Effects of Red Light Quality on Anabolism and Related Genes of Medicinal Active Components in Dendrobium Officinale Tissue Culture

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Abstract: Studies have shown that red light has a significant effect on the growth and main components of Dendrobium Officinale, but the specific pathway is not clear. In order to elucidate the effect of red light on Dendrobium Officinale from the aspects of gene and metabolic pathway, the tissue culture seedlings of Dendrobium Officinale treated with red and white light were used to determine the difference of medicinal components (Dendrobium polysaccharide, mannose, Dendrobium alkaloids, etc.) under different culture conditions, and to detect the expression level of related genes. The results show that red light has some effects on the effective components of Dendrobium Officinale, especially on the synthesis and accumulation of mannose, and can promote the accumulation of alkaloids. And under different light quality, mannose consumption and transport mode will change, thus affecting the growth and development of the plant.

1. Introduction

Dendrobium officinale is one of the most precious traditional Chinese medicinal materials in China. It is honored as the first of the “Nine Great Herbs of China”, known as “gold in medicine”. It is listed as the best medicinal material in Sheng Nong's Herbal Classic and Compendium of Materia Medica[1]. Modern pharmacological studies showed that the main medicinal components of Dendrobium Officinale were polysaccharides phenanthrene bibenzyl nuclei amino acids alkaloids and so on[2]. Among them, Dendrobium polysaccharide and mannose contribute more to the therapeutic mechanism (included in Chinese Pharmacopoeia). Dendrobium alkaloids were the first to be isolated and identified, and Dendrobium alkaloids were the main components of Dendrobium Officinale alkaloids. It has been shown that light quality has a certain impact on the content of effective components of Dendrobium Officinale [3], but the specific reasons are not clear. There is no transcriptome level to explore the changes of effective components of Dendrobium Officinale until now. Therefore, this topic is the first time to explore the specific reasons for changes in pharmaceutical components under different light quality at the transcriptome level, and to explain the mechanism of changes in pharmaceutical components from the transcriptome level.

According to the existing literature [7], the main components of Dendrobium Officinale were different under different light quality. However, the specific reasons for the changes are not clear. The production of these components is mainly related to glucose metabolism in vivo, and a large number of enzymes will participate in the synthesis of these substances. Therefore, the detection of the changes in the expression of enzymes in the metabolic process can further explain the reasons for the changes in the composition of the drug. There are many genes related to mannose, Dendrobium polysaccharide and alkaloid, including GMPase, PMM, Mans, PPC and HDR, which control the synthesis of mannose, Dendrobium total sugar and Dendrobium alkaloid.

Mannose is a kind of monosaccharide with immunological effect, which has anti-tumor, anti-inflammation, anti-infection and anti-cancer effect as the main effective component of Dendrobium Officinale. The synthesis of mannose is mainly related to the synthesis and metabolism of polysaccharide from Dendrobium Officinale. Phosphomannomutase (PMM), GDP-Mannose pyrophosphorylase; (GMPase) and 4-beta-mannosyl-transferase are involved. Among them, GDP-Mannose provides monosaccharides for the synthesis of polysaccharides. PMM catalyzes the tautomerism between D-Mannose-6-phosphate and D-Mannose-1-phosphate, providing a substrate for the next step in the synthesis of GDP-Mannose; GMPase catalyzes the production of mannose

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by using D-Mannose-1-phosphate as substrate. GDP-Mannose is the substrate of polysaccharide synthesis. 4-beta-mannosyl-transferase takes part in that 4-beta synthesis of mannan with GDP-Mannose as substrate.

Dendrobium Officinale polysaccharide has the effect of promoting lymphocytes of immune system to produce migration inhibitory factor, effectively counteracting the side effects of increasing migration inhibitory index caused by the addition of cyclophosphamide, and can inhibit cancer, tumor, antioxidation and hypoglycemia. At present, no genes directly controlling polysaccharide synthesis in Dendrobium Officinale have been found. We detected phosphoenolpyruvate carboxylase (PPC), a source key enzyme in facultative citric acid metabolic pathway (CAM) plant photosynthetic carbon metabolism,

Dendrobium Officinale alkaloids can protect neurons, relieve pain and fever, protect gastrointestinal tract, and have certain effects on Alzheimer's disease [References]. It can be synthesize via that methylethritol phosphate (MEP) pathway. 4-hydroxy-3-methyl-2-(E)-butenyl-4-diphosphate reductase (HDR) is the last enzyme in the MEP pathway, which catalyzes the production of IPP and DMAPP from HMBPP. As precursors of alkaloid synthesis, these two substances are directly involved in alkaloid synthesis. The HDR gene is use for monitoring that alkaloid anabolism of Dendrobium Officinale

2. Materials and Methods

2.1 Materials

Tissue culture seedlings of Dendrobium officinale Kimura et Migo (Yunnan Agricultural Research Institute). In this experiment, seedlings of Dendrobium Officinale were cultured in bud tip tissue culture.

Light quality is irradiated with both red and white light (white light).

The medium was MS + 2 mg/L peptone + 0.4 mg/L MAA, 20g sucrose as carbon source, pH 5.8

2.2 Experimental Treatment

2.2.1 Seedling Raising

The tissue culture plantlets were cultured in thermostat and illuminated light tube. The temperature was controlled at 25°C±3°C, the humidity in the incubator was kept at 50%, the light intensity was 1600-2000lux, the white light temperature was 6000 k, and the red light temperature was 4500 k. The light time was adjusted according to the growth condition. The initial average time was 4.5 h/d and gradually increased to 8 h/d.

2.2.2 Sampling

Three representative growth stages of Dendrobium Officinale tissue culture seedlings were used to extract medicinal components and qPCR RNA, which were divided into white-30d, white-90d, white-120d, red-30d, red-90d and red-120d groups according to the difference of irradiated light quality, and the samples were recorded as white-30d, white-90d, white-120d, red-30d, red-90d and red-120d respectively

2.2.3 Extraction and Determination of Medicinal Components

2.2.3.1 Determination of Dendrobium Polysaccharide

Refer to *Pharmacopoeia*, the content standard curve of polysaccharide in Dendrobium Officinale was made and determined by phenol-sulfuric acid method.

The standard curve equation of Dendrobium polysaccharide content was: $C = (0.13175 \cdot A448 + 0.09285) \cdot 90$ (μg/ml), $r = 0.9968$

2.2.3.2 Determination of Mannose

Refer to *Pharmacopoeia*, the determination was performed using a high performance liquid chromatography method under correction factor determination conditions

Chromatographic Conditions and System Applicability Test Octadecylsilane Bonded Silica Gel as Filler; The mobile phase was acetonitrile -0.02 mol/L ammonium acetate solution (20: 80). The detection wavelength was 250nm. The number of theoretical plates shall be not less than 4000 according to the mannose peak. According to the calibration factor determination method, the supernatant was poured into the liquid chromatograph from “filling 0.5 mol/L PMP methanol solution” to obtain the calibration factor.

2.2.3.3 Determination of Alkaloids in Dendrobium Officinale

The standard curve and alkaloid extraction of the sample were made according to the methods of Ding Yaping et al.

The standard curve equation is: C= (A630-0. 0002)/0.0803 (g/ml), r=0. 9996. Take 2mL of the prepared solution to be determined, add the separation funnel, supplement chloroform to 10mL, and determine the alkaloids according to the standard method

2.2.4 Extraction of Rna from Plant Samples, Reverse Recording of Cdna, and Fluorescent Quantitative Pcr

RNA was extracted by TRIzol Reagent method. Then, reverse transcription and qPCR amplification were performed using SYBR Green Mix (with ROX) kit, and cDNA was synthesized according to the steps of TaKaRaPrimeScript RT reagent Kit.

The qPCR reaction system was as follows:

Table 1: qPCR reaction system recipe

TOTAL	20UL
PRIMER	0.4ul each
SYBR	10ul
DEPC WATER	8.2ul
TEMPLATE	1ul

As use in qPCR, the primers are as follow:

Table 2: the Primers for the Real-Time Pcr

Primer name	Primer sequence
18S rRNA-F	CCTGAGAAACGGCTACCACAT
18S rRNA-R	CACCAGACTTGCCCTCCA
PPC-F	TTCTGCCACTACTGAATCGG
PPC-R	TGACTGTGTTGGATGTGCTG
HDR-F	TTCTCGTTGTTGGTGGG
HDR-R	GCTTATCTTGTTTCCTGGTC
PMM-F	AGGACCGATGAGACGAAAACC
PMM-R	CTCGCTTCTACGTGATGGCT
GMPase-F	TGCTGGTCCTCTTGCTTTGG
GMPase-R	TGTGTGGTCCTGAAGCCAAT
Mans1-F	CAGGGCAATGGTTGTGCTTC
Mans1-R	AATCTGGACAAGGACAGCGG
Mans2-F	CCCATCGAAAGTGTACCGCT
Mans2-R	AGACGATCAGACGGCCAAAG
Mans3-F	TCATCATGCTGCTCGTCTCC
Mans3-R	ACTAGGCACAGTGACACAGC
Mans4-F	ACTGGAAGGGCGACTGAATG
Mans4-R	AAACCCAACGACGAGGAAGG
Mans5-F	TTATGTGGGCACCTTCGTCC
Mans5-R	ACCCTCACTGGAACCTCCTT

3. Results and Analysis

3.1 Quantitative Analysis of Pharmacologically Active Constituents of Dendrobium

Compared with red light, white light had a positive effect on the accumulation of total

polysaccharides during the rooting stage of Dendrobium Officinale, so the content of medicinal polysaccharides was the highest in all samples after 30 days of white light. However, combined with the accumulation of mannose, white light inhibited the mannose accumulation of Dendrobium Officinale and the conversion of polysaccharides from Dendrobium Officinale to mannose. It was also observed that the growth and development of Dendrobium Officinale seedlings treated with white light were different.

The total sugar accumulation ability of red light to Dendrobium Officinale was weaker than that of white light, so the polysaccharide content of Dendrobium Officinale was relatively low, but the accumulation of mannose was better than that of white light. Combined with the analysis of total sugar content, the content of mannose in Dendrobium Officinale under red light decreased abruptly during the 90-day vigorous growth period, but the total sugar content increased steadily, so it can be inferred that part of mannose was converted into polysaccharide in the vigorous growth period of Dendrobium Officinale. The alkaloids content of Dendrobium Officinale treated with white light was lower than that of Dendrobium Officinale treated with red light in each growth cycle, which was related to the effect of red light on Dendrobium Officinale producing round bulbs at the early stage of growth and development. The development of round bulbs was generally synchronized with alkaloids synthesis.

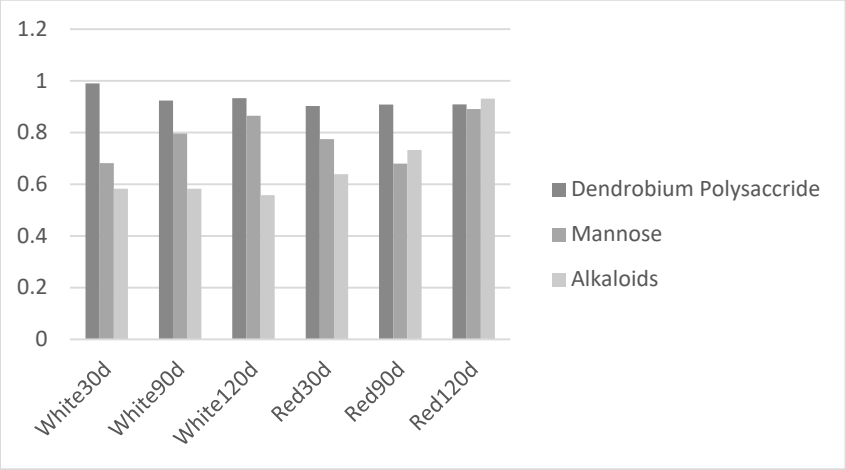

Fig.1: Effects of Red and White Light on the Contents of Medicinal Active Constituents in Dendrobium Officinale

3.2 Gene Related to Anabolism of Medicinal Active Ingredient in Dendrobium Officinale

The expression of PMM and GMPase, two enzymes related to mannose synthesis, changed significantly at different stages (Figure 2). The tissue culture seedlings of Dendrobium Officinale treated with red light had higher expression of related genes at each growth stage, which was consistent with the fact that the accumulation of mannose was more than that of the seedlings treated with white light.

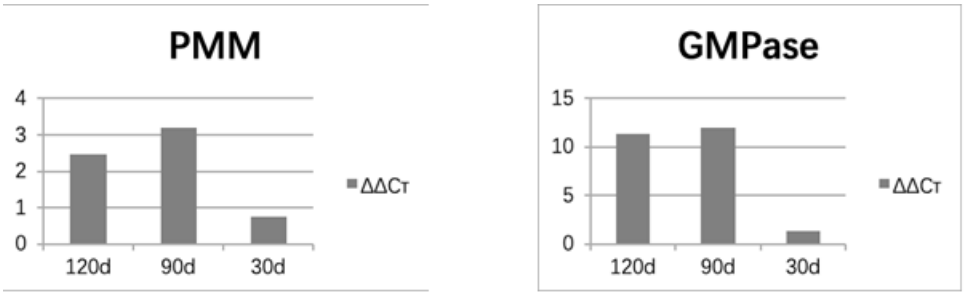

Fig.2: Mannose-Related Gene Expression

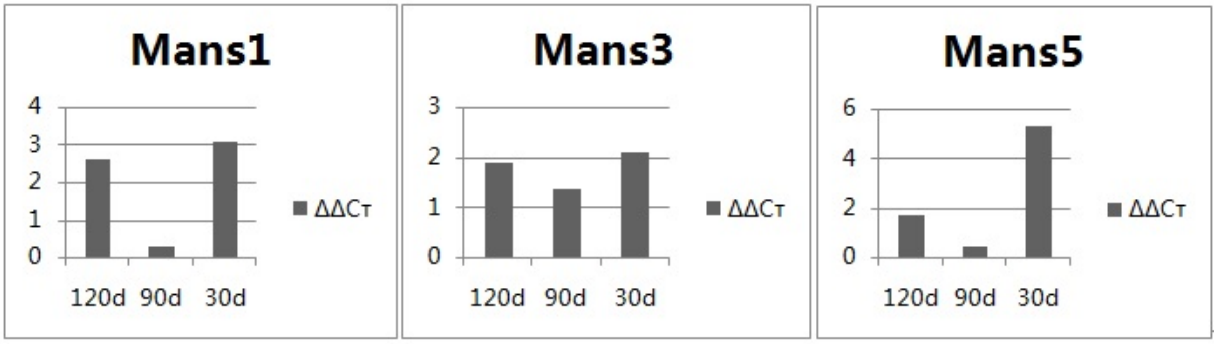

Fig.3: Mannose Transport-Related Gene Expression a

Although the relative expression of genes related to mannose synthesis was significantly correlated with light quality, the relative expression of synthetic genes at five transmembrane sites of Mans protein related to mannose transport was not consistent with light quality. As shown in that above three figure, Mans1, Mans3, and Mans5 were expressed in high amounts in general under red light quality treatment; As shown in that follow two figures, Mans2 and Mans4 were express higher overall in white light quality.

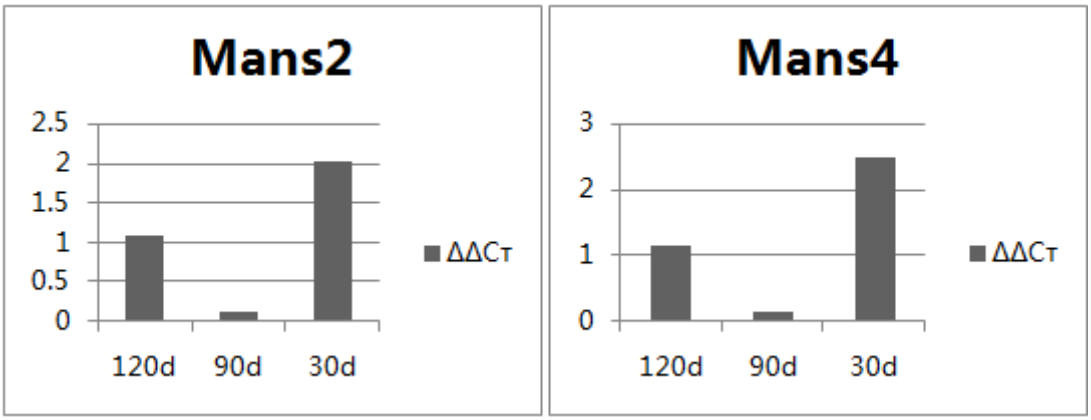

Fig.4: Mannose Transport-Related Gene Expression B

3.2.1 Relative Expression of Genes Related to Polysaccharide Synthesis in Dendrobium Officinale

Because of the lack of genes directly regulating polysaccharide synthesis in Dendrobium Officinale, the relative expression of C4 photosynthetic PPC enzyme was studied in this experiment. It was found that the relative expression of C4 photosynthetic PPC enzyme in red light treatment was higher than that in white light treatment at all stages of growth.

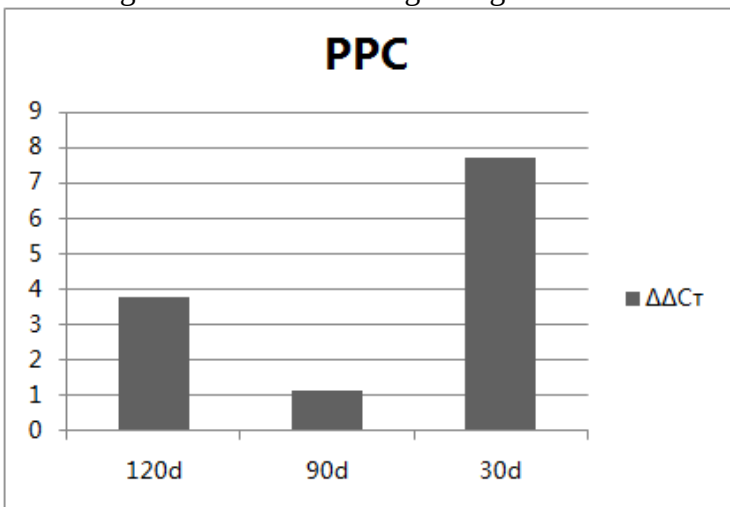

Fig.5: Carbohydrate-Related Gene Expression

3.2.2 Relative Expression of Genes Related to Alkaloid Synthesis in Dendrobium Officinale

HDR gene controlled alkaloid synthesis and accumulation in Dendrobium Officinale. In this experiment, the expression of HDR gene in Dendrobium Officinale treated with red light was higher than that in the control group, and the maximum expression of HDR gene appeared at the 90th day of white light treatment, but the total expression of HDR gene in Dendrobium Officinale treated with red light was higher than that in the control group.

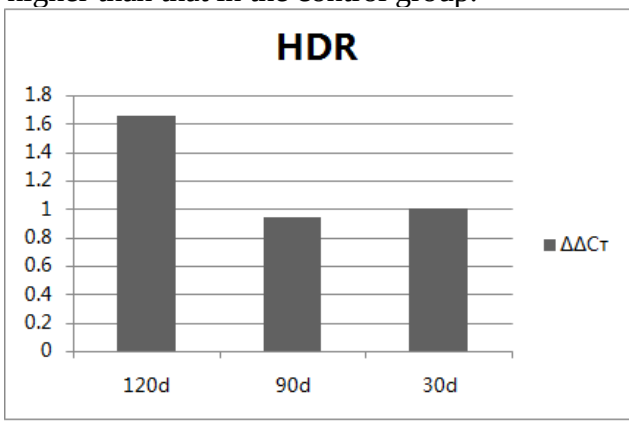

Fig.6: Alkaloid-Related Gene Expression

4. Discussions and Conclusions

4.1 Effect of Different Light Quality on Accumulation of Medicinal Active Components in Dendrobium Officinale

Combined with the above data, we can see that white light is more conducive to the accumulation of total sugar in Dendrobium Officinale plants. Under red light condition, the relative expression of PEPC enzyme was increased because of stress resistance, so although white light was more beneficial to photosynthesis and accelerated metabolism of sugar accumulation, there was no significant difference between red light treatment and white light treatment in the amount of total medicinal sugar. During the vigorous growth period, we observed the slow increase of the total sugar content and the decrease of the mannose content in the seedlings treated with red light. It can be inferred that some mannose was transported to the polysaccharide of Dendrobium Officinale due to the need of growth and development. The results showed that red light had no significant effect on the polysaccharide content of Dendrobium Officinale, but the mannose content of Dendrobium Officinale under red light was higher than that under white light.

4.2 Effects of Different Light Quality on Genes Related to Anabolism of Medicinal Active Components in Dendrobium Officinale

White light is more beneficial to the accumulation of polysaccharide and red light is more suitable for the accumulation of mannose. However, the effects of these two genes on Mans (glycosyltransferase), which assists in mannose synthesis and transport, were different. The relative expression of Mans (glycosyltransferase) at sites 1, 3 and 5 encoding the transmembrane site of the protein was higher under red light than that under white light, while that at sites 2 and 4 was higher under white light. . Because changes in light quality can cause changes in plant photosynthesis, and then cause the change of mannose content, At that same time, There have also been changes in the way in which they are transported, In view of the lack of reports on specific synthesis and transport mechanisms, It can only be inferred that the proteins encoded by 1, 3 and 5 loci and 2 and 4 loci represent the components of different synthesis and transport strategies of Dendrobium Officinale seedlings under high mannose content and low mannose content, that is, the changes of light environment will affect the mannose transport pathway.

Because of the lag of biological effect, the mannose content of Dendrobium Officinale was not the highest in 90 days, but it was the highest in 120 days after treatment, that is to say, the red light

treatment had the highest mannose content in Dendrobium Officinale.

As for that alkaloid synthesis of Dendrobium Officinale, the red light has larger gain effect than the white light, In the morphological observation, it has been found that red light can promote the development of protocorm in the early stage of Dendrobium Officinale plant growth, And this process is often associated with alkaloid synthesis, so in each growth cycle, the red light treatment of Dendrobium Officinale plants have higher alkaloid content than the white light treatment, and the relative expression of related synthetic genes is also relatively high, consistent with the actual alkaloid accumulation.

The relative expression of some genes was also affected by the content of their corresponding synthetic products at the same time. Except for the increase of the expression caused by stress resistance, this may also be related to the negative feedback mechanism. Some related synthetic genes also play the role of synthetic rate-limiting genes, which is more obvious in white light treatment plants.

The relative expression of genes could also reflect the synthesis rate of the corresponding synthetic products in this period to a certain extent. Considering that the relative expression of synthetic genes of all kinds of medicinal active components in red light treatment plants was relatively high at 120 days, it could be judged that red light treatment was more suitable for the synthesis and accumulation of three kinds of medicinal active components in Dendrobium Officinale plants. Although red light is superior to white light, considering that red light did not show the advantage of synthesis and accumulation of medicinal polysaccharides at the initial stage of rooting, we can also consider using white light culture at the rooting stage, and gradually turn to red light at the end of the rooting stage, and the rooting stage is also the stage when the white light treatment plant seedlings begin to have great differences in morphology, so the timely light-quality conversion theory can be used as a good practical reference.

Based on the fact that the red light quality is beneficial to the morphological growth of Dendrobium Officinale, The effect of red light quality treatment on the effective components of Dendrobium Officinale was studied, and the genes controlling the anabolism of the related components were detected. The molecular biological mechanism and pathway in the study period could play a guiding role in the quality of Dendrobium Officinale in vitro and in vivo seedlings and the culture strategy of Dendrobium Officinale seedlings.

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