	61.78 ± 19.13 %[a]

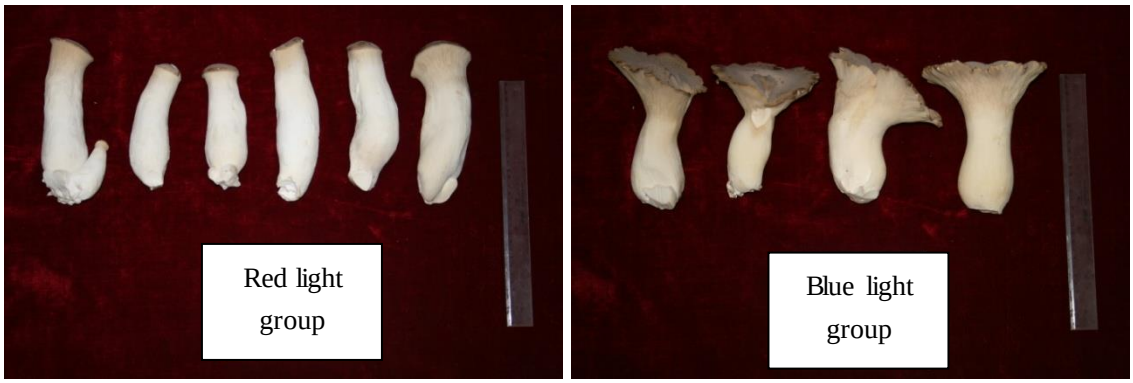
Note: R=Red light treatment; B=Blue light group treatment

Table 3 Summary of transcriptome sequencing

Sample ID	Raw reads	Clean reads	Base number (G)	Q30	Total Mapped reads (% mapped)	Uniquely mapped (% mapped)
R-1	49585572	48567346	7.28	95.08%	43068679(88.68%)	42565283(87.64%)
R-2	50890728	49925846	7.49	95.30%	44397657(88.93%)	43870773(87.87%)
R-3	49442760	48676204	7.30	95.60%	43513221(89.39%)	42995114(88.33%)
B-1	49684352	48925314	7.34	95.65%	43174297(88.25%)	42673118(87.22%)
B-2	50593548	49748650	7.46	95.51%	44376225(89.20%)	43813588(88.07%)
B-3	50093872	49276330	7.39	95.49%	44068245(89.43%)	43552344(88.38%)

Note: R=Red light treatment; B=Blue light group treatment

a



b

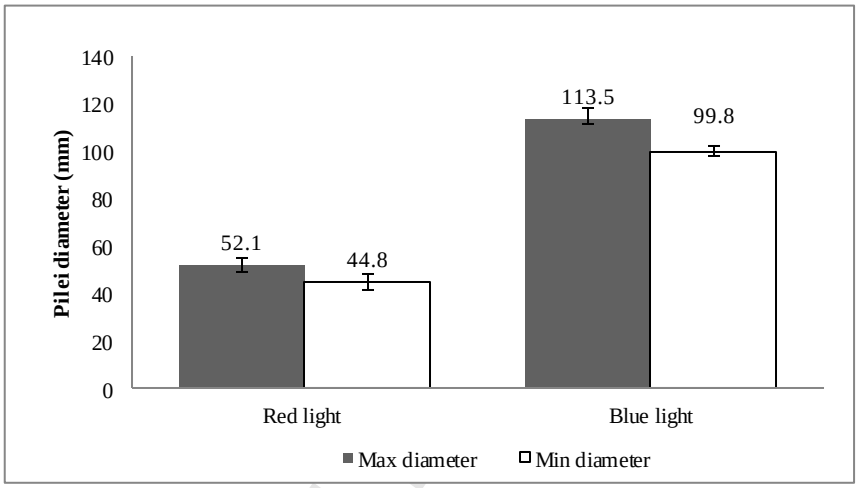


Figure 1

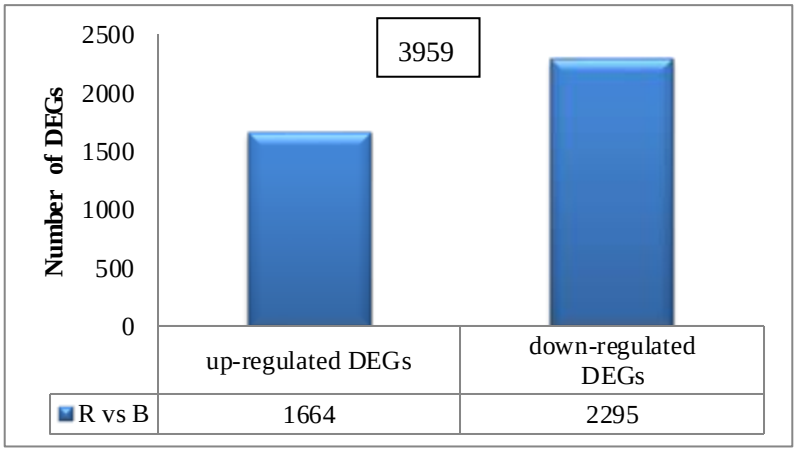
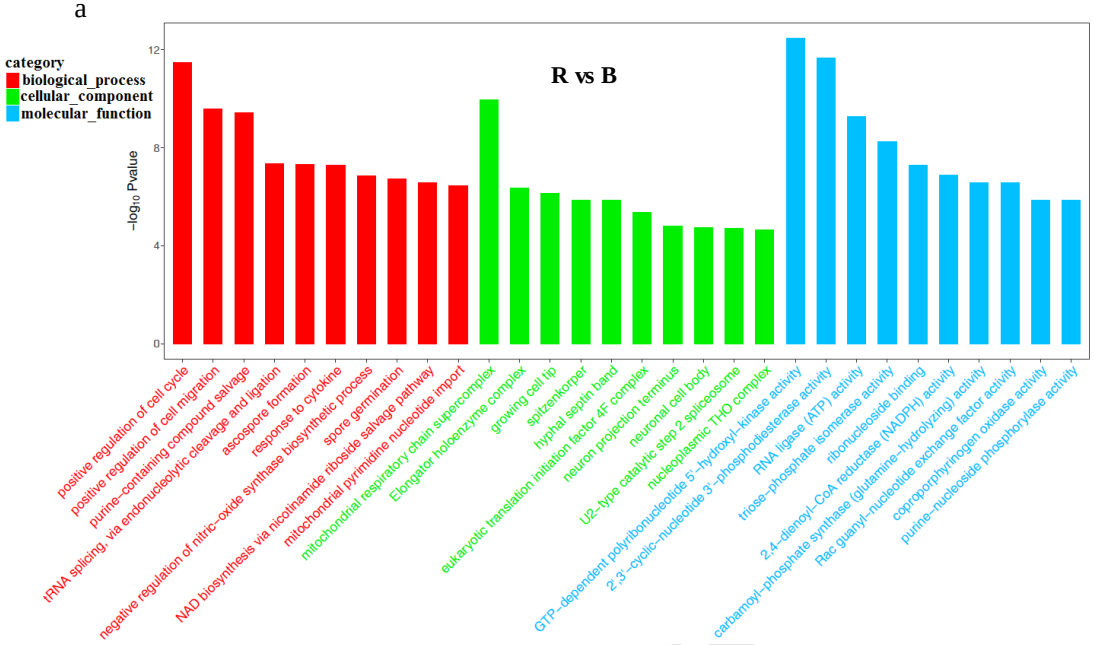


Figure 2

a


R vs B
category
biological_process
cellular_component
molecular_function
−log10 Pvalue
0
4
8
12
positive regulation of cell cycle
positive regulation of cell migration
purine−containing compound salvage
tRNA splicing, via endonucleolytic cleavage and ligation
ascospore formation
response to cytokine
spore germination
negative regulation of nitric−oxide synthase biosynthetic process
NAD biosynthesis via nicotinamide riboside salvage pathway
mitochondrial pyrimidine nucleotide import
mitochondrial respiratory chain supercomplex
Elongator holoenzyme complex
growing cell tip
spitzenkorper
hyphal septin band
eukaryotic translation initiation factor 4F complex
neuron projection terminus
neuronal cell body
U2−type catalytic step 2 spliceosome
nucleoplasmic THO complex
GTP−dependent polyribonucleotide 5'−hydroxyl−kinase activity
2,3'−cyclic−nucleotide 3'−phosphodiesterase activity
RNA ligase (ATP) activity
triose−phosphate isomerase activity
ribonucleoside binding
2,4−dienoyl−CoA reductase (NADPH) activity
carbamoyl−phosphate synthase (glutamine−hydrolyzing) activity
coproporphyrinogen oxidase activity
Rac guanyl−nucleotide exchange factor activity
purine−nucleoside phosphorylase activity

b

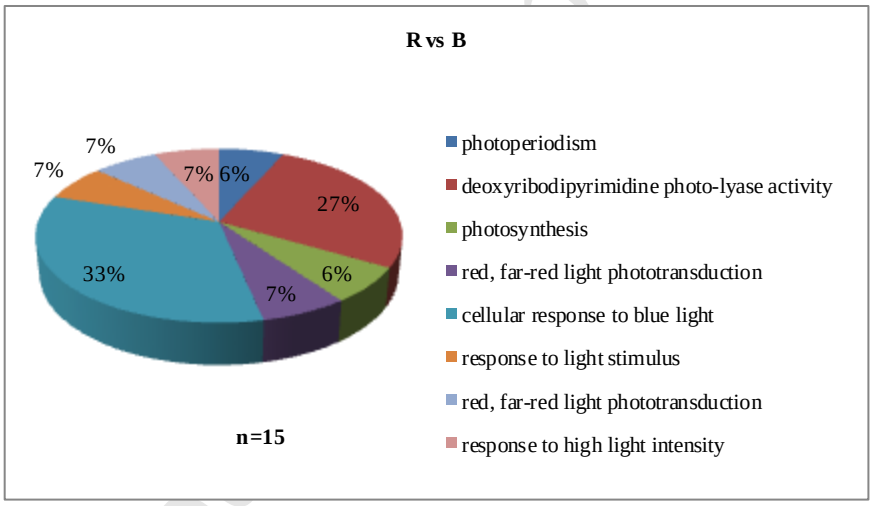

R vs B
7%
7%
7%
6%
27%
6%
7%
33%
n=15
photoperiodism
deoxyribodipyrimidine photo-lyase activity
photosynthesis
red, far-red light phototransduction
cellular response to blue light
response to light stimulus
red, far-red light phototransduction
response to high light intensity

Figure 3

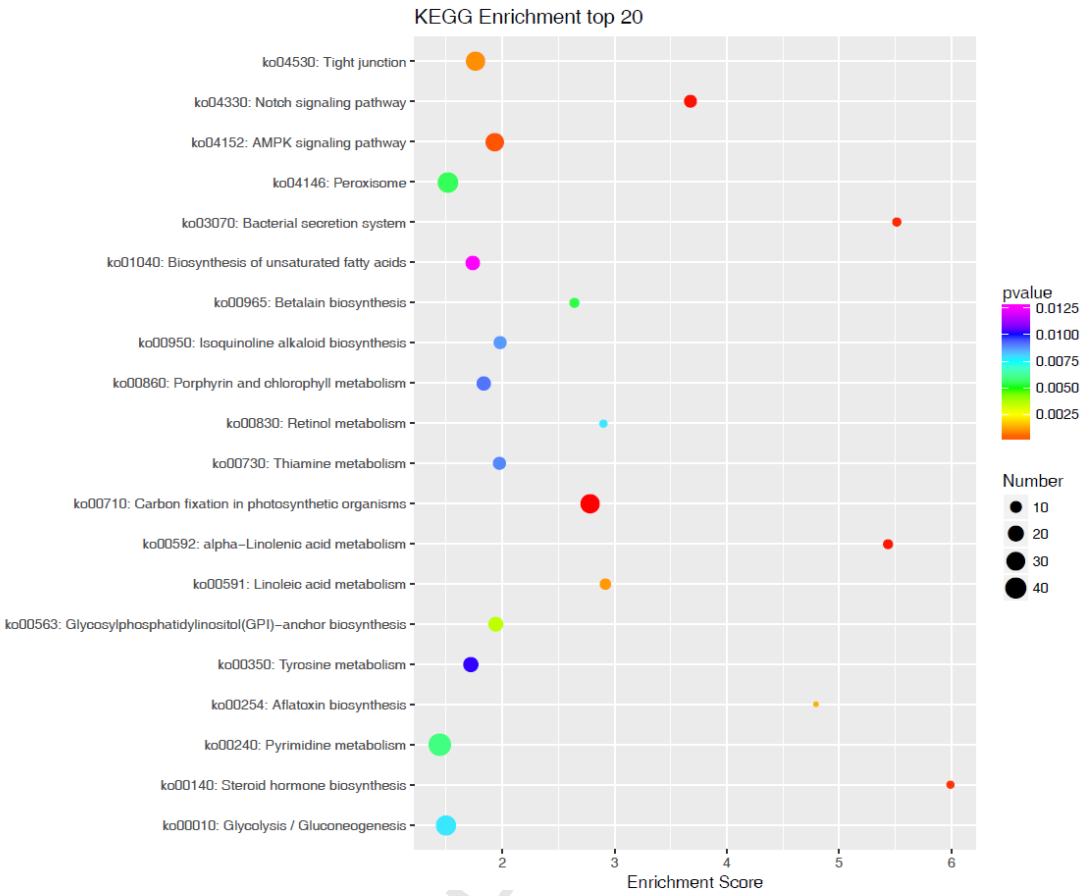


Figure 4

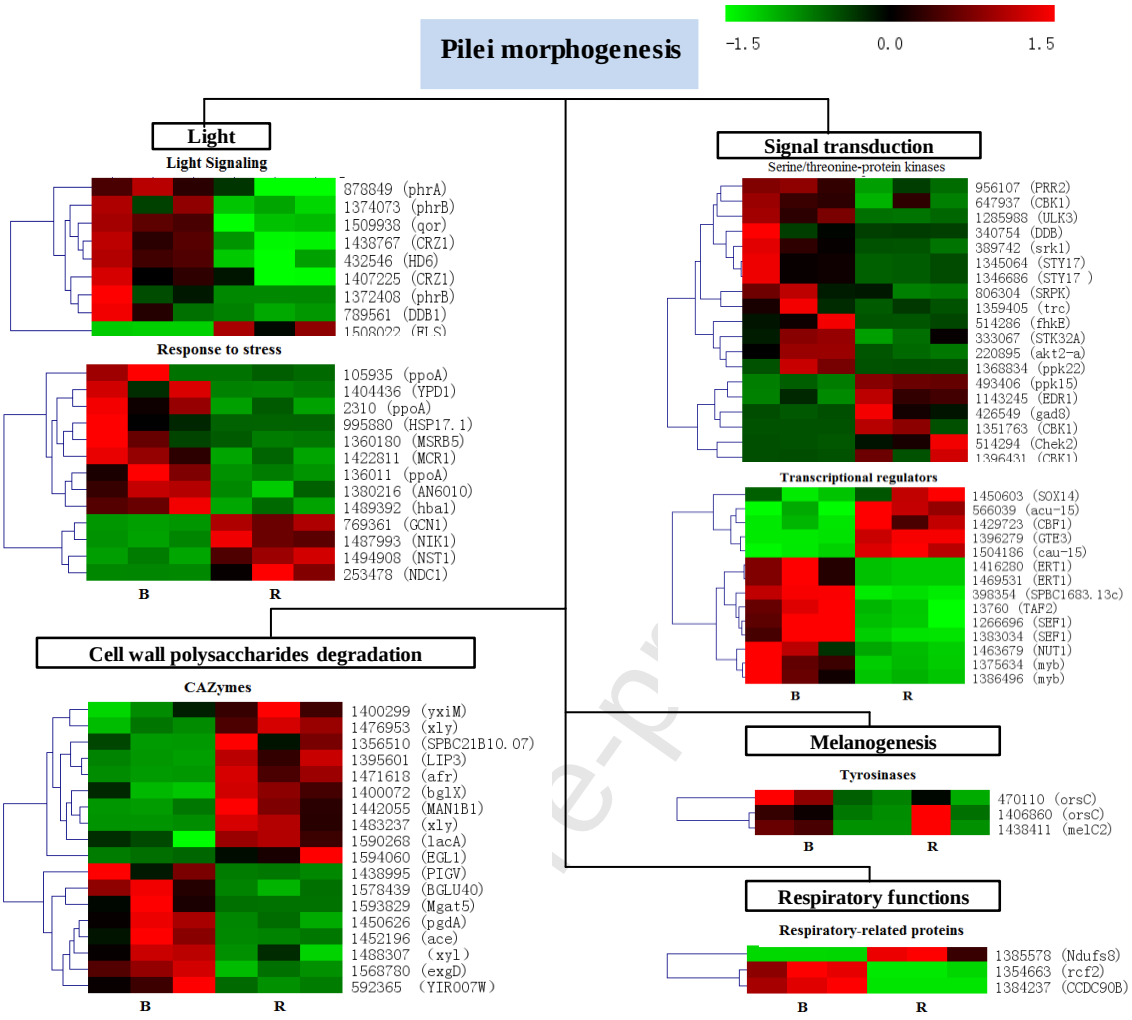

Pilei morphogenesis
-1.5
0.0
1.5
Light
Light Signaling
878849 (phrA)
1374073 (phrB)
1509938 (qor)
1438767 (CRZ1)
432546 (HD6)
1407225 (CRZ1)
1372408 (phrB)
789561 (DDB1)
Response to stress
105935 (ppoA)
1404436 (YPD1)
2310 (ppoA)
995880 (HSP17.1)
1360180 (MSRB5)
1422811 (MCR1)
136011 (ppoA)
1380216 (AN6010)
1489392 (hba1)
769361 (GCN1)
1487993 (NIK1)
1494908 (NST1)
253478 (NDC1)
B
R
Signal transduction
Serine/threonine-protein kinases
956107 (PRR2)
647937 (CBK1)
1285988 (ULK3)
340754 (DDB)
389742 (srk1)
1345064 (STY17)
1346686 (STY17)
806304 (SRPK)
1359405 (trc)
514286 (fhkB)
333067 (STK32A)
220895 (akt2-a)
1368834 (ppk22)
493406 (ppk15)
1143245 (EDR1)
426549 (gad8)
1351763 (CBK1)
514294 (Chek2)
Transcriptional regulators
1450603 (SOX14)
566039 (acu-15)
1429723 (CBF1)
1396279 (GTB3)
1504186 (cau-15)
1416280 (ERT1)
1469531 (ERT1)
398354 (SPBC1683.13c)
13760 (TAF2)
1266696 (SEF1)
1383034 (SEF1)
1463679 (NUT1)
1375634 (myb)
1386496 (myb)
B
R
Cell wall polysaccharides degradation
CAZymes
1400299 (yxiM)
1476953 (xly)
1356510 (SPBC21B10.07)
1395601 (LIP3)
1471618 (afr)
1400072 (bglX)
1442055 (MAN1B1)
1483237 (xly)
1590268 (lacA)
1594060 (EGL1)
1438995 (PIGV)
1578439 (BGLU40)
1593829 (Mgat5)
1450626 (pgdA)
1452196 (ace)
1488307 (xyl)
1568780 (exgD)
592365 (YIR007W)
B
R
Melanogenesis
Tyrosinases
470110 (orsC)
1406860 (orsC)
1438411 (melC2)
B
R
Respiratory functions
Respiratory-related proteins
1385578 (Ndufs8)
1354663 (rcf2)
1384237 (CCDC90B)
B
R

Figure 5

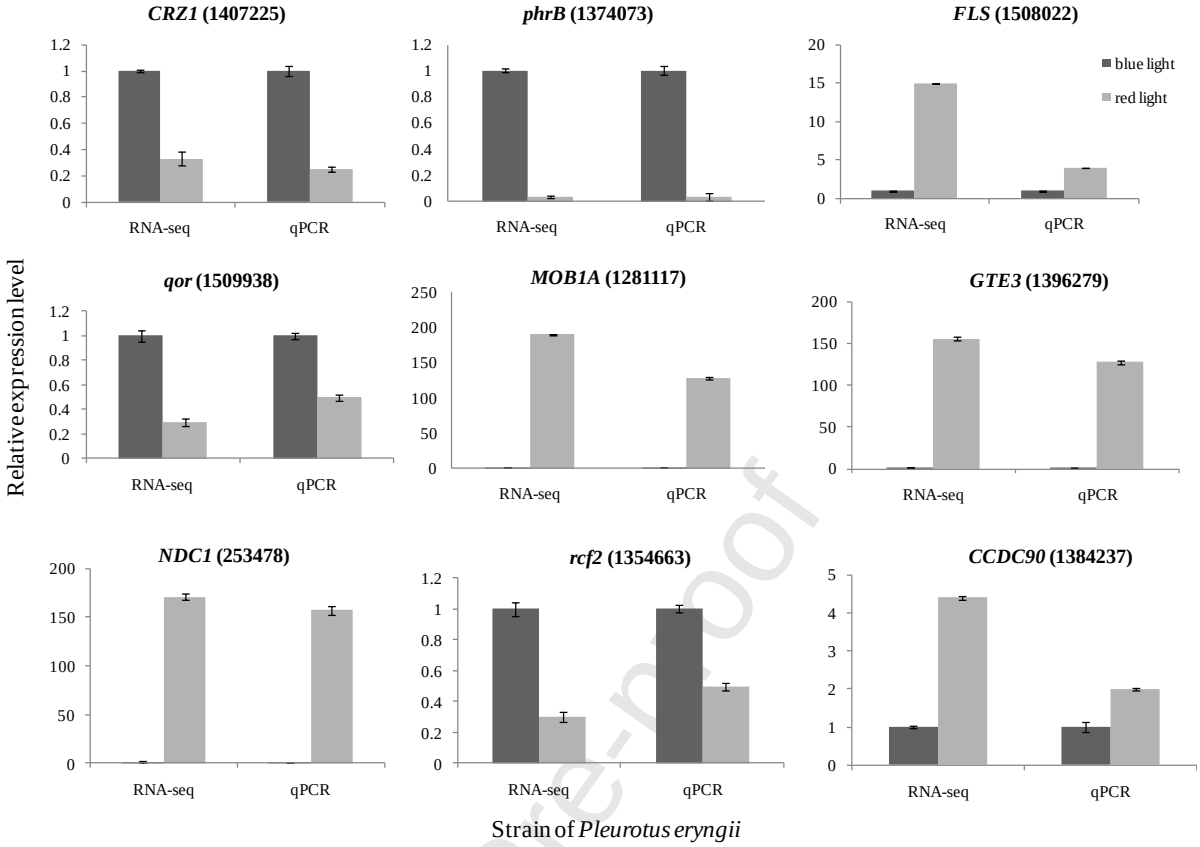


Figure 6

Highlights

1. The variations in metabolic and physiological processes underlying pileus morphogenesis under different light qualities were revealed at the transcriptional level.

2. Genes involved in sensing and transducing blue light signals were significantly up-regulated in blue light-treated pilei, while red light–responsive genes displayed high expression in red light groups of pilei.

3. The expression level of tyrosinase associated with pigment formation was significantly up-regulated in blue light-treated pilei with deeper color.