AcademicPres

Wang Y *et al.* (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 2, Article number 13606
DOI:10.15835/nbha52213606
Research Article

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Sequencing and analysis of transcriptome to reveal regulation of gene expression for polysaccharide synthesis in *Dendrobium officinale* under different light quality

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Abstract

Light is a key factor affecting the growth and quality formation of *Dendrobium officinale* Kimura et Migo. In this study, we used *D. officinale* under different light conditions as experimental materials to explore the key genes that regulate its polysaccharide content. In addition, we cloned the *UGPase* gene and validated the gene by bioinformatics analysis and real-time quantitative PCR. Compared with the natural light control group, 494, 1630 and 599 differentially expressed genes were screened under different light quality conditions of red, blue and yellow, respectively. GO and KEGG enrichment analyses of these differentially expressed genes showed that the differentially expressed genes were enriched in the light signal transduction process under different light quality conditions. Bioinformatics analyses showed that the sequences of the genes were highly conserved with the sequences of the *UGPase* genes of other species, and had the highest sequence similarity with Phalaenopsis, which is also a member of the orchid family. Real-time quantitative PCR results showed that red light had the most significant promoting effect on the *UGPase* gene of *D. officinale*. In this study, we applied high-throughput sequencing technology to establish a transcriptome database of *D. officinale* under different light conditions and obtained a large amount of transcriptional information about *D. officinale*. The related genes affecting the quality of *D. officinale* were analysed at the transcriptional level, and the *D. officinale UGPase* gene was cloned, and its expression was analysed in different tissues and under different light conditions.

Keywords: *Dendrobium officinale*; light qualities; polysaccharide content; transcriptome analysis; *UGPase*

Received: 04 Jan 2024. Received in revised form: 18 Apr 2024. Accepted: 06 Jun 2024. Published online: 26 Jun 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Dendrobium officinale (*D. officinale* Kimura et Migo) is an herbaceous plant of the genus *D. officinale* in the family of Orchidaceae, which is a medicinal, food and rare and valuable plant with high medicinal value and has a very high value of application in in the medicine auxiliary treatment, health care and nutrition (Jin *et al.*, 2013; Xiang *et al.*, 2013). It has the efficacy of liver protection, anti-tumour, hypoglycaemia, gastric ulcer protection and enhancement of human organism immunity *D. officinale* is rich in saccharides, of which polysaccharides (*D. officinale* polysaccharide, DOP) are its main active ingredient and the highest content of them, mainly including glucose, xylose, arabinose, galactose, Rhamnose, mannose and trace fructose, polysaccharide is the main component of *D. officinale* antioxidant, scavenging free radicals and other pharmacological effects, it was found that the higher the polysaccharide content of *D. officinale*, the higher the quality of its germplasm. *UGPase* is a key enzyme in the glucose metabolic pathway of plants, which can catalyse the reaction between glucose 1-phosphate and uridine triphosphate to produce UDP-glucose and pyrophosphate, thus providing precursors for the synthesis of cellulose, hemicellulose, pectin, glycolipids, glycoproteins and so on in medicinal plants, and therefore it is one of the key enzymes in the pathway of polysaccharide synthesis. Studies have shown that the *UGPase* gene is widely distributed in a variety of plants. At present, this gene has been studied and reported accordingly in rice, astragalus, barley, potato and other plants, but the study on the function of *UGPase* gene in the biosynthetic pathway of sugar metabolism has been less reported in *D. officinale*.

Light is one of the important conditions in plant growth and development, light signals provide energy for plant growth and development, and also regulate plant secondary metabolism, light factors affecting plant growth are mainly light intensity (Li *et al.*, 2013), light quality and light time (Goto *et al.*, 2003; Chory and Wu, 2001). Light quality is crucial for plant growth and development, it not only serves as an energy source to control photosynthesis, but also as a trigger signal to affect plant growth. Different light quality triggers different photoreceptors, which in turn affects the photosynthetic properties, growth and development, stress resistance and senescence of plants, etc (Tatusov *et al.*, 2000) However, no in-depth research has been carried out on the role of different light qualities in the growth regulation of *D. officinale*. The group's previous research found that the polysaccharide content of two-year old *D. officinale* is the highest, and light is one of the important conditions in plant growth and development, to provide energy for plant growth and development, but also can regulate the secondary metabolism of the plant, is a key factor affecting the growth and development of *D. officinale* and the quality of the formation of *D. officinale*, on the through the review of the literature, for the time being, did not find the relevant literature of the study of the effect of different light qualities on the quality of *D. officinale* (Meng *et al.*, 2011).

Therefore, this experiment took *D. officinale* biennial as the research object and used different light quality as the experimental conditions to study the changes of polysaccharide content of *D. officinale* under different light quality and used transcriptomics method to study the changes of polysaccharide content in *D. officinale* under different light quality conditions as an important evaluation index affecting the quality of *D. officinale* medicinal use. Through the comparative transcriptome analysis of the genes that affect the quality of *D. officinale*, we cloned the *UGPase* gene of *D. officinale*, analysed it by bioinformatics, and detected its expression by RT-PCR, so as to explore the role of this gene in the biosynthesis of glucose metabolism pathway and its impact on the synthesis of polysaccharides in *D. officinale*, and provide some theoretical basis for the further research on the biosynthesis of polysaccharides in *D. officinale*. Synthesis of polysaccharides in *D. officinale* the effect of different light conditions on the quality of *D. officinale* is revealed at the molecular level, which provides a theoretical foundation and experimental basis for the quality improvement of *D. officinale*.

Materials and Methods

Plant materials and light treatment

The 2-year-old *D. officinale* were used and cultivated in the *D. officinale* planting base under Guangxi Nong Feng Mountain *D. officinale* Technology Co., Ltd. 90 clusters were planted per 1 m^2 with the spacing of 10cm*10cm. The experimental group was covered with three different coloured films of red (600~700 nm), yellow (520~650 nm), and blue (410~540 nm) in the greenhouse, to create different light quality and environment as red light group (R), yellow light group (Y), and blue light group (B) and *D. officinale* cultivated under uncovered natural light conditions as the control group (N) (Figure 1). *D. officinale* branches were randomly taken from each sample of each treatment group, and the middle part of the stems with even growth was taken from each branch, and then 3-5 samples were randomly taken from the samples of the different groups for the experimental sectioning, and later, the samples from different groups, and then observe and measure them.

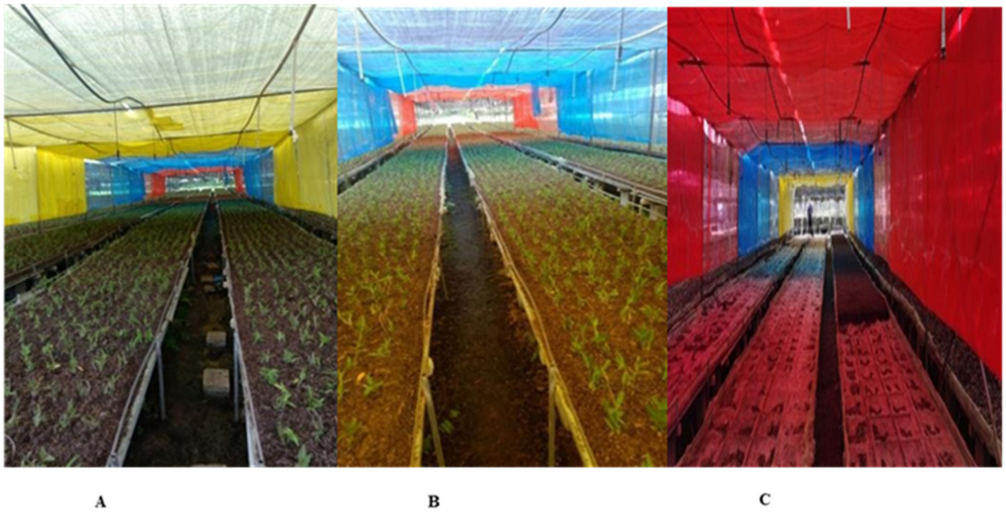

Figure 1. Different light quality treatments for *D. officinale*. The experimental group created different light quality environments for *D. officinale* cultivation by covering the greenhouse with three different coloured films, red (600-700 nm), yellow (520-650 nm) and blue (410-540 nm), as the red-light group (R), the yellow light group (Y), and the blue light group (B), and used *D. officinale* cultivated in uncovered natural light conditions as the control group (N)

Equipment

PCR instrument (Shanghai Bole Biomedical Products Co., Ltd.), freezing centrifuge and micropipette (Eppendorf Company); Gel electrophoresis instrument (American Bole Company), real-time fluorescence quantitative PCR instrument (Shanghai Roche diagnostic products); Ultra-micro nucleic acid analyzer (Beijing Tiangen Biochemical Technology Co., Ltd.); Biosafety cabinet (Haier Group); High pressure steam sterilizer (Huayue Enterprise Group Co., Ltd.); One ten thousandth electronic balance (Beijing Presys Instrument Co., Ltd.); Ultraviolet-visible spectrophotometer (Shimadzu Instrument Co., Ltd.); Constant temperature metal bath (Hangzhou Bori Technology Co., Ltd.); Vortex hybrid oscillator (Haimen Qilinbeier Instrument Manufacturing Co., Ltd.); Electrophoresis instrument (Beijing Liuyi Instrument Factory); Ice maker (Matsushita Electric Industry Co., Ltd.).

D. officinale of polysaccharide content with different light quality

Sulfuric acid-phenol method was used to determine the polysaccharide content, pipette 1 mL of the sample solution to be tested, placed in a 10 mL clean stoppered glass tube, add 1 mL of 5% phenol solution, then add 5 mL of concentrated sulfuric acid, shake well. The same amount of 1 mL of distilled water in a 10 mL stoppered glass tube, and then add the same amount of phenol and concentrated sulfuric acid, as a blank control. Then put them together in a boiling water bath and heat for 20 min, take them out after the time, put them in ice water and cool them for 10 min, and then measure the absorbance of the samples at the wavelength of 488 nm according to the UV-visible spectrophotometric method. The concentration of *D. officinale* polysaccharide in the sample solution was read out from the standard curve, and the content of *D. officinale* polysaccharide was calculated by the polysaccharide content calculation method.

Polysaccharide content = $(C \times V \times D)/M \times 100\%$

Note: C is the polysaccharide concentration read from the standard curve; V is the total volume of the extract; D is the dilution factor; M is the mass of the herb powder, g.

Extraction of sample RAN, preparation of library and sequencing of transcriptome

The total RNA was extracted from each group of samples by liquid nitrogen cryomill, and the A260 and A280 values of the extracted RNA samples were measured by ultra-micro nucleic acid detector to calculate the concentration of the samples, and the quality and concentration of the qualified RNA were detected by denaturing agarose gel electrophoresis, and the library construction and sequencing work were completed by Maiwei Metabolic Biotechnology Co. After sequencing, the quality-controlled data were spliced and assembled, and the spliced and assembled sequences were de-redundantly clustered, and finally, the functional annotation of the genes was performed, and all the gene sequences were subjected to GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genome) enrichment analyses according to the functional annotations and Genome enrichment analyses based on their functional annotations to determine which biological processes the obtained genes are mainly involved in.

The primer screening and expression analysis of UGPase gene in samples

In this experiment, based on the known *UGPase* gene sequence of *D. officinale* (GenBank: KF711982.1) and literature review, the primer sequences in the following table were obtained, which were synthesized by Sheng gong Bioengineering (Shanghai) Co., Ltd., and then fluorescence quantitative PCR was carried out. According to the experimental results, the dissolution curve was analysed to screen out the target gene suitable for fluorescence quantitative PCR. UGPase-F : 5'- TGGTTGATGGCTTCGTAG-3', UGPase-R: 5'-AATGCTAGGAATGGACTTGA-3'

According to the cloned *UGPase* cDNA sequence, the transcription level of *UGPase* in different parts of *D. officinale* and under the influence of different light quality was determined by using the fluorescent quantitative primers obtained above and GAPDH as the internal reference gene. The target gene and internal reference gene of each experimental group were set with 3 accessory pores, and the CT value of the experimental results was calculated by the $2^{-\triangle\triangle CT}$ formula.

Establishment of prokaryotic expression system of UGPase gene and its gene expression

The cDNA was synthesized from *D. officinale* RNA with high quality, concentration, and integrity following the instructions of PrimerScript TMⅡ 1st Strand cDNA Synthesis Kit. Primers Primer 1: ATGGCGGCCGTCGCCTCT and Primer 2: Primes 1: ATGGCGGCCGTCGCCTCT were utilized for the reaction template (<200 ng) from the synthesized cDNA reaction solution. TTAGATATCCTCAGCTCT (primer synthesized by Sheng gong Bio Engineering (Shanghai) Co., LTD.) was used in a 50 μl reaction system. PCR amplification conditions included denaturation at 98 ℃ for 10 sec, annealing at 55 ℃ for 5 sec, extension at 72 ℃ for 5 sec over a span of thirty cycles. The product was then analyzed using agarose-gel electrophoresis, followed by cutting and recovery of the target strip, purification, and A-Tailing reaction. Subsequently, pMD19-T Vector (0.5 μL) was added before being introduced into JM109 receptive cells for transformation. After screening blue and white spots, several positive bacteria were randomly selected and cultured in a constant temperature shaker at overnight at 37°C using universal primers M13-47(F): AGGGTTTTCCCAGTCACG and M13-47(R): GAGCGGATAACAATTTCACAC.

PCR (primer synthesized by Shenggong Bioengineering (Shanghai) Co., LTD.) was employed to validate the bacterial solution under pre-denaturation conditions of 98 °C for 10 sec; Annealing at 55 °C for 30 sec; within a PCR reaction system consisting of 50 μL PremeixTaq including 25 μL PremeixTaq ,1 μL bacterial solution ,1 μL M13-47(F), and 1 μL M13-47(R), extending at 72 °C for one minute over thirty cycles.

Bioinformatics analysis of UGPase gene in samples

The amino acid sequences of eight species with high homology were compared by BLAST alignment in NCBI database, and the phylogenetic tree analysis of *UGPase* gene sequences in different plants was carried out by using MEGA7.0 software and NJ method.

Mathematical statistics and analysis

The experimental data were statistically analysed by SPSS 20.0, and the measurement data were expressed by $\bar{X} \pm SD$, When the data conformed to normal distribution and homogeneity of variance, One-Way ANOVA test was used, and LSD test was used for pairwise comparison between groups. However, when the data didn't not conform, Kruskal-Wallis H test analysis was used, and Mann-Whitney U test analysis was used for comparison between groups. $P<0.05$ is considered to be significant.

Results and Discussion

Polysaccharide content of each sample under different light treatments

The polysaccharide content in three different tissues of *D. officinale* roots, stems and leaves was determined under natural light, and the results showed that *D. officinale* stems had the highest polysaccharide content of 28.76%, followed by leaves with 10.49%, and roots had the lowest polysaccharide content of 2.39% (Table 1).

Table 1. Results of the determination of polysaccharide content of *D. officinale* under different light qualities (n=3)

Sample number	Absorbance	Concentration(mg/mL)	Average concentration (mg/mL)	Quantity contained	Average content	RSD
Natural light	0.2407	0.00358	0.00357	26.08%	26.01%	0.77%
	0.2379	0.00354		25.78%		
	0.2414	0.00359		26.16%		
Yellow light	0.3101	0.00460	0.00464	33.55%	33.81% a	1.00%
	0.3161	0.00469		34.19%		
	0.3113	0.00462		33.68%		
Red light	0.3166	0.00470	0.00468	34.25%	34.12% a	1.24%
	0.3111	0.00462		33.65%		
	0.3187	0.00473		34.47%		
Blue light	0.2875	0.00427	0.00425	31.12%	31.00% a	0.49%
	0.2848	0.00423		30.83%		
	0.2870	0.00426		31.06%		

Note: Comparison of polysaccharide content between different light quality treatment groups and the control group, a indicates $P<0.05$.

The results of the polysaccharide content of *D. officinale* stems in different treatment groups showed that compared with the control group, the polysaccharide content of *D. officinale* was significantly increased under red, blue and yellow light conditions, and the promotion of polysaccharide content of *D. officinale* was more obvious under red and yellow light conditions. The three light qualities of red light, blue light and yellow light had significant promotion effects on polysaccharide content in *D. officinale* stems, which were 34.12% (red light group), 33.81% (yellow light group), 31.00% (blue light group) and 26.01% (natural light group), respectively, and the polysaccharide content of the red-light group (R) was higher than that of the natural light group (N) by 8.11%. The polysaccharide content of *D. officinale* stems under different light qualities was in the order of R > Y > B. We found that the three light qualities, red light, blue light and yellow light, had significant promotion effects on polysaccharide content in *D. officinale* stems ($P>0.05$), indicating that the different light qualities had a significant effect on the polysaccharide content of *D. officinale* stems, and that, using the natural light as a control, the effects of red light and yellow light were more significant among the three light qualities.

Transcriptome analysis

Sample RNA quality

Extracting high-quality RNA is the foundation of the success of the whole project. To obtain high-quality RNA, the database can only be established after passing the test. After the ultra-micro nucleic acid concentration and purity test, the A260/A280 ratio of the extracted *D. officinale* RNA is between 1.8 and 2.1, indicating that there are few impurities in the RNA sample, and the A260/A280 ratio reading is 2.0, indicating that the extracted RNA is of high quality. Furthermore, the results of gel electrophoresis showed that the bands of 28S rRNA, 18S rRNA and 5S rRNA in the extracted RNA electrophoresis map were clearly visible, and the brightness of 28S band was about twice that of 18S, which indicated that the extracted RNA had high integrity and good quality and met the requirements of subsequent sequencing analysis (Figure 2).

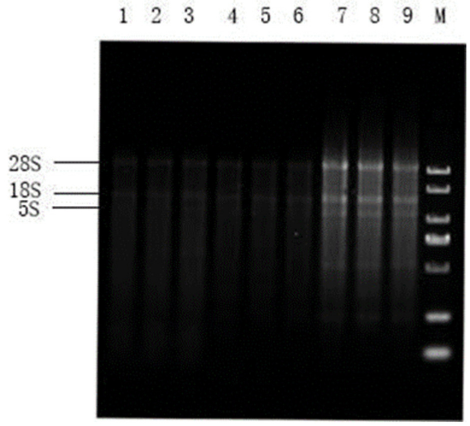

Figure 2. The extracted RNA gel electrophoresis map

After sequencing by high-throughput sequencing technology of llumina HiSeq, the high-quality reads of 10 groups of *D. officinale* transcription groups accounted for 93.83% ~ 97.45% of the original data respectively, and the overall sequencing error rate was within an acceptable range. In terms of quality evaluation, the number of bases with mass ≥20 was screened out, which accounted for 97.40% ~ 97.69% of the total number of bases, and the number of bases with mass ≥30 was selected (Table 2).

The proportion of base numbers ranged from 92.57% to 93.28%, and the G and C numbers in each group were selected to account for 44.85% ~ 46.09% of the total bases.

Table 2. Comparison statistics table

Sample Number	Raw Reads	Clean Reads	Error Rate (%)	Q20(%)	Q30(%)	GC Content (%)
Natural light	46391758	44926594	0.03	97.54	92.95	44.97
	51657062	48761730	0.03	97.59	93.10	45.75
Red light	46684204	44522982	0.03	97.40	92.57	45.42
	48269584	46396444	0.03	97.56	93.00	44.85
	47188398	44275732	0.03	97.67	93.28	45.58
Yellow light	47683936	45733816	0.03	97.49	92.78	45.46
	48279320	47046690	0.03	97.57	92.99	45.64
	47197000	45945640	0.03	97.69	93.22	46.09
Blue light	55573458	53767384	0.03	97.62	93.09	45.70
	47890534	46137546	0.03	97.56	93.03	45.32

Screening of differential genes

The heatmaps of the expression profiles of red-light VS natural light, blue light VS natural light, and yellow light VS natural light showed that both the control and experimental groups were clustered in one cluster separately, indicating good reproducibility between the three replicates of different light quality treatments. The volcano plot shows the overall distribution of differential genes. The number of differential genes in red light VS natural light was 494, including 275 up-regulated genes and 219 down-regulated genes; the total number of differential genes in blue light VS natural light was 1,630, including 847 up-regulated and 783 down-regulated differential genes; the total number of differential genes in yellow light VS natural light was 599, including 294 up-regulated genes and 305 down-regulated genes (Figures 3-4).

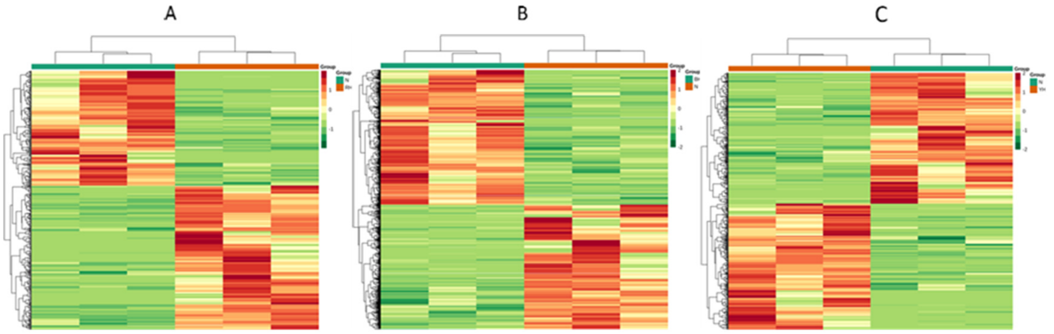

Figure 3. Differential gene heat maps for red (A), blue (B) and yellow (C) light qualities compared with natural light, respectively

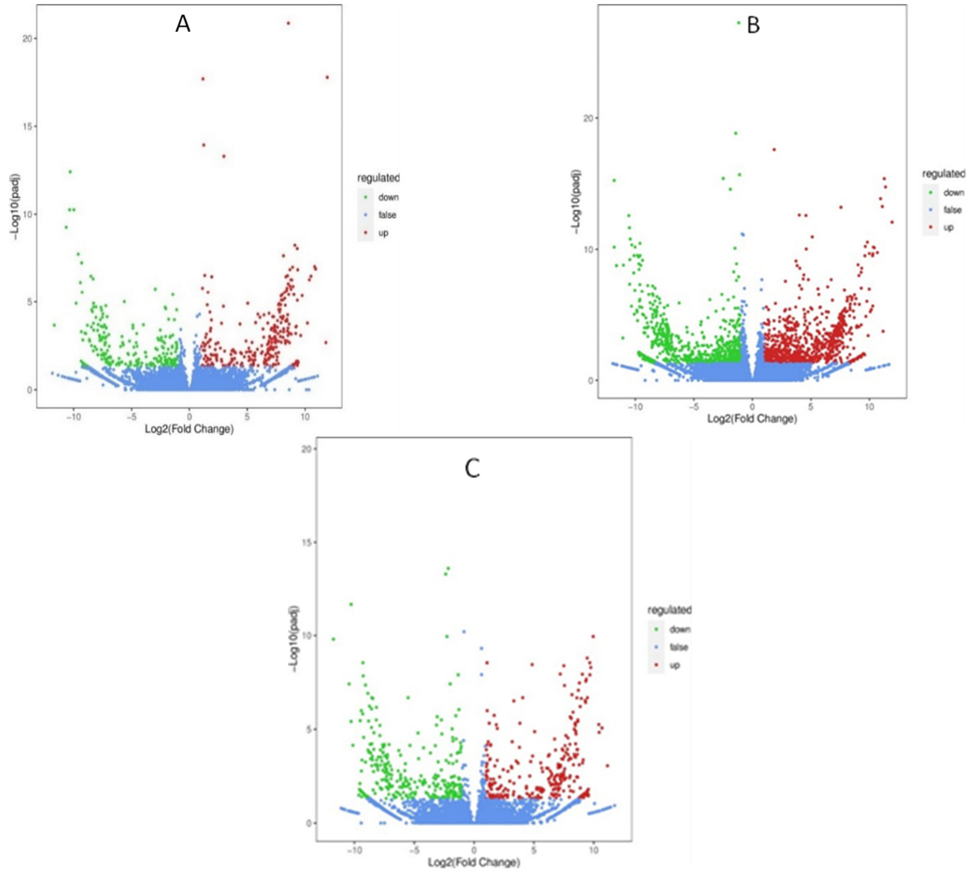

Figure 4. Differential gene volcano plots of red (A), blue (B) and yellow (C) light qualities compared with natural light, respectively

Differential gene GO analysis

The results of GO functional enrichment of red light vs. natural light differential genes showed that the differential genes were mainly enriched in the biological processes of response to light, especially red light, and to a lesser extent in the processes of stimulation of external abiotic stresses, light signalling and hormone biosynthesis; there was no enrichment of cellular components; and the molecular functions were enriched in the functions of the transmembrane transport proteins, histone acetyltransferase activity, RNA decoding enzyme activity, and exogenous transmembrane transporter activity. The molecular functions were mainly enriched in chlorophyll catabolic transmembrane transporter protein activity, histone acetyltransferase activity, RNA deconjugating enzyme activity, exogenous transmembrane transporter activity, and other functions (Figure 5).

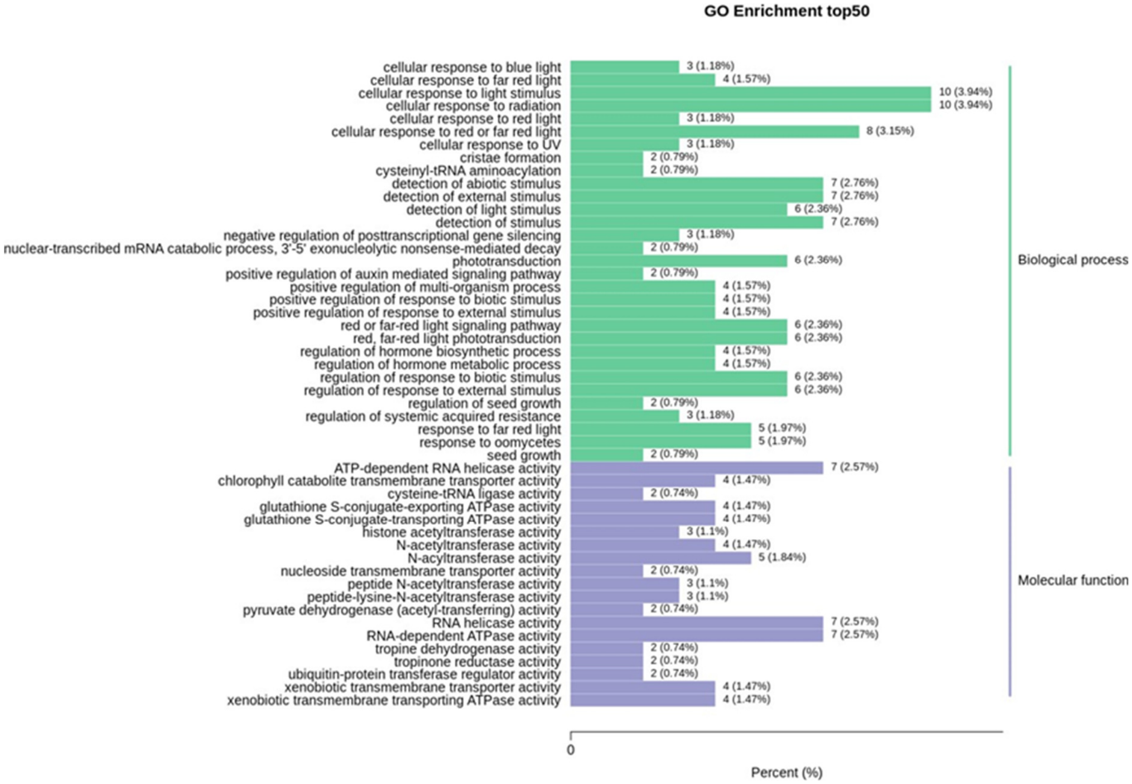

Figure 5. Red light vs. natural light differential gene GO analysis

The results of GO functional enrichment of blue light vs. natural light differential genes showed that the differential genes were mainly enriched in biological processes in response to light, especially blue light, in addition to cell wall macromolecule catabolism, gibberellin-stimulated cellular response, disaccharide biosynthesis, gibberellin-mediated signalling pathway, photoperiod, seedling development and algal glucose metabolism, etc. The cellular components were mainly enriched in endoplasmic reticulum, photosystem Ⅰ, SCF ubiquitin ligase complex. The cellular components are mainly enriched in the endoplasmic reticulum, photosystem Ⅰ, SCF ubiquitin ligase complex; the molecular functions are mainly enriched in the carboxylic ester hydrolase activity, lipase activity, mannosidase activity and other functions (Figure 6).

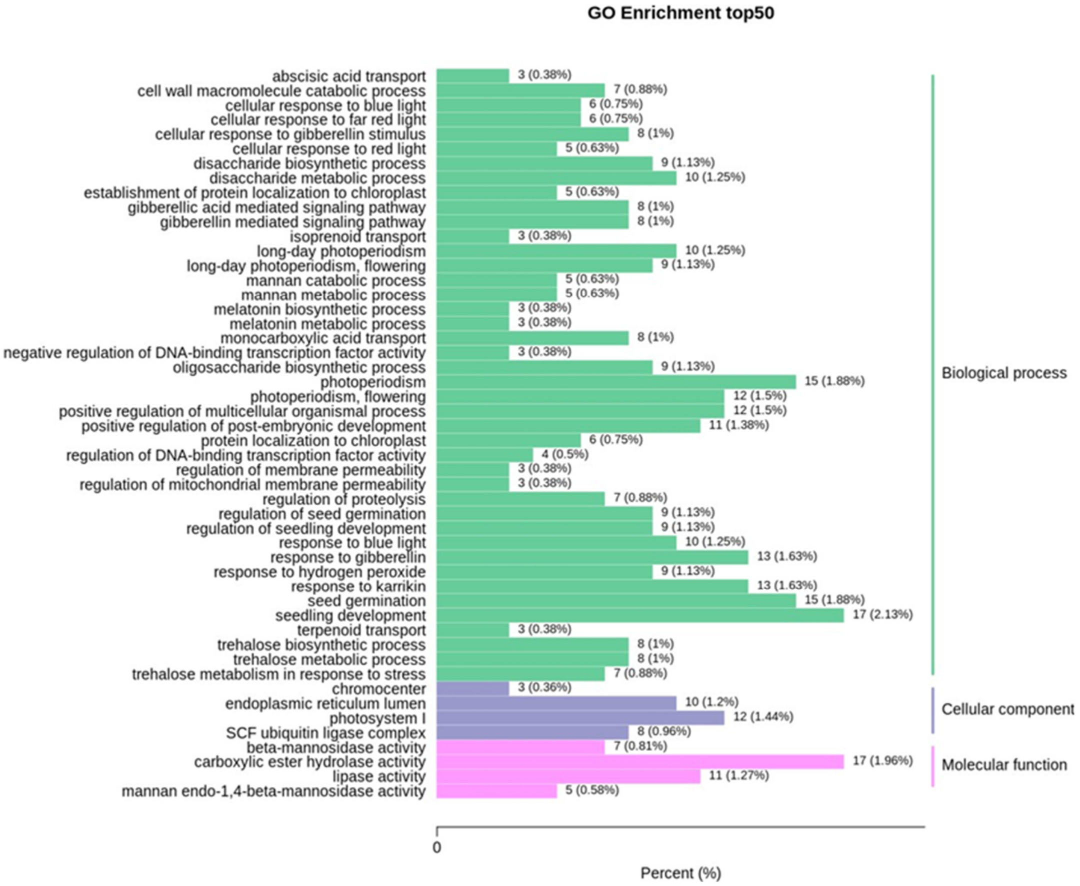

Figure 6. Blue light VS natural light differential gene GO analysis

The results of GO functional enrichment analysis of yellow light VS natural light differential genes showed that the differential genes were mainly enriched in biological processes such as response to light, in addition to plant organ senescence, plant organ formation, response to gibberellins, response to hydrogen peroxide, and response to Karrikins (KARs), etc.; there was no enrichment of cellular components; and the molecular functions were mainly enriched in functions such as lysosomes, senescent-associated vesicles, and the cytoplasmic side of endoplasmic reticulum membranes. -accompanied vesicles, cytoplasmic side of the endoplasmic reticulum membrane, and other functions (Figure 7).

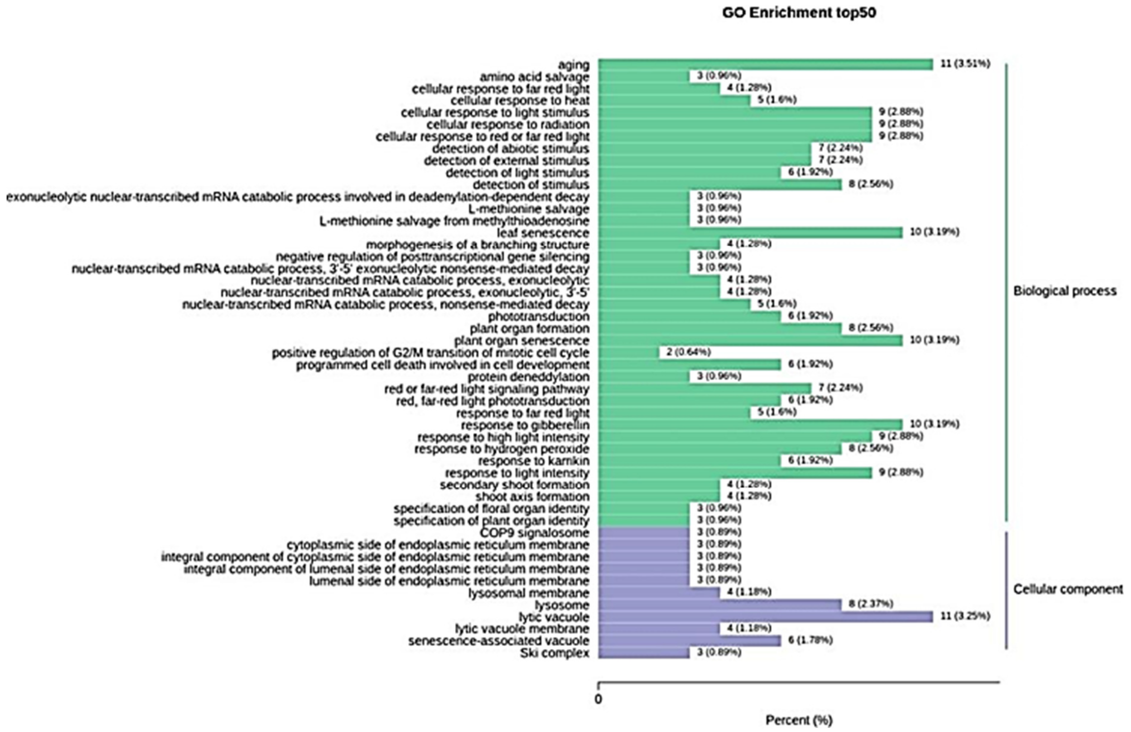

Figure 7. Yellow light VS natural light differential gene GO analysis

Enrichment analysis of differential gene KEGG Pathway

KEGG enrichment analysis of red-light VS natural light differential genes revealed that the differentially expressed genes were mainly annotated to secondary metabolism biosynthesis, such as zeatin biosynthesis, terpenoid backbone biosynthesis, flavonoid biosynthesis, carotenoid biosynthesis, and other pathways as well as phytohormone signal transduction (Figure 8).

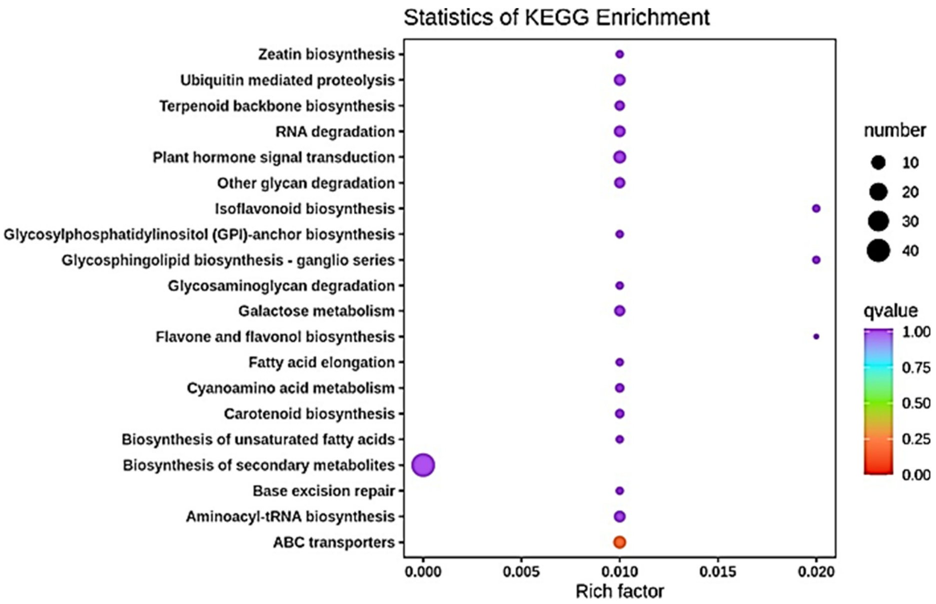

Figure 8. KEGG analysis of red light vs. natural light differential genes

KEGG enrichment analysis of blue light vs. natural light differential genes showed that the differentially expressed genes were mainly annotated to the biosynthetic pathways of starch and sucrose metabolism, pyruvate metabolism, pentose phosphate pathway, flavonoid metabolism, porphyrin and chlorophyll metabolism, as well as to phytohormone signalling and carbon sequestration in photosynthetic organisms (Figure 9).

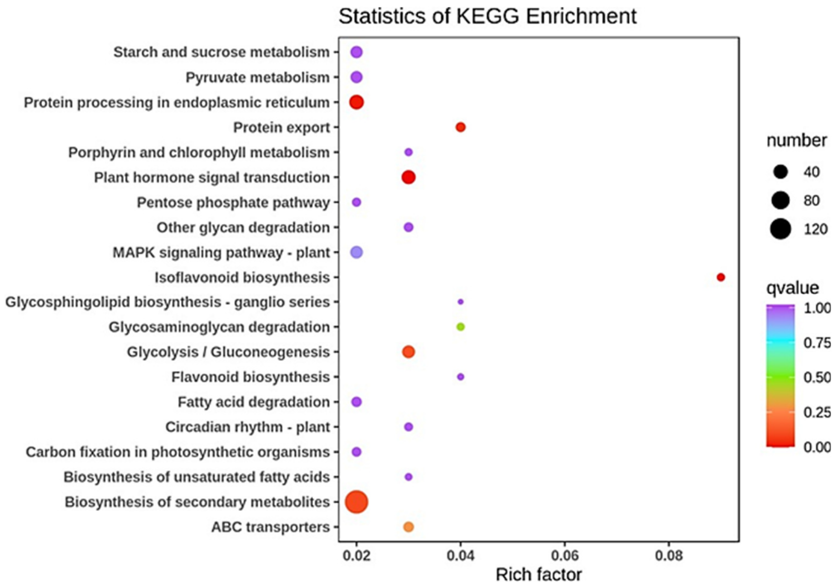

Figure 9. KEGG analysis of blue light vs. natural light differential genes

KEGG enrichment analysis of yellow light vs. natural light differential genes showed that the differentially expressed genes were mainly annotated to starch and sucrose metabolism, riboflavin metabolism, pentose-phosphate pathway, carbon metabolism, folate synthesis, carotenoids biosynthesis pathway, as well as plant-pathogen interactions and protein processing in the endoplasmic reticulum (Figure 10).

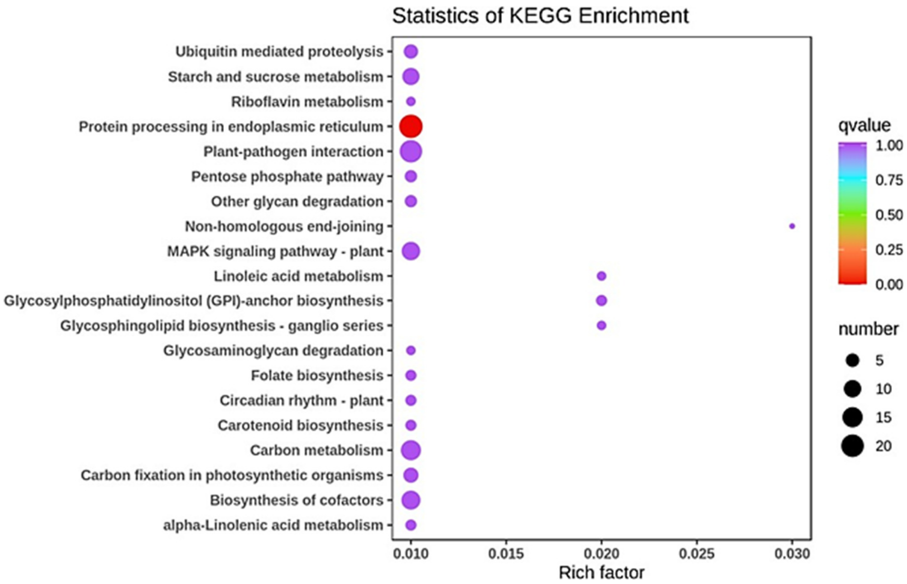

Figure 10. Yellow light vs. natural light differential gene KEGG analysis

Analysis of differentially expressed genes for polysaccharide synthesis

Given the large number of key genes in the polysaccharide synthesis pathway, we selected a few of the more critical and representative enzyme genes for analysis. From the transcriptome results, we found transcripts of 2 *SUS*, 1 *SPS*, and 8 *UGPase* genes. The transcripts of one *SUS* gene showed no significant change in expression under red and blue light conditions and a significant decrease in expression under yellow light conditions (Cluster-57138.114965); the other *SUS* gene transcript was up-regulated in expression under red and blue light conditions and did not show any significant change in expression under yellow light conditions; the *SPS* gene was induced to express under red and blue light conditions and did not show any significant change in expression under yellow light conditions; the *UGPase* gene was up-regulated in expression under red and blue light conditions and did not show any significant change in expression under yellow light conditions. One transcript of *UGPase* gene was specifically induced by red light (Cluster-57138.128140); Cluster-57138.174233 was specifically induced by yellow light; Cluster-57138.98087 was specifically expressed in response to blue light; and two transcripts were expressed in response to red light and blue light. Two transcripts showed no significant change under red and blue light conditions and were significantly repressed under yellow light conditions (Cluster-57138.128141, Cluster-57138.130769); two transcripts showed no significant change under red and yellow light conditions and were significantly down-regulated under blue light conditions; and one transcript showed a significant down-regulation of expression under three different light qualities, namely, red, blue, and yellow (Cluster-57138.128141, Cluster-57138.130769). The expression of one transcript was significantly down-regulated under different light qualities of red, blue and yellow (Cluster-57138.18049) (Figure 11).

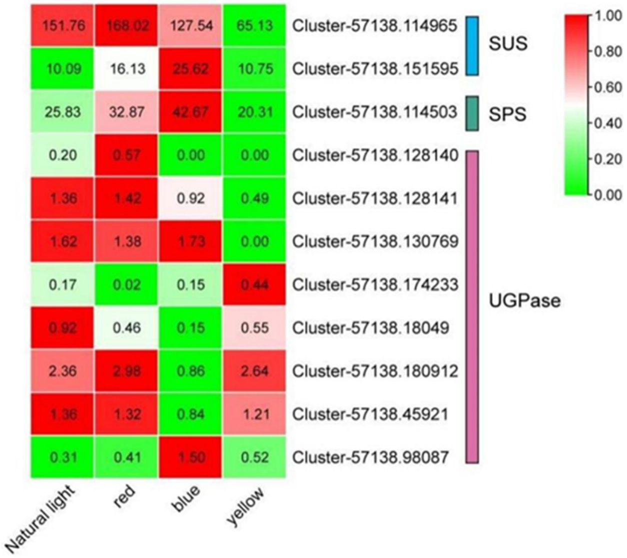

Figure 11. Heat map of different transcripts of key genes for polysaccharide biosynthesis under different light quality conditions

Expression of UGPase gene in each sample

Firstly, the expression of *UGPase* gene in different tissues of *D. officinale* under natural light was analyzed. The experimental results showed that the expression of *UGPase* gene in stems was higher, followed by leaves and the lowest in roots. Then, the expression of *UGPase* gene in *D. officinale* was analyzed under different light quality. The results showed that both red light and yellow light could significantly promote the expression of *UGPase* gene, and red light had a stronger promotion effect, while blue light had no significant effect on the expression of *UGPase* gene (Figures 12-13).

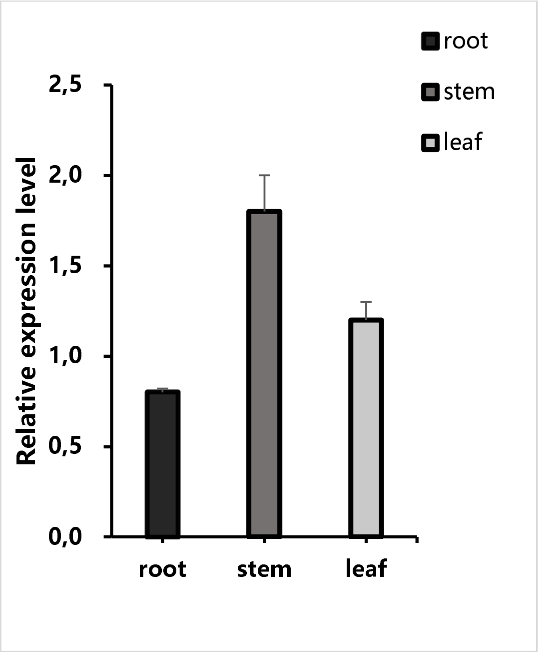

Figure 12. Relative expression of *UGPase* gene in different parts

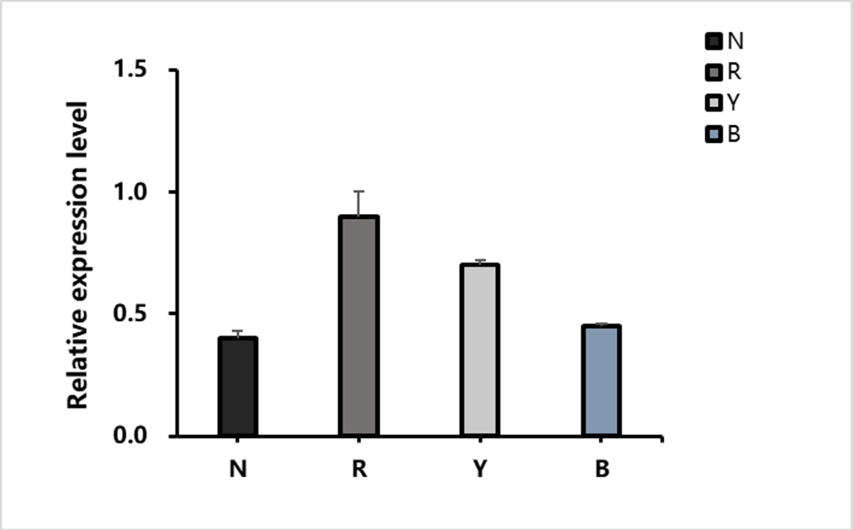

Figure 13. Relative expression of *UGPase* gene in different light qualities

Cloning and expression of UGPase gene

Put the agarose gel block soaked in GelRed dye into a gel imager for observation and compare the bands with Marker to determine whether the bands are consistent with the target gene sequence length. After querying in NBCI, the full length of *UGPase* gene of *D. officinale* is 1419 bp (Figure 14), and the gel diagram shows that the band position is about 1500 bp, which is relatively consistent with the sequence length of *UGPase* gene.

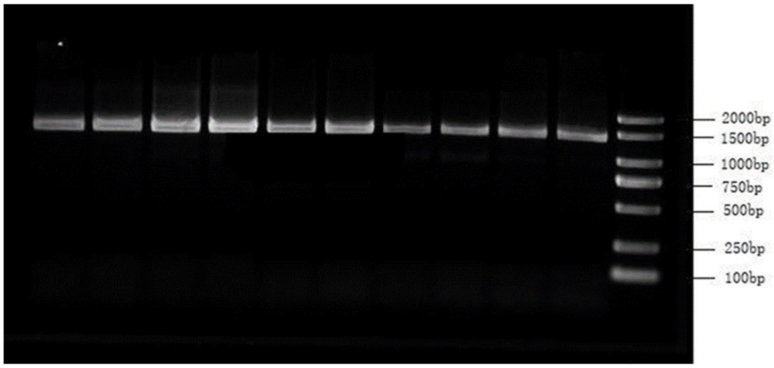

Figure 14. PCR agarose gel electrophoresis of prokaryotic expression of UGPase gene

BLAST amino acid sequence ratio and cluster analysis of UGPase gene in samples

The amino acid sequence of *D.officinale* was very similar to that of *Phalaenopsis equestris* (Schauer) Rchb.f. (XP_ 020596934.1), *Ananas comosus* (Linn.) Merr. (OAY68359.1), *Sorghum bicolor* (L.) Moench (XP_ 002453185.1), *Amorpha fruticosa* (AF435969.1), *A. membranaceus* （AF281081.1）, *C. pilosula* （AJA91184.1） and *Arabidopsis thaliana* （AT3G03250） by BLAST amino acid sequence comparison,all of which reached more than 82%. Among them, the amino acid sequence of UGPase of *D.officinale* had the highest homology with *Phalaenopsis aphrodite* Rchb. f. (Figure 15)

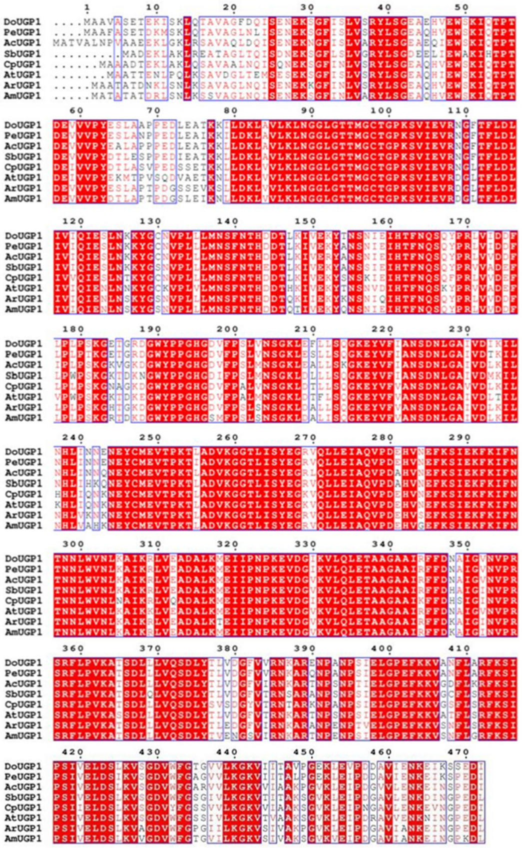

Figure 15. Sequence comparison diagram

The amino acid sequences of the *UGPase* genes of the eight different species mentioned earlier were used to construct a phylogenetic tree using MEGA 7.0 software, and molecular phylogenetic analyses were carried out. Phylogenetic tree results showed that *D.officinale* was clustered with *Phalaenopsis equestris* (Schauer) Rchb. f., *Ananas comosus* (Linn.) Merr. （OAY68359.1） and *Sorghum bicolor* (L.) Moench (Figure 16).

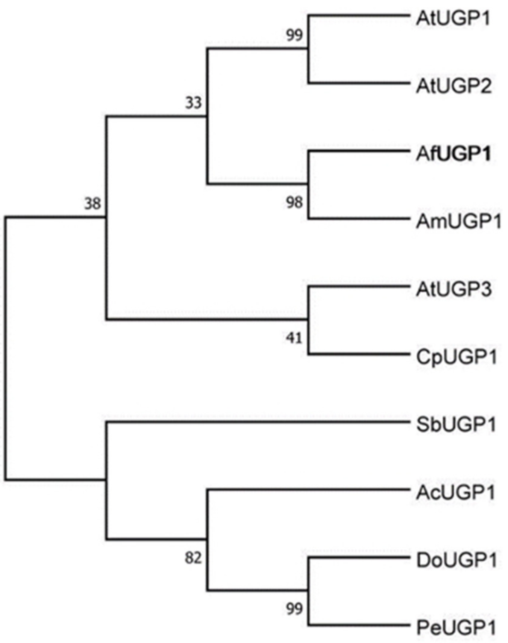

Figure 16. Phylogenetic tree diagram

Differential genes in different light qualities are enriched in light signaling processes

The adaptation of *D. officinale* to light is related to the unique photosynthetic metabolic pathway it contains, which can regulate plant metabolism as well as increase the accumulation of major plant components, etc., while light contains light intensity, light quality, and light cycle. Light signalling provides energy for plant growth and development and regulates plant secondary metabolism. Light quality is crucial for plant growth and development, which not only serves as an energy source to control photosynthesis, but also as a trigger signal to influence plant growth. Different light quality triggers different photoreceptors, which in turn affects the photosynthetic properties, growth and development, stress tolerance and senescence of plants.

Enrichment of differentially expressed genes in major molecular metabolic and information transduction pathways through KEGG function is conducive to further functional identification and study of genes. Transcriptomics can be applied to related gene mining, differential gene mining, gene function prediction, and gene analysis of key enzymes of biological metabolic pathways, etc. The transcriptome process mainly includes RNA extraction, quality testing (Coleman *et al*., 2008; Escamilla-Treviño *et al*., 2010; El-Sharkawy *et al*., 2015), mRNA enrichment, library construction, and sequencing. The biological function of an organism is accomplished through the interaction of different gene products, and the annotation and analysis of differentially expressed gene pathways facilitates a more in-depth interpretation of gene functions (Bräutigam and Gowik, 2010; He *et al*., 2017; Li *et al*., 2017).

In this study, the comparative transcriptome analysis of *D. officinale* under different light quality conditions was carried out by means of transcriptomics: the stems of control and experimental samples were subjected to transcriptome sequencing (Liu *et al*., 2017), and the different genes of different light quality were compared with those of the control group, from which the different genes related to *D. officinale* polysaccharides content were screened out, and the effects of different light quality conditions on *D. officinale*'s medicinal qualities were investigated from the transcriptional level; and a more in-depth analysis was carried out for the effects of different light quality on the polysaccharides content of *D. officinale*. The effect of different light quality on the polysaccharide content of *D. officinale* was analysed in depth (Luo *et al.*, 2016). The RNA agarose gel results and transcriptome quality control ensured that the transcriptome data used in this experiment were real and reliable. Under the three different light conditions of red, blue and yellow, 494, 1630 and 599 differentially expressed genes were screened, among which 275 genes were up-regulated and 219 genes were down-regulated under red light condition; 847 and 783 differentially up-regulated and down-regulated genes were up-regulated and down-regulated respectively under blue light condition; 294 up-regulated genes and 305 down-regulated genes were up-regulated and down-regulated under yellow light condition. Significant enrichment analysis of GO and KEGG for these differential genes showed that the differential genes were enriched in the process of light signal transduction under different light qualities. In addition, the differential genes were enriched in biosynthesis of secondary metabolism and hormone signalling under red light condition; while the differential genes were enriched in biosynthesis of primary metabolism, phytohormone signalling and carbon fixation in photosynthetic organisms under blue light condition; and the differential genes were enriched in secondary metabolism, phytohormone signalling and carbon fixation in photosynthetic organisms under yellow light condition. The differential genes were significantly enriched in secondary and primary metabolism under yellow light, and in response to endogenous plant hormones, plant organ senescence and plant organ formation.

Red and yellow light significantly promotes the expression of UGPase gene in D. officinale.

Polysaccharide is an important quality evaluation index in *D. officinale*, which has significant pharmacological effects in immunomodulation and anti-tumour. Polysaccharides are a class of biomolecules with complex structure, various types and combinations (He *et al.*, 2016), and their biosynthetic pathway mainly consists of three parts: sucrose conversion, uridine diphosphate (UDP)-glucose to other NDP monosaccharides conversion, and polysaccharide polymerization (Qiao *et al.*, 2019). The key enzymes involved in this synthesis process include sucrose synthase (SUS), fructose-6 phosphate in sucrose phosphate synthase (SPS), invertase (INV), hexokinase (HK), and fructokinase (FK).), fructokinase (FRK), UDP-glucose dehydrogenase (UGDH), UDP-glucose pyrophosphorylase (UGPase), UDP-rhamnose synthase (RHM), UDP-glucose pyrophosphorylase, UDP-rhamnose synthase (RHM), phosphomannose isomerase (PMM), GDP-mannose pyrophosphorylase (GMPP), glycosyl transferases (GTs), and so on[30]. The transcripts of SUS, SPS, and *UGPase*, which affect the biosynthesis of *D. officinale* polysaccharides, were mostly induced by red and blue light, and yellow light also up regulated the expression of transcripts of some *UGPase* genes (Vogt, 2010).

In this study, using the reported *UGPase* gene sequences of other plants, we cloned the *UGPase* gene from *D. officinale* with an open reading frame of 1416 bp, encoding 472 amino acids, and performed online BLAST sequence comparison on NCBI. The *UGPase* amino acid sequence homology comparison and phylogenetic tree analysis showed that the *D. officinale UGPase* amino acid fragment had high homology (82% to 88 60%) with other plants, of which the highest homology was with orchids such as *Phalaenopsis*. plants (82.43%-88.60%), with the highest homology with orchids such as Phalaenopsis, indicating that the coding region of the *UGPase* gene is highly conserved, which is very similar to the reported results. In this study, the *D. officinale UGPase* gene was cloned, and bioinformatics analysis showed that the sequence of this gene was highly conserved with the *UGPase* gene sequences of other species and had the highest sequence similarity with that of *Phalaenopsis*, which is also an orchid. In addition, the *D. officinale UGPase* gene was most highly expressed in stems, followed by leaves, suggesting that the gene functions mainly in leaves and stems. Finally, we also detected that red light and yellow light had obvious promotion effects on the expression of *D. officinale*

UGPase gene, and the results of fluorescence quantitative PCR showed that red light had the most significant promotion effect on *D. officinale UGPase* gene, which provided the necessary preparations for the study of the anabolic pathway of *D. officinale* polysaccharides and laid the foundation for the future increase of polysaccharides content by means of molecular biology. content by means of molecular biology.

Conclusions

In this study, we applied high-throughput sequencing technology to establish the *D. officinale* transcriptome database under different light conditions and obtained a large amount of transcriptional information related to the medicinal quality of *D. officinale*. The effects of different light qualities on the polysaccharide production content of *D. officinale* were deeply investigated by using three different light qualities, red, blue and yellow, as the experimental group and natural light as the control group, and the experimental results showed that red and blue light were the main influences to promote the polysaccharide synthesis of *D. officinale*. Yellow light, on the other hand, had less influence on polysaccharide synthesis in *D. officinale*. The genes related to the quality of *D. officinale* were analysed at the transcriptional level, and the promotion of polysaccharide synthesis in *D. officinale* under different light conditions was explained at the molecular level. The *UGPase* gene was cloned, and its expression was analysed in different tissues and under different light conditions. We found that red light has the most significant effect on the *UGPase* gene and polysaccharide content of *D. officinale*, which provides the theoretical and experimental basis for the quality improvement and standardised cultivation of *D. officinale*, and also lays a good foundation for the subsequent research on the molecular mechanism of light affecting the quality of *D. officinale*. However, the effects of light cycle and environmental factors on *D. officinale* quality need to be further explored.

Authors' Contributions

Conceptualization, YW, XL and LC.; presented ideas and supervision, MZ, ZHD, JBT, XXW and HZ.; performed the experiments, YW and XJD； analyzed data and formal analysis, YW, DH, XL and LC.; writing-original draft, YW and XL.; revised the manuscript, YYB, MZ and LC.; project administration, MZ and XXW.; All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

We thank Professor Zhu Hua, Wang Xiaoxun, Zhang Miao and Teng Jianbei. This work was supported by the National Natural Science Foundation of China (Grant No. 8206140326).

Funding

This study was supported by the National Natural Science Foundation of China (No. 82060695); GuangXi Key Laboratory of Zhuang and Yao Ethnic Medicine [Gui Ke Ji Zi (2014) No. 32]; Collaborative Innovation Center of Zhuang and Yao Ethnic Medicine [Gui Jiao Ke Yan (2013) No. 20]; Guangxi Zhuang Autonomous Region Ethnic Medicine Resources and Application Research Center of Engineering [Gui Fa Gai Gao Ji Han (2020) No. 2605]; Guangxi Science and Technology Base and Talent Special Project (Gui Ke AD21238031); Guangxi Key Research and Development Program Project (Gui Ke AB21196016); Guangxi First-class Discipline of Traditional Chinese Medicine (Ethnopharmacology) (Gui Jiao Ke Yan [2018] No. 12); Doctoral Starting up Foundation of Guangxi University of Chinese Medicine in 2018 (2018BS019).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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