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Comparative proteomic analysis reveals differential protein expression of *Hypsizygus marmoreus* in response to different light qualities

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ABSTRACT

Light is important environmental stress that influences the growth, development, and metabolism of *Hypsizygus marmoreus* (white var.). However, the molecular basis of the light effect on *H. marmoreus* remains unclear. In this study, a label-free comparative proteomic analysis was applied to investigate the global protein expression profile of *H. marmoreus* mycelia growing under white, red, green, and blue light qualities and darkness (control). Among 3149 identified proteins in *H. marmoreus*, 2288 were found to be expressed in all tested conditions. Data of Each light quality was compared with darkness for further analysis, numerous differentially expressed proteins (DEPs) were identified and the white light group showed the most. All the up-regulated and down-regulated DEPs were annotated and analyzed with the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The KEGG enrichment analysis revealed that light stress was associated with primary metabolism, glycolysis/gluconeogenesis, MAPK, proteasome, and carbohydrate-active enzyme pathways. This study advances valuable insights into the molecular mechanisms underlying the role of different light qualities in mushroom growth and development.

1. Introduction

Hypsizygus marmoreus, one of the mushroom-forming basidiomycetes, is wildly cultivated in Asia with high commercial values due to their organoleptic and medicinal properties [1,2]. Recently, more and more molecular genetics studies have been conducted, which have facilitated *H. marmoreus* a model for studying the developmental process of mushrooms [1,3–6]. Further deep understanding of the fruiting process of *H. marmoreus* will consequently have a commercial impact on non-model mushrooms. The process of mushroom formation is one of the most important research filed in mycological research, which is closely related to mushroom quality and regulated by complex cellular processes. Multiple environmental factors have been proved to affect the fruiting process of mushrooms. Among them, light is a key environmental factor that determines the behavior of fungi, e.g. light controls developmental decisions (fruiting formation and sporulation), stress response, physiological adaptations, metabolism, circadian rhythms, and so on [7,8]. Light effects are often complex and can be influenced by biotic or abiotic factors. Moreover, light signaling may be tightly linked with other biological pathways in fungal cells [9]. Research on the light signal mechanism was comprehensively performed in *Neurospora crassa*, *Aspergillus nidulans*, and other filamentous fungi and thus revealed that the fungi can respond to different light intensities and wavelengths by discrete receptor proteins, such as the White Collar proteins and cryptochromes for blue light, opsins for a green light, and phytochromes for red light [10,11]. Then the light was used as an information signal to perceive and interact with the environment [8,9,12–16]. However, the research on the light effect on edible mushrooms is still in the initial stage.

Light can influence the growth and development of mushrooms. It has been proved that the occurrence of fruiting bodies of *Basidiomycetes* is facilitated by light and light wavelength is found to be the crucial action factor [17,18]. The effect of dark or light at a different wavelength on the growth and development of mycelia, reproduction, and formation of primordia has been studied in a few mushrooms, such as *Coprinopsis cinerea* (used as a model to study the molecular mechanism for photomorphogenesis) [19], *Lentinus edodes* (as one of the most important edible mushrooms worldwide with light-induced brown film formation by the mycelium) [2,20–22], *Pleurotus ostreatus* (popularly cultivated all over the world) [23,24], *Pleurotus eryngii* [25–27] and *Cerrena unicolor* (as a wood-degrading basidiomycete with ecological and biotechnological importance) [28–30]. Due to the limitation of

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molecular and genetic manipulation, these studies were formerly focused on the improvement of growth of the fruiting body or certain metabolic production or proteinase at different light wavelengths, and most recently, the transcriptome technology related to the expression level of RNA was favored to illustrate the mechanisms of light-induced effect on different mushrooms [21,23,25,28]. However, the associated changes in proteins have been poorly understood.

As a rapid-developed powerful tool used for sensitively detection and rapid identification of changes in protein expression in response to various stress conditions, the proteomic has become increasingly relevant to the study of fungal biology and physiology [2,31]. And it has also been applied to study the growth and development of various types of mushrooms, such as *Flammulina velutipes* [32], *Hericium erinaceus*, *Sparassis crispa* [33], and L. *edodes* [2]. When it comes to *H. marmoreus,* the proteomic has been applied to investigate the changes in protein expression responding to heat stress and different developmental stages [34,35]. However, little is known regarding the influence of light on the mycelium growth stage of *H. marmoreus*. Until now, only one study related to different light qualities was reported, which was performed to identify a suitable light colour for the development of the fruiting body but no illustration of molecular mechanism was involved [3].

In this work, we performed a comparative proteomic analysis of *H. marmoreus* at the early mycelium growth stage cultivated in different lighting conditions. Our results provide a comprehensive dataset for analysis of protein expression profile changes occurring in fungal cells in response to variable lighting qualities, contributing to a better understanding of *H. marmoreus* photobiology and light-dependent metabolism and behavior of this commercially editable mushroom.

2. Materials and methods

2.1. Strains, cultivation conditions and sample collection

Hypsizygus marmoreus (white var.) strain G12 used in this study was provided by Shandong Provincial Key Laboratory of Applied Mycology. The strain was inoculated from potato dextrose agar (PDA) slants to an 11 cm PDA plate for 10 days at 25 °C, which was used as inoculum for further studies. The mycelial plugs (5 mm diameter) were cut from the margin of seed agar cultures and placed in the center of new PDA plates, which were covered with cellophane. The mycelia were cultivated at 25 °C in darkness for 5 days (the diameter of mycelia was ~3 cm) and 12 days (the diameter of mycelia was ~10 cm), respectively. Then, the plates were transferred into boxes (40 cm × 30 cm × 30 cm) with different light qualities (dark, white light, red light, green light or blue light), respectively. The following light qualities were applied: white (W group) (400–760 nm), green (G group) (400–480 nm), blue (B group) (492–572 nm), and red (R group) (620–760 nm) light and darkness (D group) as a control. Each group was prepared with three biological repeats. The box was placed at 25 °C and the mycelia were exposed to different light qualities (12 h on and 12 h off, two cycles). In addition, the cover glasses were pre-inserted into the edge of the medium to collect the mycelia for micromorphology observation. After treatment, the colonies of all the plates were batch photographed, and only the mycelia with a growth up to 14 days were scraped from the plate under faint light within 5 min, and immediately snap-frozen in liquid nitrogen and stored at −80 °C until protein extraction.

2.2. Microscopic observation of the H. marmoreu mycelia

To determine the micromorphological structures of *H. marmoreus*, the mycelia collected by cover glasses were stained with Congo Red for 10 min in darkness. Stained mycelia were washed with PBS for 5 min and then observed with confocal laser scanning microscope (CLSM) (Leica TCS SP5II, Bensheim, Germany) for micromorphology visualization.

2.3. Protein extraction and peptide digestion

Total protein extraction from the frozen *H. marmoreus* samples was performed using the method previously described [35]. Briefly, 0.1 g of each frozen sample was transferred into the centrifuge tube with two 5-mm stainless steel beads. The mycelia were frozen using liquid nitrogen and lysed with JXFSTPRP-24 TissueLyser (Jingxin, Shanghai, China) at 60 Hz for 120 s. Then, 1 mL UDT (8 M urea, 0.1 M DTT, 0.1 M Tris-HCl pH 8.5) buffer containing HaltTM Protease Inhibitor Cocktail (Thermo Fisher, Shanghai, China) was added into the centrifugation tube. After vortexed for 5 min, the DNA was broken using water bath ultrasonication for 30 min. Tissue debris was removed by centrifugation (12,000 ×*g* for 10 min at 4 °C), and the supernatant was transferred into a new tube. Protein concentration was determined using the Bradford method.

Thereafter, enzymolysis was performed according to the FASP method as previously described [36].

The initial protein sample for each treatment is 100 μg, and alkylation was performed using iodoacetamide. 2 μg of modified trypsin (Promega, Madison, WI, USA) was used for the enzymolysis process, and enzymolysis was performed with gentle shaking at 37 °C for 12 h. Peptides were eluted with 50 mM NH_4HCO_3 and desalted using C18 SPE dicks (Merck, 66,883-U) according to the methods of Rappsilber et al. [37]. Desalted peptide sample was lyophilized on an RVC 2–25 CD plus vacuum concentrator (Christ, Osterode am Harz, Germany).

2.4. Label-free LC-MS/MS quantitative proteomics analysis

The peptides were reconstituted in 0.1 % formic acid, and the concentration was determined using Nanodrop ONE spectrophotometer (Thermo Fisher Scientific Inc.). The concentration of peptides was adjusted to 0.6 μg/μL using 0.1 % formic acid. LC-MS analysis was performed using a Nano-LC system coupled with Orbitrap Fusion™ Tribrid™ (Thermo Fisher Scientific, San Jose, CA, USA). The MS instrument was operated in data-dependent acquisition (DDA) mode, with full MS scans over a mass range of *m/z* 350–1500 with detection in the Orbitrap (120 K resolution) and with auto gain control set to 100,000. Different chromatographic gradient lengths from 60 to 240 min were tested for peptides separation. All gradient started at 5 % (*v*/v) ACN (0.1 % formic acid) and went up to 32 % (v/v) ACN (0.1 % formic acid). Three biological repeats for each group were applied.

2.5. Identification and quantitation of proteins

The MS raw data of each sample were combined and searched using Proteome Discoverer software suite version 2.0 (Thermo Fisher Scientific, San Jose, CA, USA) against the *H. marmoreus* proteome database (proteome ID UP000076154) from Uniprot. Protein identification was supported by at least two unique peptides with a false discovery rate lower than 0.05.

2.6. Bioinformatic analysis

Raw data obtained from Proteome Discovery software was normalized as follows. Firstly, missing values were supplemented (three sets of data, data with only one set was deleted, and missing values in data with two or three sets were supplemented, which is the k-proximity method). Then, median standardization was executed on intensity data. TBtools was used to construct heatmaps and Venn diagrams [38]. Gene Ontology (GO) [39] (http: //geneontology.org/ (accessed on 18 March 2022)) and Kyoto Encyclopedia of Genes and Genomes (KEGG) [40] (http: //www.kegg.jp/ (accessed on 20 March 2020)) were used to analyze the function and metabolic pathways of differentially abundant proteins. The number of differentially expressed proteins allocated to each category was counted, and the significance of the enrichment was calculated using the hypergeometric distribution test. OmicShare online tools (htt

ps://www.omicshare.com/tools) were applied to plot enrichment diagrams of GO annotations or KEGG pathway annotations. DbCAN for carbohydrate-active enzyme (CAZyme) families (based on CAZyDB07/15/2016) were used to annotate the identified proteins.

3. Results

3.1. Micromorphology visualization of the H. marmoreus

In this study, the mycelia of *H. marmoreus* (white var.) strain G12 were treated with different light qualities comprising white (W), red (R), green (G), blue (B) light, and darkness (D) (as a control), respectively. The morphological changes were detected and assessed at a microscopic scale (Fig. S1). Growth characteristics of the fungus and micromorphological observations of the mycelia structure were carried out using mycelium under controlled light qualities.

On the PDA plate, *H. marmoreus* exhibited typical radical growth with a center circle and formed a pure white mat, which grew faster and more compact in the darkness condition than the light conditions. Accordingly, the fastest growth was observed in darkness. However, obvious growth retardation and weakened central circle during colony formation occurred in the green light and blue light groups (Fig. S1a). After being strained by Congo red, CLSM was used to observe the micromorphology of mycelia. *H. marmoreus* exhibited differences in the morphological features of the mycelia in different conditions tested in this work (Fig. S1b). In general, unbranched skeletal hyphae and hyphae with single septa predominated and were the only types observed in all conditions. It is worth noting that rare branched hyphae and low mycelia crosslinking degree were formed only in darkness.

3.2. Proteomics statistics of H. marmoreus samples for growth in different light qualities and the correlation among the five groups of samples

A label-free LC-MS/MS quantitative proteomics approach was employed to gain insight into the protein expression profile of 14-day-old mycelia of *H. marmoreus* G12 with exposure to different light qualities in the later period. Five groups of samples, each with three replicates, were prepared from *H. marmoreus* mycelia and a total of 3149 proteins were identified based on the database generated from *H. marmoreus* proteome. Analysis of the shared proteins (Fig. 1) showed that 2288 proteins were identified in all five groups of samples. The second-largest subgroup contained 135 proteins distributed in all the groups except the darkness group, which will be analyzed in the section below.

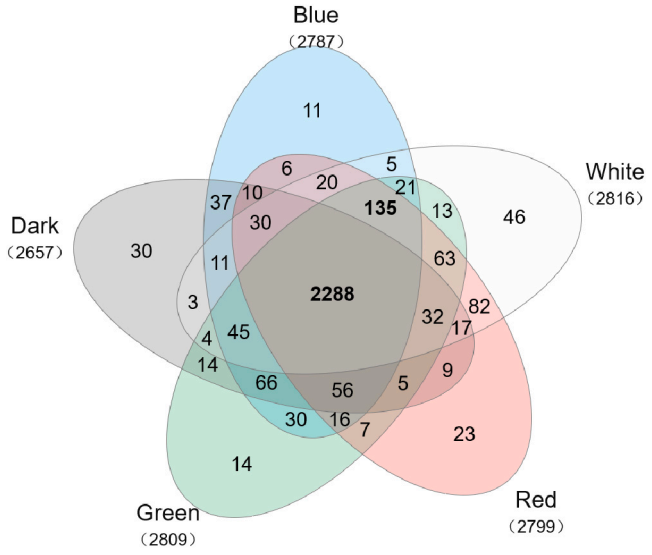

Fig. 1. Venn diagram demonstrating the number of expressed proteins obtained from the tested samples. Proteins found uniquely during *H. marmoreus* growth in white light (light grey), red light (red), green light (green), blue light (blue), and darkness (without light) are marked. The number of expressed proteins common for two, three, four, and all five tested groups are indicated.

Pearson Correlation Coefficient (PCC) analysis (Fig. 2a) indicated that the protein expression profile of the blue light group showed a higher correlation with that of the green light group (0.96) and the darkness group (0.93), respectively, while the white light group displayed a higher correlation with the red light group (0.97). Besides, the white light and darkness groups took the lowest correlation which is 0.63. What is more, the principal component analysis (PCA) exhibited similar results as shown in Fig. 2b, indicating that there was a little difference between the blue light and green light groups, which was the same between the red light and white light groups. In addition, PCC and PCA analysis results revealed samples of all groups were reasonable with good correlations among biological repeats.

The samples were further executed for cluster analysis by heat map. The result revealed that samples from the same group clustered in one cluster, respectively, and a division was observed between two units, one of which was composed of white and red groups and the other was composed of other three groups (Fig. 2c).

3.3. Identification of differentially expressed proteins (DEPs)

The analysis of *H. marmoreus* proteomics revealed numerous differentially expressed proteins (DEPs) between the dark group (control) and each of different light groups used. A 2.0-fold change cut-off and *p*-value BH < 0.01 were used in the volcano plot to categorize DEPs among the common proteins in each comparison (Fig. 3a). A total of 3835 DEPs (including repeat count of the same protein in different comparisons) were identified and the up-regulated proteins predominated in all the tested conditions versus darkness (Fig. 3b). In general, when compared to the control darkness condition, the greatest number of DEPs (375 up-regulated and 268 down-regulated) was observed when the *H. marmoreus* was grown in the white light group. In contrast, the lowest number of DEPs (181 up-regulated and 78 down-regulated) was observed in the blue light group. The red light group was observed with 407 up-regulated and 224 down-regulated and the green light group was with 249 up-regulated, and 75 down-regulated. Besides, the unique proteins expressed under individual light quality were designated as up-regulated DEPs and those only expressed in the darkness group in each comparison were designated as down-regulated DEPs. Numbers of DEPs for each comparison were shown in Fig. 3b. Finally, 1758 nonredundant DEPs were obtained after merging the shared DEPs between each comparison by the Venn diagram (Fig. 3c).

3.4. GO analysis for the DEPs in each comparison to molecular function (MF), cellular component (CC), and biological process (BP) categories

To determine the functions of these DEPs, a GO enrichment analysis was performed to classify the annotated DEPs for each comparison. Generally, these profiled DEPs were categorized into three main GO categories, CC, MF, and BP. In both the white and red light groups, compared to the darkness group, up-regulated DEPs groups were enriched in the BP category while the down-regulated DEPs had the least terms in BP. On the contrary, the blue and green light groups showed remarkably decreasing go terms. Among them, the down-regulated DEPs of the blue light group were mostly enriched in the BP category, and the green light group had the least GO terms (Fig. 4a).

Level 2 of the GO terms enrichment for each comparison was further analyzed for in-depth functional illustration and the results were shown in Fig. 4b. In the comparison of white light group and darkness group (W vs D), the 760 up-regulated and 495 down-regulated DEPs were assigned in 38 GO terms, including 15 terms in the BP category, 13 terms in the CC category, and ten terms in the MF category (Table S1). In the

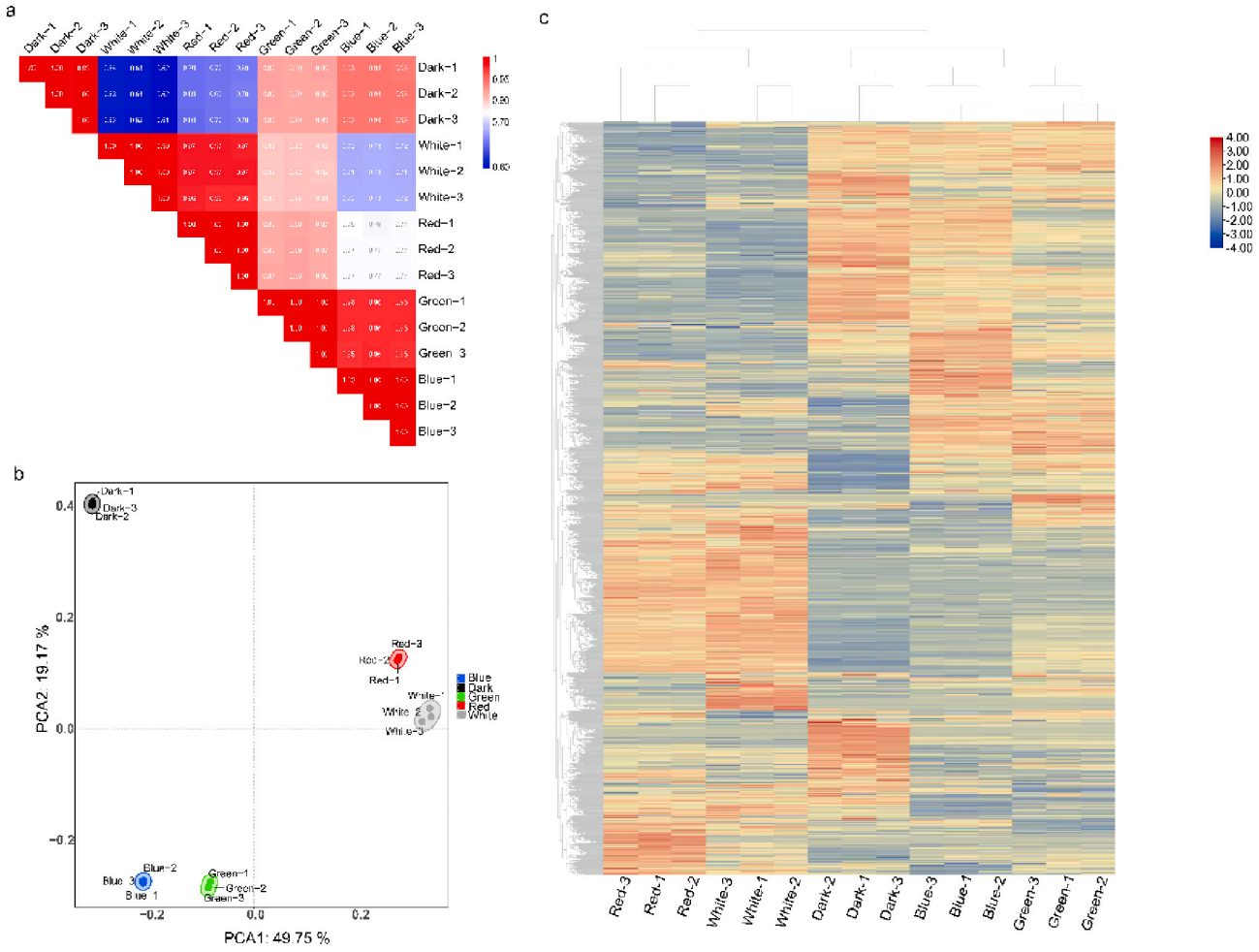

Fig. 2. Correlation analysis and cluster analysis of the proteomic dataset of five groups of *H. marmoreus* samples. (a) PCC analysis for pair-wise comparisons of proteome data. (b)Principal component analysis (PCA) of proteome data from the five groups of samples. (c) Heat map generated from 3149 proteins identified in all samples by hierarchical clustering using paired Euclidean distance. The value is treated by using the log2 scale and using Row Scale in the normalized method. The White, Red, Green, Blue and Dark indicate the samples of the group treated with corresponding light qualities and each with three repeats marked as −1, −2 and − 3.

comparison of red light group and darkness (R vs D), the 759 up-regulated and 434 down-regulated DEPs were assigned in 40 Go terms, including 16 terms in the BP category, 13 terms in the CC category, and 11 terms in the MF category (Table S2). In the comparison of green light group and darkness group (G vs D), the 548 up-regulated and 222 down-regulated DEPs were assigned in 37 GO terms (level 2), comprising 13 BP, 13 CC, and 11 MF terms (Table S3). In the comparison of blue light group and darkness group (B vs D), the 425 up-regulated and 192 down-regulated DEPs were assigned in 36 GO terms, including 13 BP, 13 CC, and ten MF terms (Table S4). Based on the number of DEPs classified into each category, the largest up-regulated GO terms and down-regulated GO terms enriched in the three categories were in accordance in all the comparisons, those were the cellular process, metabolic process, single-organism process, and cellular component organization or biogenesis for BP; the cell, cell part, organelle, membrane, membrane part, macromolecular complex and organelle part for CC; catalytic activity, binding, structural molecule activity and transporter activity for MF. However, the B vs D comparison displayed the least corresponding number of that, which may lead to the obvious proteomic differences with other comparisons.

Besides, the top 20 of GO enrichment were illustrated in a circle map as shown in Fig. 4c and detailed in Fig. S2. All the comparisons had six GO terms all belonged to the CC category in common, those were GO:0005737 (cytoplasm), GO:0005622 (intracellular), GO:0044424 (intracellular part), GO:0005623 (cell), GO:0044464(cell part) and GO:0032991 (macromolecular complex). Besides, another six common CC terms were found in the W vs D, G vs D and B vs D comparisons, those were GO:0043229 (intracellular organelle), GO:1990904 (ribonucleoprotein complex), GO:0043226 (organelle), GO:0043234 (protein complex), GO:0030529 (intracellular ribonucleoprotein complex), and GO:0000786(nucleosome). Thus, it is speculated that the general influence of light may be exerted mainly through the biological functions of the cellular component (CC) to make the differences from the darkness. In addition, GO:0016853 (isomerase activity) as the only MF term, together with the other two CC terms GO:0044444 (cytoplasmic part) and GO:0016469 (proton-transporting two-sector ATPase complex) and two BP terms (GO:0072521 (purine-containing compound metabolic process) and GO:1901564 (organonitrogen compound metabolic process) only existed in W vs D and R vs D comparisons, which may contribute the similarity for the white and red light groups. The common five CC enrichment terms of GO:0043228 (non-membrane-bounded organelle), GO:0043232 (intracellular non-membrane-bounded organelle), GO:0000785 (chromatin), GO:0044815 (DNA packaging complex) and GO:0032993 (protein-DNA complex) existing only in the G vs D and B vs D comparisons may contribute to the similarities for the green and blue light groups. However, the unique top enriched Go terms for each comparison may make the specific differences for each light effect, those were (1) GO:0006397 (mRNA processing) in BP category for W vs D; (2) 9 of nucleoside phosphate associated metabolic process in BP category for R vs D; (3) GO:0006333 (chromatin assembly or disassembly) in BP

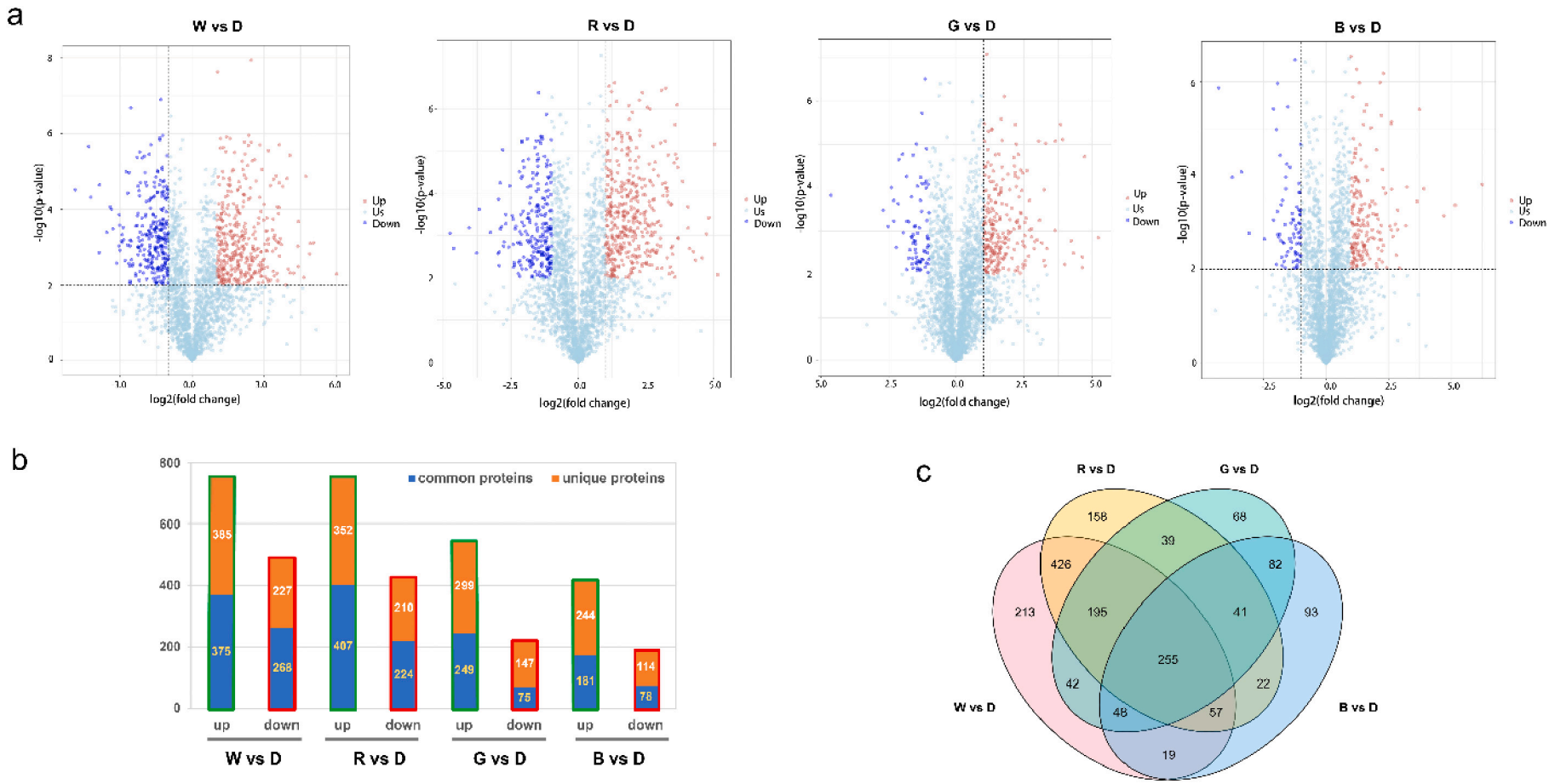

Fig. 3. Analysis of DEPs of each comparison group between the light and darkness conditions. (a) Volcano plot showing changes in protein expression from White, Red, Green and Blue light groups comparing to Darkness group, respectively. Up stands for up-regulated proteins, Down stands for down-regulated proteins and Us means proteins with unsignificance. (b) Statistics of the up-regulated (marked with green square frame) and down-regulated (marked with red square frame) DEPs profiles in four comparisons, W vs D, R vs D, G vs D, and B vs D, respectively. The blue columns indicate common proteins existing in both light and darkness conditions, while the orange ones indicate unique proteins only expressed in light qualities (defined as up-regulated) or in darkness (defined as down-regulated). (c) Venn diagram of the intersection and the number of all nonredundant 1758 DEPs among four comparisons.

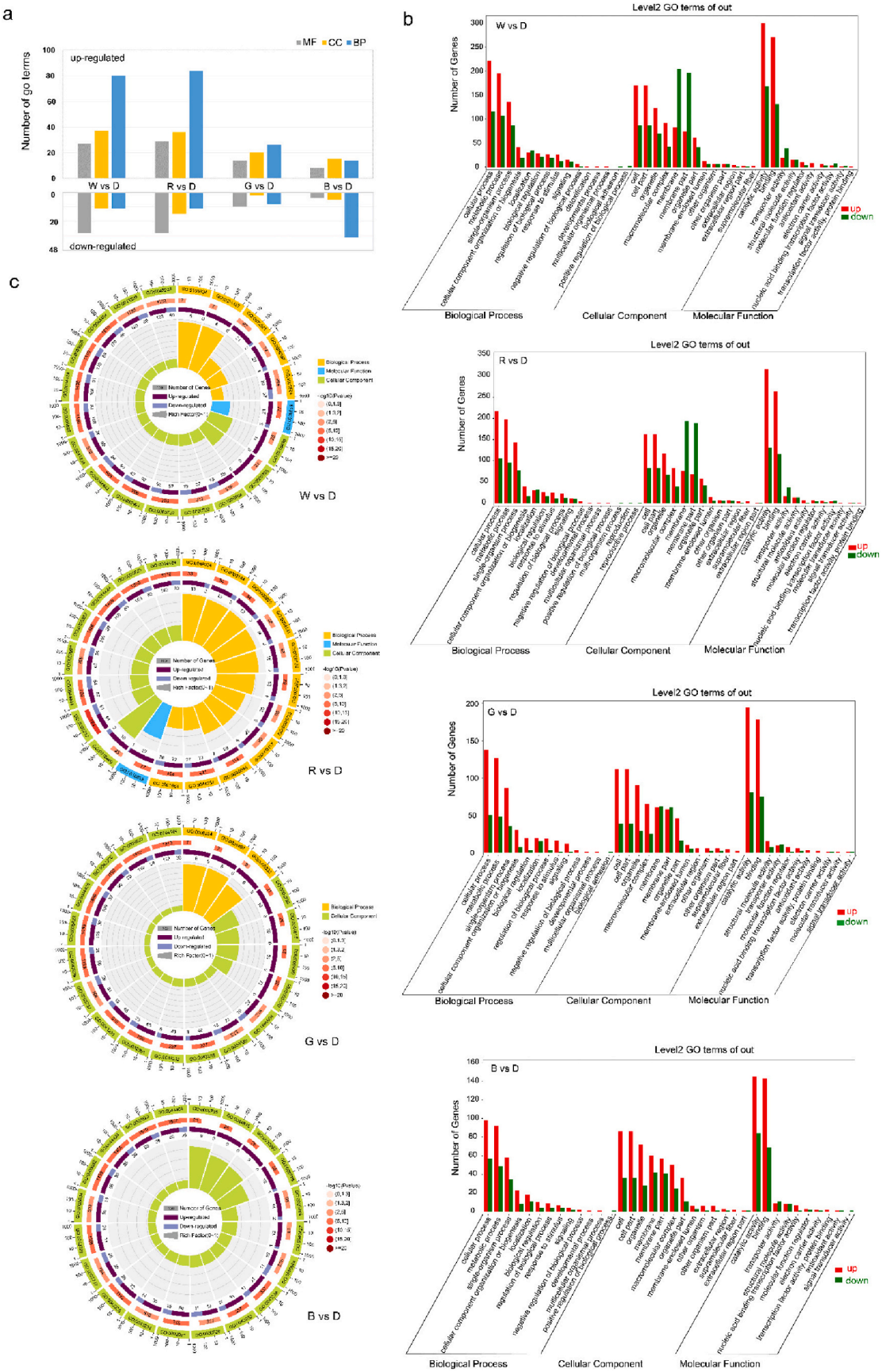

Fig. 4. Go enrichment analysis of the DEPs from each comparison, W vs D, R vs D, G vs D, and B vs D, respectively. (a) Quantity assignment of Go terms for the up-regulated DEPs and down-regulated DEPs of each comparison into MF, CC, and BP categories. (b) Level 2 of the go terms enrichment for each comparison. The red columns indicate the up-regulated DEPs and the green ones indicate the down-regulated DEPs.

category for G vs D; (4) GO:0005694(chromosome), GO:0044427 (chromosomal part) and GO:0005634 (nucleus) in CC category for B vs D.

Overall, various light qualities induced up-regulated and down-regulated DEPs in the proteome of *H. marmoreus* with common terms and differential terms in all the three GO categories. When focusing on the top 20 enrichment, the blue light mainly affected the CC function, the red light mainly conducted the change in BP function, the green light worked in between, and the white light took an overall effect.

3.5. KEGG pathway enrichment analysis of DEPs

KEGG pathway analysis was performed for the DEPs of each comparison. Generally, the DEPs in the W vs D comparison were associated with 110 specific KEGG pathways (104 for the upregulated DEPs and 74 for down-regulated DEPs) (Table S5). The DEPs in the R vs D comparison were associated with 111 specific KEGG pathways (101 for the upregulated DEPs and 71 for down-regulated DEPs) (Table S6). The DEPs in the G vs D comparison were associated with 98 specific KEGG pathways (83 for the upregulated DEPs and 58 for down-regulated DEPs) (Table S7). The DEPs in the B vs D comparison were associated with 91 specific KEGG pathways (75 for the upregulated DEPs and 54 for down-regulated DEPs) (Table S8). Only a few KEGG categories were found as unique in individual light group when down-regulated genes were considered in the pathway analysis. Results of the distributions of mainly enriched KEGG pathways based on the number count (≥8 for W vs D and R vs D; ≥4 for G vs D and B vs D) were shown in Fig. 5 and the distributions of the KEGG B class were shown in Fig. S3. Generally, the DEPs encoding various pathways were mostly up-regulated in each of the analyzed light groups, and most of the DEPs were ascribed into the "Metabolic pathways (ko01100)" category in all the lighting conditions. The proteins representing KEGG pathways related to Biosynthesis of secondary metabolites (ko01110), Spliceosome (ko03040), RNA transport (ko03013) and Ribosome (ko03010) were fairy abundant in all the light groups as well. However, proteins related to the environment information processes category were only identified in the MAPK signaling pathway – yeast (ko04011), which was associated with lots of environmental stresses. It was speculated that the MAPK signaling pathway played an important role in responding to different light qualities. Among all the comparisons, the W vs D as well as R vs D showed a large number of DEPs related to MAPK signaling pathway – yeast, while the B vs D as well as G vs D showed much less, which was in accordance with PCC analysis mentioned above and implied the significant difference caused by while/red light and blue/green light.

We focus on the top 20 pathways (Fig. 6) and the DEPs significantly enriched in the KEGG pathways of Protein export (ko03060), Spliceosome (ko03040), Protein processing in the endoplasmic reticulum (ko04141) and the Purine metabolism (ko00230) were found in all the four comparisons. Changes of protein expression in these common pathways might reveal the light effect on the mycelium growth of *H. marmoreus*. The individual enriched pathways in each comparison displayed the influence degree contributed by the relevant proteins under corresponding light conditions, *e. g.* Glycerophospholipid metabolism (ko00564), Cysteine and methionine metabolism (ko00270), etc., in white light; Pyruvate metabolism (ko00620) in red light; Riboflavin metabolism (ko00740) and Cyanoamino acid metabolism (ko00460) in green light; and Alanine, aspartate and glutamate metabolism (ko00250) and Proteasome (ko03050), etc., in blue light.

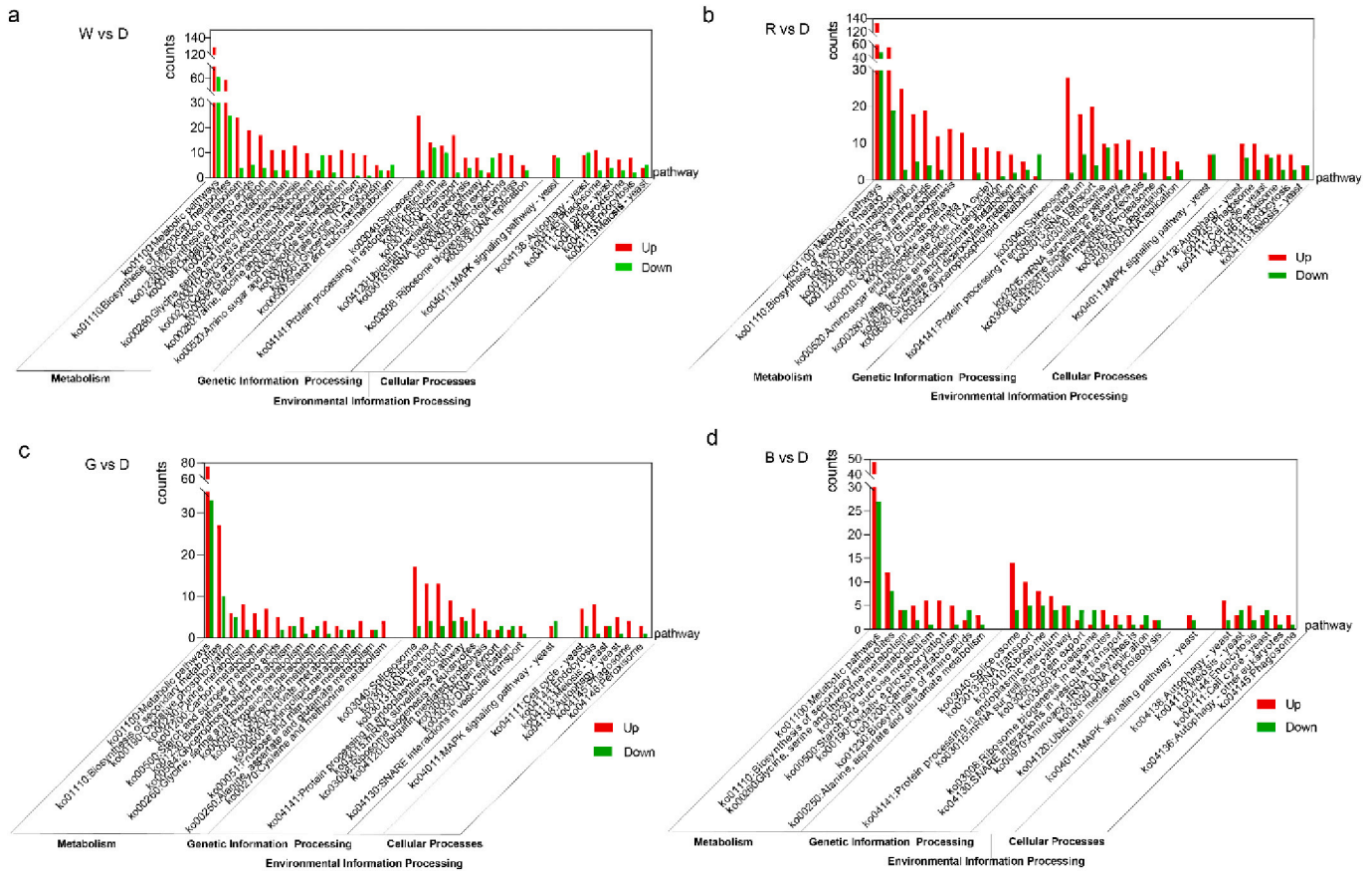

Fig. 5. Quantity statistics of the DEPs in the KEGG pathway analysis were obtained for the following light culturing conditions: (a) white vs dark; (b) red vs dark; (c) green vs dark; and (d) blue vs dark. The mainly enriched pathway categories were shown as Metabolism, Genetic Information Processing, Environmental Information Processing, and Cellular Processes. The number of specific up-regulated (red bar) and down-regulated (green bar) proteins are indicated.

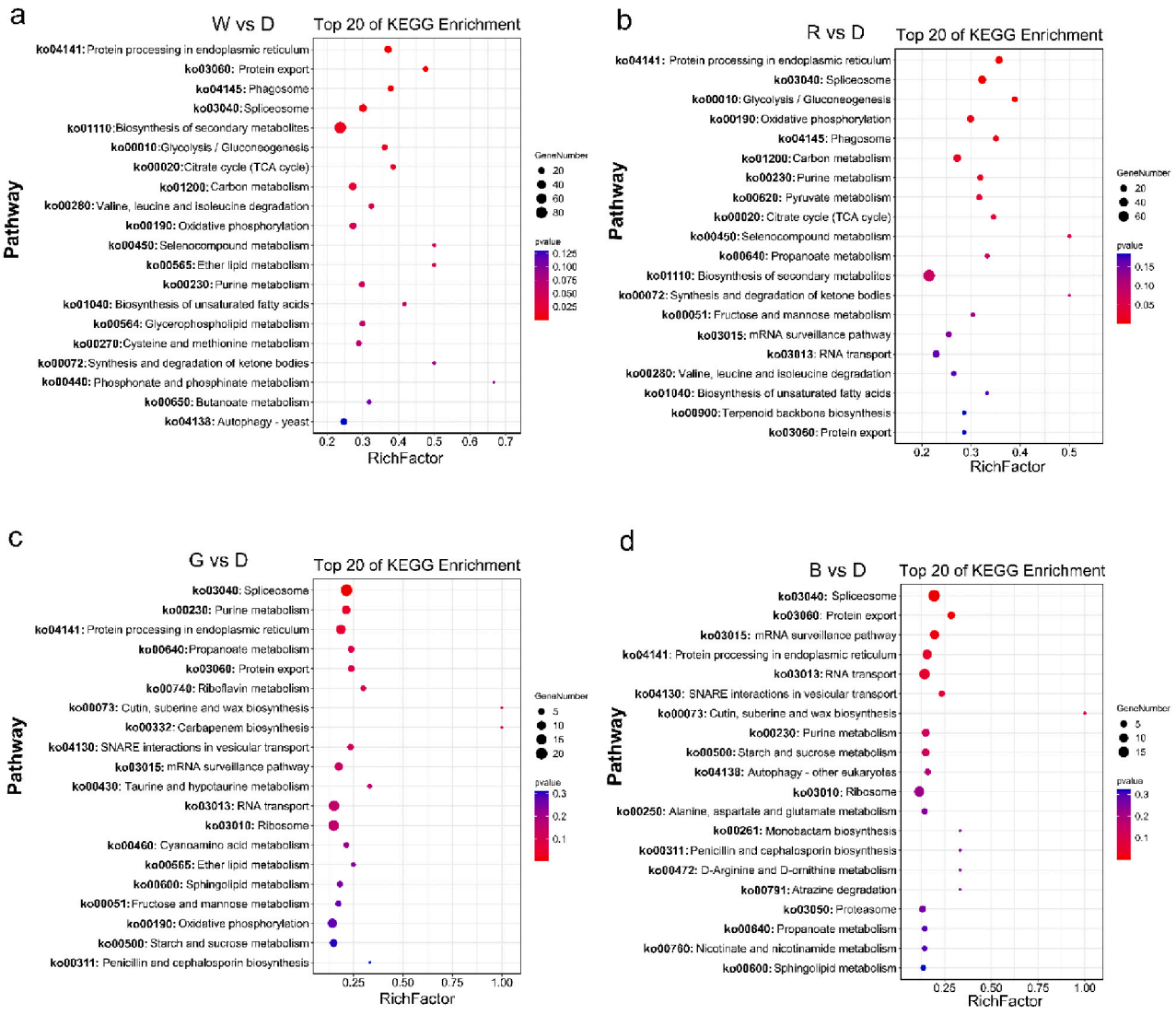

Fig. 6. Top 20 enriched KEGG pathways of the DEPs for each comparison group: (a), white vs. dark; (b), red vs. dark; (c), green vs. dark; and (d), blue vs. dark. The colour intensity is proportional to the enrichment significance, and the circle size indicates the number of enriched proteins.

3.6. *Analysis of the unique proteins under all light conditions*

As mentioned above in Section 3.1, 135 proteins were identified in all the groups except the darkness group, which all belonged to the DEPs identified Table S9. These proteins were mostly enriched in the Go terms of mRNA processing, mRNA metabolic process, RNA splicing, Isomerase activity as shown in Fig. 7a, and only 34 proteins among them were enriched in KEGG pathways and mostly in the spliceosome (ko03040) pathway (Fig. 7b). The spliceosome composed of several proteins and multiple small RNA molecules is involved in assembly of exon-based mRNA by removal of introns from pre-mRNAs. It is obvious that the light, no matter what colour, generally promoted the expression of mRNA levels which lead to the activation of proteins of *H. marmoreus*.

All the 135 proteins in different light groups were further executed for cluster analysis by heat map, and a significant discrepancy was displayed between the red light group and the blue light group (Fig. 7c). Expression of the top 20 of the 135 proteins in different light qualities-was shown in Fig. 7d, which can be divided into three groups according to the expression trend. The first group showed the highest expression in the red light group and lowest expression in the blue light group, which conferred to the Oxidative phosphorylation pathway (A0A369JR33) and the Purine metabolism pathway (A0A369K836). Particularly, the A0A369JGZ2 related to a heat shock 9/12 family protein and A0A369J818 as an uncharacterized protein showed an obviously high expression level under the white and red light. The second group showed a descending expression from the white light to the blue light group, which conferred the RNA transport (A0A369JYQ0) and Endocytosis (A0A369JX46) pathway. And the AOA369JKDX4 as an uncharacterized protein showed an obviously high expression level under the white and red light. The third group showed the lowest expression in the red light group and a much higher expression in the blue light group, which conferred to the RNA polymerase pathway (A0A369JK48) and Cutin, suberine and wax biosynthesis pathway (A0A369JRW6). In a word, light-induced an expression of the special proteins compared to the darkness, but with a different level when responding to different light qualities. The function of the uncharacterized proteins with high expression needs to be further experimentally identified.

3.7. *Light regulates primary metabolism of H. marmoreus*

According to the KEGG pathway analysis, light exerted a global influence on the primary metabolism of *H. marmoreus*. First of all, in comparison to darkness group, fungus growth in the white and red lights showed a great in common. Substantially increased expression of various proteins associated with primary metabolism pathways such as carbon metabolism (ko01200) and glycolysis/gluconeogenesis

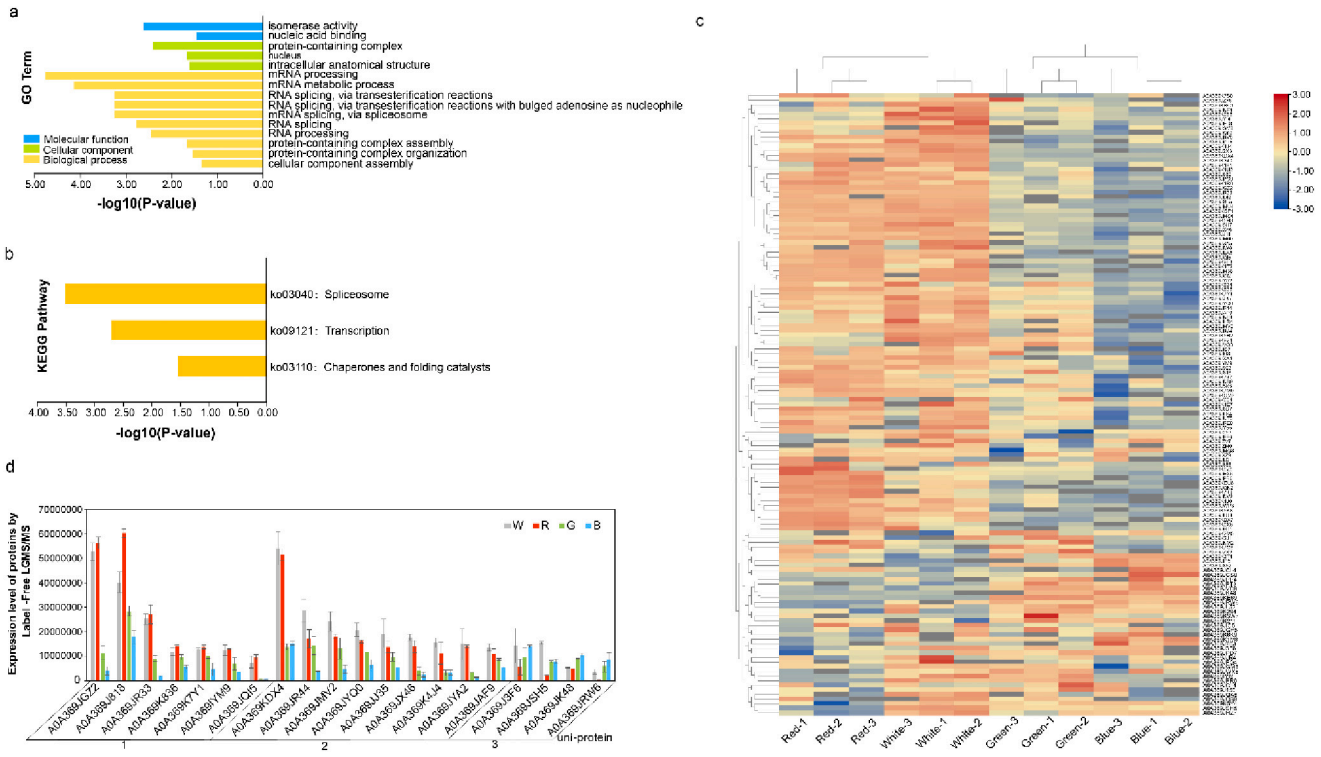

Fig. 7. Analysis of the 135 proteins expressed under lighting conditions. (a) GO enrichment of the 135 proteins. The abscissa axis indicates the enrichment index using -log10(*P*-value), and the ordinate axis indicates the go terms for each category, MF, CC, and BP. (b) KEGG enrichment of the 135 proteins. The abscissa axis indicates the enrichment index using -log10(P-value), and the ordinate axis indicates the KEGG pathways. (c) heatmap analysis of expression of 135 proteins in all light groups. (d) top 20 expression levels of the proteins under different light, W for the white light, R for the red light, G for the green light, and B for the blue light.

(ko00010) were highly concentrated in these two light conditions. On the other hand, up-regulated proteins corresponding to D-lactate dehydrogenase [EC:1.1.1.28], L-lactate dehydrogenase (cytochrome) [EC:1.1.2.3], (*R*)-2-hydroxyglutarate—pyruvate transhydrogenase [EC:1.1.99.40], pyruvate dehydrogenase E1 component [EC:1.2.4.1], pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase) [EC:2.3.1.12], acetyl-CoA *C*-acetyltransferase [EC:2.3.1.9], homocitrate synthase [EC:2.3.3.14] may enhance the pyruvate metabolism (ko00620). Most of the DEPs with a depressed expression during the white or red lighting condition encoded putative enzymes that are involved in tryptophan metabolism (ko00380), glycerophospholipid metabolism (ko00564), and sphingolipid metabolism (ko00060). In addition, the white or red light-induced expression of putative enzymes that engaged in valine, leucine and isoleucine degradation (ko00280), and enhanced the acetyl-CoA acyltransferase [EC:2.3.1.16] or enoyl-CoA hydratase [EC:4.2.1.17] in the fatty acid degradation (ko00071). However, starch and sucrose metabolism (ko00500) in all the lighting conditions except the white light, seemed to be significantly influenced by up-regulating the β-glucosidase [EC:3.2.1.21] that generates D-glucose.

The fungus cultivation in the blue light showed the least differences in comparison with darkness. Up and down-regulated putative enzymes were enriched in pathways involved in ribosome (ko03010), purine metabolism (ko00230), spliceosome components (ko03040), mRNA surveillance pathway (ko03015), and RNA transport (ko03013), however, proteins engaged in the proteasome (ko03050) were down-regulated.

Similar to the blue light, the green light resulted in decreased expression of proteins responding to the ribosome, tryptophan metabolism, and beta-Alanine metabolism. Moreover, the protein A0A369K3S8 as an enzyme pyridoxamine 5′-phosphate oxidase [EC:1.4.3.5] related to Vitamin B6 metabolism (ko00750) was detected with no expression in the green light, which was the same with darkness.

4. Discussions

Light is an important factor that influences the growth and development of mushrooms, which may induce divergence due to response to different light wavelengths [25,28]. Although many transcriptomes of light effect on fungi, such as *H. marmoreus*, *Letinula edodes*, *Cerrena unicolor*, *Pleurotus eryngii*, have been reported [1,2,25,28], few studies reveal the changes of proteins under different light wavelength by proteomes. In this work, the protein profiles of *H. marmoreus* responding to different light qualities were studied to characterize its reaction to illumination. Referring to expression data, from 11 to 46 proteins were found to be unique in each of the ligh conditions, suggesting that *H. marmoreus* can respond to specific light wavelengths in a certain manner. This observation was in agreement with the previously reported transcriptome studies on different basidiomycetes [25,28]. The analysis of protein expression under different light qualities revealed numerous DEPs in comparison with darkness. The KEGG pathway analysis of DEPs revealed significant enrichment of metabolic pathways. Light signaling may be tightly linked with other pathways such as sporulation, primary metabolic pathways, or the production of secondary metabolites or hydrolytic enzymes [9]. However, the light receptor proteins of this *H. marmoreus*, such as white collar 1 protein (A0A369KBB3) and cyanobacterial phytochrome B (A0A369J697 and A0A369JC30), showed no expression in all the five groups, suggesting a low expression level of these light receptor proteins at the mycelia stage.

4.1. Proteins correlated to glycolysis/gluconeogenesis pathway

Among the central carbon metabolism pathways, the glycolysis/gluconeogenesis pathway (ko00010) is crucial to providing energy and biochemical precursors for living organisms and tightly associated with several carbon metabolism pathways [41,42]. Most of the enzymes on the glycolytic pathway catalyze reversible reactions that also contribute

to gluconeogenesis [43,44]. In this study, 13 DEPs (corresponding to 11 key reversible enzymes) of glycolysis/gluconeogenesis pathway were significantly enriched in *H. marmoreus* under different light qualities, especially the white and red light (Fig. 8). Briefly, the enzymes responding to the transformation of glucose-1P to glycerate-2P, including putative phosphoribomutase [EC:5.4.2.2], glucose-6-phosphate isomerase [EC:5.3.1.9], fructose-bisphosphate aldolase, class I [EC:4.1.2.13], Triosephosphate isomerase [EC:5.3.1.1], glyceraldehyde-3-phosphate dehydrogenase [EC:1.2.1.12], phosphoglycerate kinase [EC:2.7.2.3] and phosphoglycerate mutase (2,3-

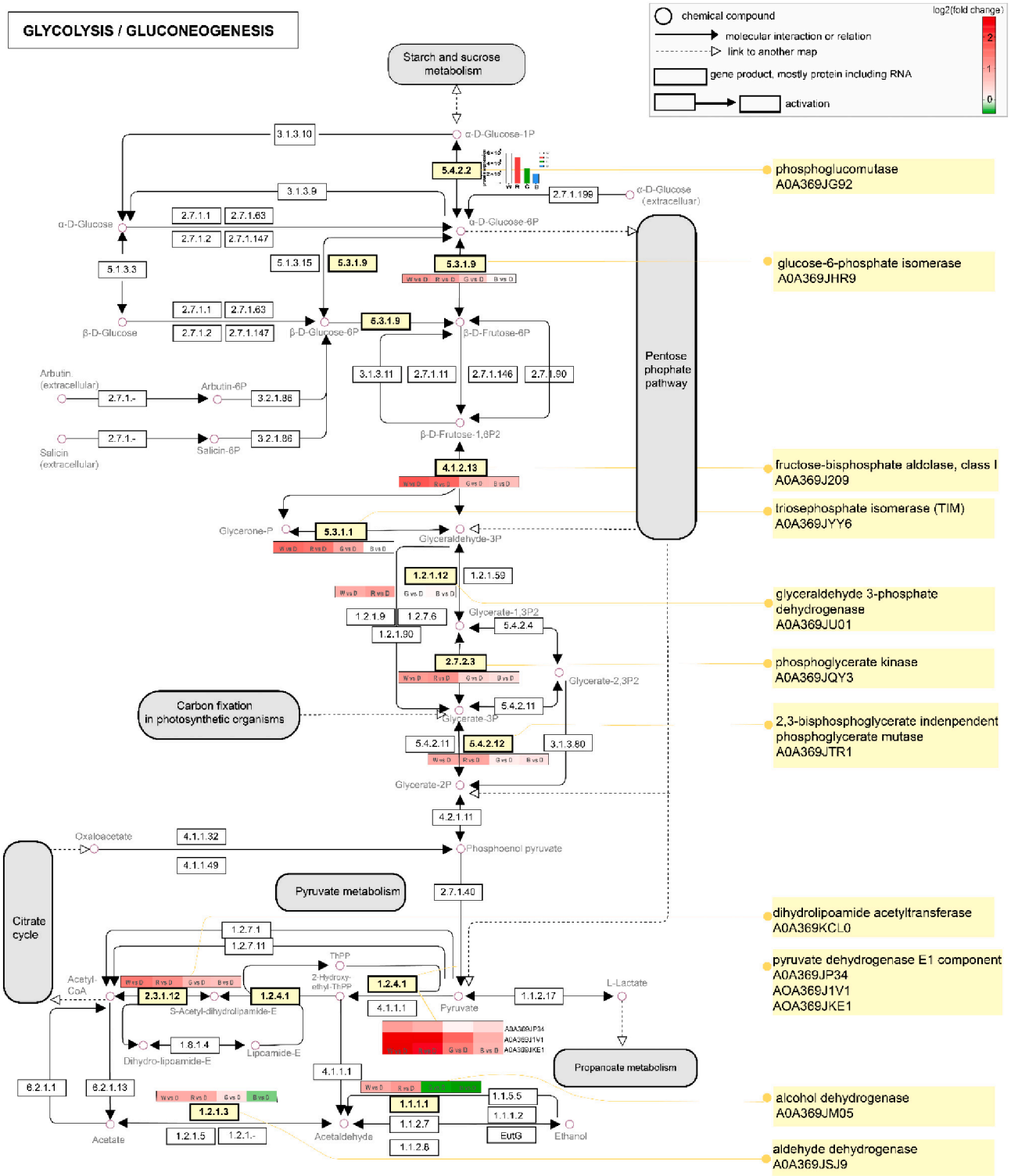

Fig. 8. The expression analysis of DEPs located in the ko00010 (glycolysis/gluconeogenesis) pathway of all comparisons. The enzymes related to 13 DEPs from all comparisons were marked with yellow box. The name and protein ID of these enzymes are indicated on the right side. The corresponding change of protein expression of each DEP in all comparisons (W vs D, R vs D, G vs D, and B vs D) is displayed by heatmap using log2(foldchange). The legends of the pathway and the colour indicator for log2(foldchange) are showed on the upper right quarter. Besides, the protein expression of the enzyme (EC5.4.2.2) is displayed in a histogram form due to no expression detected in the darkness group, W for white light group, R for red light group, G for green light group, and B for blue light group.

diphosphoglycerate-independent) [EC:5.4.2.12], were much more significantly up-regulated under the white and red light than the green and blue light, however, the blue light showed the least changes. More to this point, the phosphoribomutase [EC:5.4.2.2] was detected with no expression in the darkness group, but with a differential expression in the light groups (Fig. 8). The pyruvate dehydrogenase E1 component subunit [EC:1.2.4.1] (including three DEPs) and acetyltransferase component of pyruvate dehydrogenase complex [EC:2.3.1.12], which related to pyruvate dehydration, were upregulated under all light qualities. Aldehyde dehydrogenase [EC:1.2.1.3] andalcohol dehydrogenase [EC 1.1.1.1] were upregulated under white and red light but downregulated under the blue/green light, indicating that the blue light group may have the weakest capacity in alcohol metabolism.

The expression changes of these relevant enzymes in each comparison (light group compared with the darkness group) indicated that glycolysis/gluconeogenesis pathway was of great significance for *H. marmoreus* to adapt to different light conditions (Fig. 8). Besides, the enrichment of genes in the glycolysis/gluconeogenesis pathway when responding to light stress has also been reported in *Pleurotus ostreatus*, *Scylla paramamosain*, *etc* [23,44]. Given all that, it is speculated that the stimulation of white/red light may promote relative pathways of carbon metabolism by significantly accelerating the glycolysis/gluconeogenesis pathway and thus facilitate the mycelium growth of *H. marmoreus*.

4.2. Proteins correlated to stress response by HOG pathway in the MAPK signaling pathway

MAPK signaling pathways are critical for the adaption of eukaryotic cells to environmental stresses [45]. In multicellular fungi, it has been approved that MAPK pathways are important signal transduction pathways involved in regulating various physiological activities such as cell function, cell wall integrity, fruiting body development, stress response, and melanogenesis [7,35,46].

Among 87 proteins that engaged in MAPK signaling pathways in the *H. marmoreus*, a total of 24 DEPs were identified under different light qualities. Five of them corresponding to hyperosmotic stress deserved our attention of their differential expression in each group (Fig. 9a), especially three of which belonged to the HOG pathway. They are as follows: the Sko1 (also named as Aft1) belongs to ATF/CREB family transcription factor, the general transcriptional corepressor Tup1, and the p38 family kinase Hog1 as a mitogen-activated protein kinase (EC 2.7.11.24) (Fig. 9b). The Sko1 and Tup1 enzymes were highly up-regulated in white and red light groups but showed no obvious difference in green or blue light group when in comparison to darkness group. The expression of Hog1 MAP kinase in these four light groups was upregulated to different degrees from 0.84 to 0.31 of the log2 (fold-change) (Fig. 9a). The yeast HOG pathway related to osmo-adaptive responses has been well illustrated [47]. Generally, the Sko1 as a transcriptional repressor of the ATF/CREB family, regulates several genes by recruiting the Tup-Ssn6 corepressor complex to target promoters. The activated Hog1 MAP kinase could phosphorylate Sko1, convert Sko1 into a transcriptional activator and disrupt the association of Sko1 with Ssn6 and Tup1 proteins [47,48]. The *H. marmoreus* samples with trace expression of Sko1and Tup1 under blue and green light, and high up-regulated expressions under the red and white light (Fig. 9a), indicated that the red and white groups may have a reduced tolerant to osmotic stress. Considering a higher expression of Hog1 in the white light group can counteract partial inhibition of Sko1, the red light group

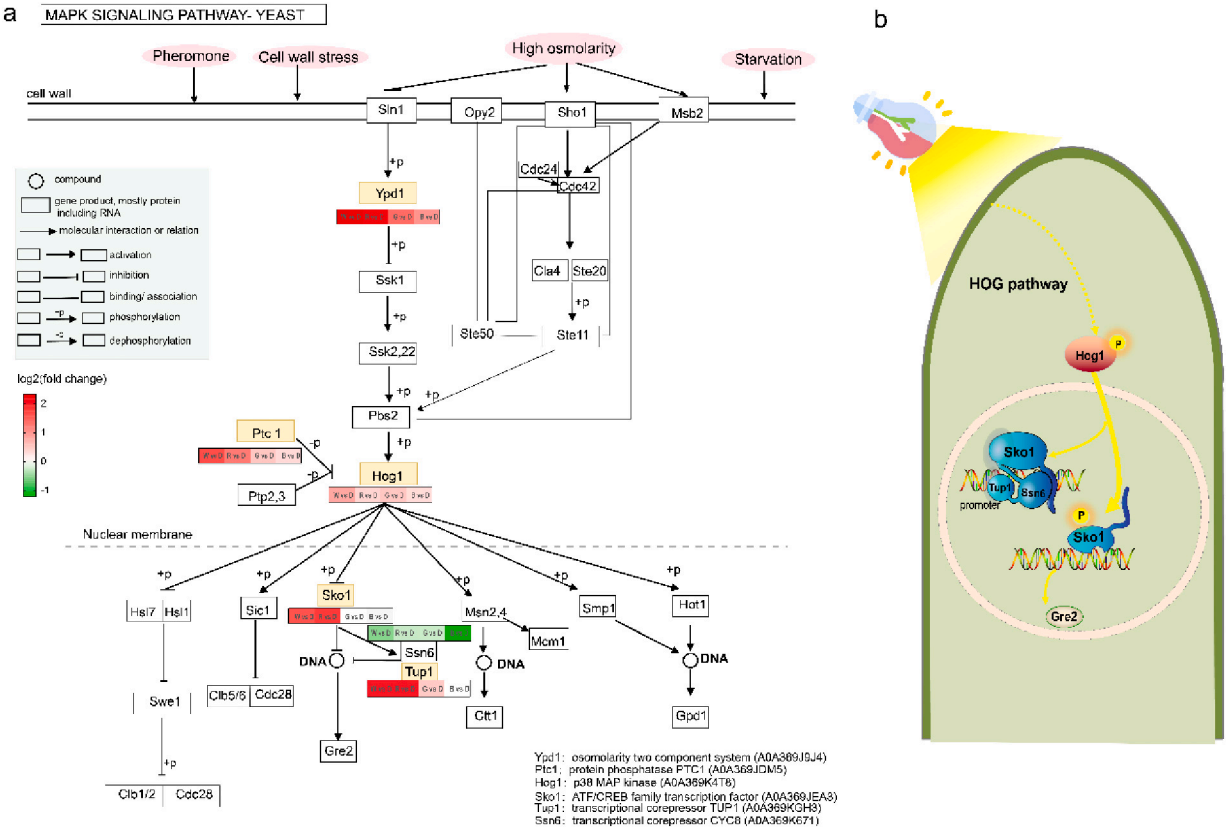

Fig. 9. The expression analysis of DEP in high osmolarity pathway located in ko04011 (MAPK signaling pathway – yeast) of all comparisons . (a) Flow chart of the high osmolarity pathway of the MAPK signaling pathway. The enzymes related to five DEPs from all comparisons in the high osmolarity pathway were marked with a yellow box and indicated at the bottom. The corresponding change of protein expression of each DEP in all comparisons (W vs D, R vs D, G vs D, and B vs D) is displayed by heatmap using log2(foldchange). The legends of the pathway and the colour indicator for log2(foldchange) are showed on the upper left quarter. (b) Schematic diagram of the light-induced HOG pathway. The relationship of Sko1, SSn6 and Tup1 during the phosphorylation (P) processes is documented.

may take the least tolerance. Coincidentally, it was reported that conidia of *Metarhizium robertsii* produced in the dark were the most tolerant and conidia produced under red light were the least tolerant to that [49]. The link between light and stress signaling was also discovered in *Botryti cinerea* (associated with cell wall stress) [50] and *Aspergillus nidulans* (associated with the HOG pathway) [51]. It is worth noticing that, the main response output of light signaling is the control of gene expression, hence the light signaling has to be transmitted into the nuclei. Under this circumstance, cytoplasmic phytochromes use the high osmolarity glycerol (HOG) signaling module for signal transduction from the cytoplasm to the nucleus (Fig. 9b). The HOG pathway is the central stress signaling module and is required for sensing oxidative or osmotic stress. In the end, the AtfA transcription factor-like Sko1 will induce gene expression as mentioned above [9].

4.3. Proteins correlated to proteasome pathway

In face of environmental changes, fungi have evolved multiple defense systems. Among them, ubiquitin-proteasome-mediated proteolysis (Fig. 10a) plays a crucial role in various cellular stress responses, leading to the degradation of proteins and changes in the rate of intracellular protein turnover [52]. The eukaryotic 26S proteasome degrades the majority of proteins in the cell under normal conditions, which is a large multisubunit complex composed of two 19S regulatory particles and one 20S core particle (Fig. 10b). Most substrates are first covalently modified by ubiquitin, which then directs them to the proteasome [53]. Light signal affects the protein stability by proteasome pathway has been studied in white-rot basidiomycetes such as *C. unicolor*, revealing various genes involved in proteolysis in response to light and the potential of proteases serving as regulatory molecules [30]. In this study, the KEGG analysis revealed a high enrichment in the proteasome pathway for the *H. marmoreus* under the blue light 15 DEPs related to the proteasome pathways in all comparisons were further analyzed and proteins were mainly reflected in 26S protease regulatory subunits and proteasome subunit (EC:3.4.25.1), most of which belong to the Go terms of protein catabolic process [GO:0030163], ubiquitin-dependent protein catabolic process [GO:0006511], and proteasome-mediated ubiquitin-dependent protein catabolic process [GO:0043161] (Table S10). The different light wavelengths could result in expression changes of the different subunits of the core 20S in the 26S protease (Fig. 10b). The proteins AOA269JPY7, A0A369JWP4, A0A369JU17 and A0A369K4H1 related to proteasome subunit α2, α6, β1, and β3 were remarkably down-regulated (−1.25 to −2.02 of log2(fold change)) under the blue light while proteins A0A369J3U0, A0A369KBQ2, A0A369JDV9 and A0A369K4N4 related to proteasome subunit α3, α5, β2 and β7 were up-regulated (0.47 to 3.61 of log2(fold change)) under the white and red light. Besides, the expression of proteins A0A369J780, A0A369J7L7, AOA369JUQ3, A0A369JTI5, and A0A369K928 related to the base of 19S regulatory particle (PA700) were all increased in all light groups only with one exception (Fig. 10b). However, the proteins AOA369JT04 and AOA369J7Y4 related to the subunits of the lid of 19S acted as up-regulated and down-regulated DEPs, respectively (both was down-regulated for the blue light group). The green light group showed no special features unless a half down-regulation proteins related to proteasome subunit α2, α6, β1, and β3 and a slightly upregulated proteins related to proteasome subunit α5 and β7 (Fig. 10b). To a certain extent, the proteasome pathway was significantly activated by the white or red light, and the blue light group committed the lowest proteolytic activity.

In *Aspergillus oryzae* cells, proteolytic activity showed sensitivity to light and production of respective enzymes were increased in darkness. In addition, a study on the regulation of protein phosphatases in *Trichoderma reesei* revealed the dependence of protease production by three phosphatases in a light-dependent manner [54]. Light control protein stability has been achieved by constructing a light-sensitive module, which is composed of a photoreceptor and a synthetic degron. The blue light induces conformational changes in the photoreceptor, which in

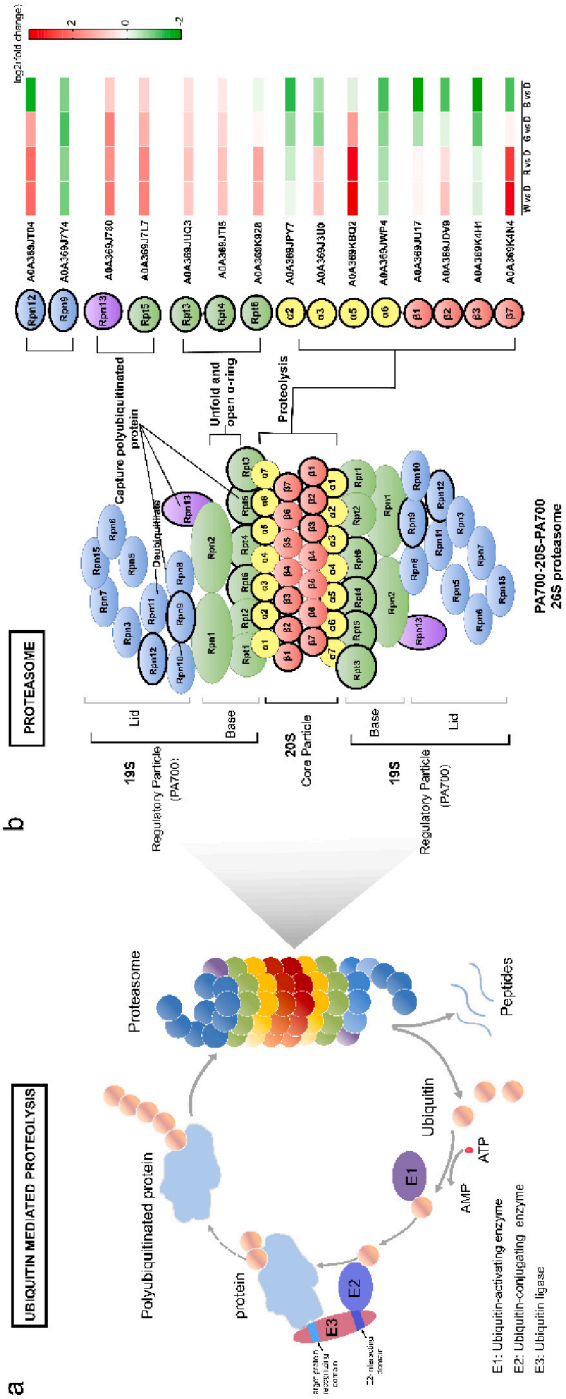

Fig. 10. The expression analysis of DEPs located in ko03050 (Proteasome pathway) of all comparisons. (a) Schematic diagram of role of the proteasome in the ubiquitin mediated proteolysis pathway. (b) Composition of the 26S proteasome and the expression analysis of the DEPs in proteasome pathway. The 15 DEPs are marked with bold outline and their expression changes in all comparisons are displayed with the colour indicator for the log2(foldchange) at the right. W vs D, R vs D, G vs D, and B vs D are short for the comparisons, in that W for white light group, R for red light group, G for green light group, and B for blue light group.

turn lead to activation of the degron and thus proteasomal degradation of the whole fusion protein [55].

Based on the observation in this study, not only the blue light can downregulate the proteasome but the red light or the white light can promote the proteasome activity, which indicates that the proteasome pathway of *H. marmoreus* was able to respond to different light wavelengths in various manners. However, the significance of regulation protease activities by light remains largely unclear and further studies should be followed up.

4.4. Proteins correlated to carbohydrate-active enzymes (CAZymes)

Mycelial growth and differentiation entail nutrient accumulation, especially the accumulation of carbohydrates. Carbohydrate-active enzymes (CAZymes) play key roles in carbohydrate metabolism, e.g. substrates degradation and nutrient absorption [56]. Based on conserved domains associated with catalytic activities, CAZymes can be divided into six categories such as glycosyl hydrolases (GHs), glycosyltransferases (GTs), carbohydrate esterases (CEs), polysaccharide lyases (PLs), carbohydrate-binding modules (CBMs), and auxiliary activities (AAs). 73 CAZymes-related proteins were identified from the DEPs list by searching against the CAZyme database, including 43 GHs, 9 GTs, 3 CEs, 1 Pls, 3 CBMs and 14 AAs (Table S11).

GHs hydrolyze the glycosidic bond between carbohydrates or between a carbohydrate and non-carbohydrate [57]. Among the 43 differentially expressed GHs, 19 were upregulated in all the comparisons, especially, the GH72 (AOA369KA55), GH152 (A0A369K6M8) and GH55 (A0A369JP91) were highly up-regulated (logFC>2). Meanwhile, GH37 (A0A369JUV5), GH5_9 (A0A369IYW8) and GH85 (A0A369JEP8) were highly down-regulated in all the comparisons. GH72 is formed exclusively from transglycosylases of fungi, and is involved in the elongation and remodeling of the 1,3-β-glucan of the cell wall [58]. The GH152 family as stress-induced thaumatin-like proteins are abundant in plants, and fungal GH152 members were reported to have β-1,3-glucanase activity in *Lentinula edodes and Ganoderma lucidum* [59,60]. GH55 family containing both *endo-* and *exo*-β-1,3-glucanases are associated with the degradation of the cell wall in mushrooms [61]. It was speculated that these up-regulated enzymes facilitated the cell wall assembling in facing light stress. GH37 can degrade the disaccharide into substituent glucose moieties for entry into metabolism [62]. GH85 is a family of endo-β-*N*-acetylglucosaminidases that cleave the *N*, *N*-diacetylchitobiose core (beta-1,4 linkage) of N-linked glycans. The deduced expression of these two enzymes represented an inefficient way of synthesis of neoglycopeptides and utilization of trehalose substrates under light. Moreover, GH5 is one of the largest GH families with a wild range of enzyme activities on a different substrate and it is most likely linked to the cellulase activity and obtaining nitrogen from the degradation of cellulose in mushrooms [27]. Seven GH5 enzymes from the DEPs included five up-regulated proteins, one down-regulated and one asynchronous protein in all the comparisons, demonstrating within enzyme sub-family variation to different light stimulations. In addition, the GH17 (A0A369K3S0), GH105 (A0A369K6X0) and GH109 (A0A369J4I9) proteins were upregulated under green and blue light and down-regulation under white and red light, which was consistent with the brown film formation stage of *Lentinula edodes* under blue light [21]. It was suggested these enzymes play important roles in providing sufficient nutrition for the growth of *H. marmoreus* under green or blue light. In fungi, approximately half of the GH5 proteins harbor a CBM1 module at the N- or C-terminus [63]. Here, three CBM13 associated with endo-1,4-beta-xylanase were identified, A0A369K5Y4 was activated by light, A0A369K0R8 was down-regulated and A0A369JXZ5 was upregulated in all light groups. CBM13 has evolved with a variety of sugar-binding specificity and is found in many carbohydrates active enzymes [64].

GTs, as the enzymes that catalyze glycosylation, are involved in the biosynthesis of disaccharides, oligosaccharides and polysaccharides [21]. GT2 is one of the largest families among GTs, however, many GTs have not been characterized. In our study, three GTs were upregulated in all light groups: GT8 (A0A369K411) related to glycogenin-1, GT61 (A0A369JD78) related to EGF domain-specific O-linked *N*-acetylglucosamine transferase, and GT2 (A0A369J0R1) related to Hyaluronan synthase. Two GTs were downregulated in all light groups: GT2 (A0A369J6L0) related to dolichyl-phosphate beta-glucosyltransferase (EC 2.4.1.117) which is responding to protein glycosylation and GT48 (A0A369K433) related to 1,3-beta-glucan synthase (EC 2.4.1.34) in starch and sucrose metabolism pathway. GT59 (A0A369K484) didn't show any expression in the white and red light groups, while GT1 (A0A369JFK1) responded for sterol 3-beta-glucosyltransferase UGT80A2 was only detected expression in the green light group. GT22 and GT90 showed a slight change in each comparison.

Only one PL14_5 (A0A369JDP4) as an uncharacterized protein was upregulated in light groups except for the blue light. The PLs family cleaves uronic acid-containing polysaccharide chains via α, β-elimination mechanism and results in the generation of an unsaturated hexenuronic acid residue and a new reducing end.

Among the CEs, CE9 (A0A369JT67) and CE4 (A0A369K1N3) were upregulated under all light qualities and the other CE4 (A0A369K6X3) was down-regulated under green and blue lights. CE4 is the largest of the CE families, including chitin and peptidoglycan deacetylases, acetylxylan esterases, and poly-*N*-acetylglucosamine deacetylases. Chitin deacetylases are involved in cell wall morphogenesis and remodeling in fungi [65]. The differential regulation of CE4 (A0A369K6X3) between the white/red and green/blue light groups indicated an activated assembly of cell walls for *H. marmoreus* under the red or white light.

The expression of enzymes of AAs showed diversity among all groups. 13 of the 14 AAs were all expressed under blue light, while 5 of the AAs were detected with no expression under white or red, or green light. Among them, AA3_2 (A0A369K7A9) and AA7 (A0A369K051) were down-regulated in all the light groups. The AA1_1(A0A369K108) related to laccase only detected with expression under the blue light and darkness, demonstrating that blue light and darkness can induce the expression of laccase. The increasing laccase activity of *H. marmoreus* is involved in mycelial growth and fruiting body initiation [66]. The enzymes with AAs accommodate a range of enzyme mechanisms and substrates, which comprise redox enzymes such as lytic polysaccharide monooxygenases, lignin-active class-II peroxidases and laccases. A large and varying group of auxiliary activities is represented by family AA3. The common feature of AA3 family members is the formation of key metabolites such as H_2O_2 or hydroquinones required for other AA enzymes [67].

5. Conclusion

In this study, the mycelia of *H. marmoreus* growing under different light qualities were collected for proteomic analysis. A total of 1758 DEPs were identified between the dark group and each light group with a threshold of 2.0-fold change cut off and *p*-value BH < 0.01. Among them, 135 common proteins were expressed under all light qualities except the dark condition. The top 20 of Go terms revealed that the blue and green light mainly affected the CC category while the white and red light mainly conducted the change in BP and CC categories. The KEGG results showed that light stress was associated with a lot of biological and metabolism pathways. Among them, proteins related to the primary metabolism, MAPK and proteasome pathways were highly up-regulated in the white and red light groups, probably leading to an active turnover of carbon and protein but low tolerance to osmotic stress. In addition, 73 CAZymes were identified and most of them associated with nutrition supply and cell wall construction were up-regulated under all light qualities and the most appeared in the white light group, which may have resulted from the overall stimulation by daylight. Besides, the blue light and darkness may induce laccase activity. Our study provided insights into the mechanism of light-induced influence from the proteome

level. This study may provide a valuable resource for further studies of this mushroom and would aid mushroom farming in the future.

CRediT authorship contribution statement

Liping Zhu: Investigation, Data curation, Writing- Original draft.
Yao Su: Methodology.
Zhiheng Ma: Methodology.
Lizhong Guo: Resources.
Song Yang: Writing- Reviewing and Editing, Funding acquisition.
Hao Yu: Conceptualization, Work design, Methodology and Validation.
All of the authors have read and approved the manuscript.

Studies in humans and animals

Not applicable.

Informed consent and patient details

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw data for the proteomic analysis reported in this paper have been deposited in the OMIX, China National Center for Bioinformation/ Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix: accession no. OMIX002332).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2022.11.037.

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