Effects of short light/dark cycles on photosynthetic pathway switching and growth of medicinal *Dendrobium officinale* in aeroponic cultivation

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Abstract: *Dendrobium officinale* has high medicinal value but grows slowly in natural environment due to its special CAM photosynthetic pathway. In this study, *D. officinale* were grown aeroponically with light/dark cycles of 12 h/12 h, 4 h/4 h, and 2 h/2 h for 150 d. The photosynthetic electron transfer characteristics, photosynthetic CO₂ fixation pathways, and accumulations of biomass and soluble polysaccharides in *D. officinale* leaves were studied. The results showed that the photosynthetic apparatus states of *D. officinale* in aeroponic cultivation under short light/dark cycles of 4 h/4 h and 2 h/2 h were better than that under 12 h/12 h. The dark net CO₂ exchange percentages of *D. officinale* were negative in short light/dark cycles of 4 h/4 h and 2 h/2 h, and the daily net CO₂ exchange amount and dry/fresh weight increases were doubled compared with those in 12 h/12 h light/dark cycle. High soluble polysaccharides content and the soluble polysaccharides yield of *D. officinale* were obtained in the shorter light/dark cycle of 2 h/2 h. Therefore, the photosynthetic pathway of *D. officinale* could be switched from CAM to C₃ by short light/dark cycles of 4 h/4 h and 2 h/2 h, and its higher biomass accumulation and soluble polysaccharides yield could be obtained by the shorter light/dark cycle of 2 h/2 h in aeroponic cultivation.

Keywords: dark net CO₂ exchange percentage, photosynthetic pathway, short light/dark cycle, soluble polysaccharides

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I Introduction

*Dendrobium officinale*, an Orchidaceae, has high medicinal value. However it grows very slowly in natural environment. It takes 3-5 years to harvest under artificial cultivation environment. This may be due to its special photosynthetic characteristics of CAM⁹. CAM photosynthetic pathway is an effective means to optimize water use, carbon balance, and photon quantum efficiency of higher plants under environmental stresses². The physiological and biochemical processes of CAM photosynthetic pathway are usually divided into four phases according to the 24 h light/dark cycle³. Phase I is the storage of malic acid after CO₂ absorption at night or dark period. Phase III is the decarboxylation of stored malic acid during daytime or light period, and the process of carbon assimilation using photon quantum. At this phase, high intracellular CO₂ partial pressure causes stomatal closure. Phases I and IV are transitions between phase I and III⁹. The C₃ photosynthetic pathway does not require malic acid to be loaded into vacuoles by active transport in dark period. Therefore, fixing CO₂ through C₃ pathway saves about 10% of the metabolic cost compared with CAM pathway⁶.

CAM and C₃ pathways of CAM plant *Kalanchoe blossfeldiana* can be induced by short light period and long dark period, and plant pigments are involved in these processes⁶-⁸. Studies on *D. ekapol* showed that short light period increases net CO₂ absorption in dark period, while long light period increases net CO₂ absorption at early and end of light period⁹. The photosynthetic pathway and CO₂ uptake of *Doritaenopsis Queen Beer “Mantefon” can also be affected by light/dark cycle¹⁰. *D. officinale* as a facultative CAM plant usually has a photosynthetic pathway of coexistence of C₃ and CAM under natural conditions¹¹. Its photosynthetic pathway can be induced into an obligate C₃ pathway in 4 h/4 h short light/dark cycle, and its daily net CO₂ exchange amount increases compared with in 12 h/12 h light/dark cycle¹¹. However, it deserves further study whether short light/dark cycles can significantly promote the growth and development of *D. officinale* in a long-term cultivation.

Artificial cultivation of *D. officinale* is mainly imitating wild cultivation and substrate cultivation in shaded greenhouses. As an advanced soilless cultivation technique, aeroponic cultivation has been applied to many horticultural crops including lettuce, tomato, cucumber, chrysanthemum, and poinsietta¹²-¹⁶. Compared with substrate cultivation, aeroponic cultivation can significantly increase the growth rate and soluble polysaccharides content of *D. officinale*¹⁷,¹⁸. Therefore, the aim of this study was to investigate the effects of short light/dark cycles on photosynthetic pathway switching and growth as well as soluble polysaccharides accumulation of *D. officinale* in aeroponic cultivation.

2 Materials and methods

2.1 Experimental materials and cultivation conditions

The high buds of *D. officinale* were domesticated and cultivated in the plant factory laboratory of China Agricultural University in Beijing for 2 years before using as experimental materials. Two hundred *D. officinale* seedlings with uniform size
were selected. The base of stem was clamped with sponge and planted in a cultivation tank (1200 mm×900 mm×120 mm). There were 117 holes in the cultivation panel. One seedling was planted in each hole. Nutrient solution was sprayed to the roots one time per hour and 10 seconds for each time. The formula of nutrient solution for aeroponic cultivation was followed by Cheng et al.[12] EC and pH of the nutrient solution were controlled at 0.6-0.7 ms/cm and 6.0-6.5, respectively.

The environmental conditions of the plant factory laboratory were controlled as follows. Artificial light source was LED lamp with red to blue light ration of 1.2 (WR-LED-16W, Beijing Lighting Valley Technology Co., Ltd., China). Photosynthetic photon flux density (PPFD) at the plant canopy was about 150 μmol/m²·s. Temperatures during the light and dark periods were 26°C±1°C and 22°C±1°C, respectively. Relative humidity was 65±5% and mean of CO₂ concentration was 670±30 μmol/mol.

2.2 Treatment design

Three light/dark cycles were set up in 12 h/12 h, 4 h/4 h, and 2 h/2 h, respectively. There were 20 seedlings of D. officinale in each light/dark cycle treatment. After 150 d of treatment, chlorophyll fluorescence parameters, net CO₂ exchange characteristics, plant morphological characteristics, biomass, and soluble polysaccharide content of D. officinale were measured under 12 h/12 h, 4 h/4 h, and 2 h/2 h light/dark cycles, respectively.

2.3 Measurement indexes and methods

2.3.1 Chlorophyll fluorescence measurement of D. officinale leaves

A multi-function plant efficiency analyzer (M-PEA, Hansatech Inc., UK) was used to measure the O-J-I-P transients of the third fully expanded leaves from top of healthy plants. Maximum quantum yield for primary photochemistry (Fv/Fm), performance index (PLS) based on absorption energy, energy absorbed by active PS II reaction center (ABS/RC), and the open degree of active reaction center (φPS II) at 2 ms were obtained by OJIP-Test. The measuring light intensity was set to 5000 μmol/m²·s, the illumination time is set to 2 s, and the light quality is 100% of the saturated pulsed red light. Following were the measuring times for the different light/dark cycle treatments: 1:00, 3:00, 5:00, 7:00, 21:00 for 12 h/12 h; 5:00, 7:00, 13:00, 15:00, 21:00, 23:00 for 4 h/4 h; and 3:00, 7:00, 11:00, 15:00, 19:00, 23:00 for 2 h/2 h. Six plants were measured at each time in each light/dark cycle treatment.

2.3.2 Continuous measurement of net CO₂ exchange rate

The photosynthetic continuous measurement system[11] used in this study consists of four cuvettes (L25 cm×W15 cm×H6 cm), a host computer, and an IRGA CO₂ analyzer (LI-7000, LICOR, Lincoln, USA). Before the measurement, pure N₂ gas and CO₂ standard gas with the concentration of 600 μmol/mol were used to calibrate the infrared ray gas analyzer (IRGA). Then the functional leaves of living plant samples were clamped into the leaf chamber, and the leaves were kept flat, then the leaf chamber was fastened.

The inlet air source of the leaf chamber was the air outside the plant factory laboratory (the average CO₂ concentration was 370 μmol/mol). The volume of buffer bottle was 20 L and the inlet air flow rate of the leaf chamber was 1 L/min. The temperature tracking mode was used to ensure that the temperature of the leaf chamber and was consistent in the plant factory laboratory. The CO₂ concentration difference of reference air and samples of each leaf chamber were recorded once every 10 min. The leaf area of each leaf chamber was measured by referring to Yang et al.[19] Then the net exchange rate of CO₂ was calculated according to the difference of CO₂ concentration between reference gas and sample gas and leaf area. The measurement method was referred to Zhang et al.[11] Dark net CO₂ exchange amount, light net CO₂ exchange amount, and daily net CO₂ exchange amount were calculated by integral method. Dark net CO₂ exchange percentage = dark net CO₂ exchange amount / daily net CO₂ exchange amount × 100%. Three replicates were conducted in this measurement for each light/dark cycle.

2.3.3 Measurement of plant morphological characteristics and biomass

The morphological indexes measured included the increases of plant height, internodes number, stem diameter, leaf area, leaf number, and leaf thickness. In order to calculate the increases of parameters above, the measurements were conducted at the beginning of the experiment and after 150 d, respectively. Plant leaf area was calculated by the ratio of leaf image obtained by scanner to the black square of 1 cm². The number of leaves was determined by the number of fully unfolded leaves. The blade thickness was obtained by measuring the transverse section of the blade with an optical microscope (BX51, Olympus Co., Japan). The plant height was measured from the connection of stem and root to growing point. The length of internodes longer than 0.5 cm was counted. The biomass indexes measured included total, stem, leaf, and root dry and fresh weight increases. Six plants were measured for each light/dark cycle treatment.

2.3.4 Measurement of soluble polysaccharide content

After 150 d, the stems of D. officinale were dried in an oven at 50°C, then crushed and screened by 40 meshes. The soluble polysaccharides content of D. officinale was measured by phenol-sulfuric acid method referenced by Li and Hirata[23]. Six plants were measured for each light/dark cycle.

2.4 Statistical analysis

SPSS 21 (IBM, Inc., Armonk, NY, USA) was used for statistical analysis. The variance analysis of the data was performed by Duncan method at the 0.05 significance level. Univariate analysis of variance (ANOVA) was used to test the difference of data mean.

3 Results and discussion

3.1 Effects of short light/dark cycles on photosynthetic electron transfer characteristics and photosynthetic pathway switching of D. officinale leaves

The Fv/Fm of all leaves grown in different light/dark cycles could recover to above 0.8 in dark period (Table 1), indicating that the photosynthetic organs of leaves were not damaged significantly[21]. The PLS of D. officinale leaves under 2 h/2 h cycle was higher than that under 4 h/4 h light/dark cycle and much higher than that under 12 h/12 h. Pls of D. officinale leaves under 2 h/2 h cycle was higher than that under 4 h/4 h light/dark cycle and much higher than that under 12 h/12 h. Pls was a more sensitive indicator than Fv/Fm. Pls synthesized a single multi-parameter expression through three main functional steps of photosynthetic activity (light energy absorption, excitation energy capture and excitation energy conversion to electron transfer) through the PSII reaction center complex[22,23]. With the shortening of light/dark cycle, Pls increased gradually, which might be related to the increase of open degree of active reaction center (φPS II) at 2 ms. There was no significant difference in the light energy absorbed by each active PS II reaction center (ABS/RC) in D. officinale leaves under different light/dark cycles.
Table 1 Leaf chlorophyll fluorescence parameters of *D. officinale* under different short light/dark cycles

<table>
<thead>
<tr>
<th>Light/dark cycle</th>
<th>Fv/Fm</th>
<th>PI_ABS</th>
<th>ABS/RC</th>
<th>ψ_o</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h/12 h</td>
<td>0.82±0.01 NS</td>
<td>4.38±0.68 c</td>
<td>1.30±0.07 NS</td>
<td>0.57±0.05 b</td>
</tr>
<tr>
<td>4 h/4 h</td>
<td>0.82±0.01 NS</td>
<td>5.41±0.57 b</td>
<td>1.21±0.10 NS</td>
<td>0.58±0.05 b</td>
</tr>
<tr>
<td>2 h/2 h</td>
<td>0.82±0.01 NS</td>
<td>7.51±0.57 a</td>
<td>1.22±0.05 NS</td>
<td>0.66±0.02 a</td>
</tr>
</tbody>
</table>

Net CO₂ exchange rate of *D. officinale* showed a multi-phase change of CAM pathway when the light/dark cycle was 12 h/12 h. When the light/dark cycle was shortened to 4 h/4 h or 2 h/2 h, net CO₂ exchange rate of *D. officinale* showed a C₃ pathway pattern, i.e. net absorption of CO₂ in light period and net release of CO₂ in dark period (Figure 1). The result is consistent with that of Zhang et al.[11]

Note: The thin black line on the horizontal axis indicates light period, and the thick black line indicates dark period.

Contrary to 12 h/12 h light/dark cycle, dark net CO₂ exchange amount and dark net CO₂ exchange percentage of *D. officinale* in short light/dark cycles of 4 h/4 h and 2 h/2 h were negative, and light net CO₂ exchange amount and daily net CO₂ exchange amount were higher (Table 2). This indicated that photosynthetic pathway of *D. officinale* could be switched to C₃ by the short light/dark cycles. The higher daily net CO₂ exchange amounts during short light/dark cycles were mainly due to the higher light net CO₂ exchange amounts during short light/dark cycles could induce the higher stomatal conductances of *D. officinale* in light period[12]. Structure features of leaf might affect CO₂ concentration in chloroplast stroma[24], thus affecting the photosynthetic capacity of *D. officinale*. Whatever it was, the photosynthetic pathway of *D. officinale* could be switched to C₃ pathway, and the daily net CO₂ exchange amount could be increased by the short light/dark cycles.

Table 2 Net CO₂ exchange characteristics of *D. officinale* under different light/dark cycles

<table>
<thead>
<tr>
<th>Light/dark cycle</th>
<th>Light net CO₂ exchange amount /mmol m⁻² d⁻¹</th>
<th>Dark net CO₂ exchange amount /mmol m⁻² d⁻¹</th>
<th>Daily net CO₂ exchange amount /mmol m⁻² d⁻¹</th>
<th>Daily net CO₂ exchange percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h/12 h</td>
<td>50.5±2.1 b</td>
<td>9.2±1.6a</td>
<td>59.7±0.4b</td>
<td>15.4±2.8a</td>
</tr>
<tr>
<td>4 h/4 h</td>
<td>87.9±5.4a</td>
<td>-11.8±1.0b</td>
<td>76.1±6.4a</td>
<td>-15.6±2.6b</td>
</tr>
<tr>
<td>2 h/2 h</td>
<td>96.6±5.5a</td>
<td>-13.5±3.9b</td>
<td>83.1±1.6a</td>
<td>-16.1±4.3b</td>
</tr>
</tbody>
</table>

Table 3 Morphological characteristics of *D. officinale* under different light/dark cycles

<table>
<thead>
<tr>
<th>Light/dark cycle</th>
<th>Plant height increase /cm</th>
<th>Increase of internodes number</th>
<th>Stem diameter /mm</th>
<th>Leaf area increase /cm²</th>
<th>Leaf number increase</th>
<th>Leaf thickness /mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h/12 h</td>
<td>10.2±0.5 b</td>
<td>8.0±1.4 b</td>
<td>5.3±0.8 b</td>
<td>4.7±2.1 b</td>
<td>3.3±0.6 b</td>
<td>0.43±0.01 a</td>
</tr>
<tr>
<td>4 h/4 h</td>
<td>19.4±4.4 a</td>
<td>16.0±3.6 a</td>
<td>6.2±0.3 a</td>
<td>15.2±4.4 a</td>
<td>6.3±0.6 a</td>
<td>0.41±0.01 b</td>
</tr>
<tr>
<td>2 h/2 h</td>
<td>15.7±1.4 a</td>
<td>10.5±1.3 b</td>
<td>5.7±0.3 ab</td>
<td>16.5±3.4 a</td>
<td>6.5±0.6 a</td>
<td>0.41±0.01 b</td>
</tr>
</tbody>
</table>

3.2 Effects of short light/dark cycles on morphogenesis of *D. officinale*

After 150 d, the plant heights of *D. officinale* treated with 4 h/4 h and 2 h/2 h short light/dark cycles were significantly higher than that treated with 12 h/12 h light/dark cycle. The colors of stem and leaf were obviously red under 2 h/2 h shorter light/dark cycle (Figure 2). Compared with 12 h/12 h light/dark cycle, *D. officinale* plants treated with 4 h/4 h and 2 h/2 h short light/dark cycles had thinner and more leaves, larger leaf area, more internodes, larger plant height, and thicker stems (Table 3).

Anthocyanins were accumulated on the back of the leaves of *D. officinale* under 2 h/2 h shorter light/dark cycle, but not with plants under 12 h/12 h and 4 h/4 h light/dark cycles (Figure 3). The biosynthesis of anthocyanins was related to cryptochrome as a blue light receptor[25]. The light-activated cryptochrome could remain in the dark for a period of time[26], which might be the reason for the high accumulation of anthocyanins in *D. officinale* treated with 2 h/2 h shorter light/dark cycle.
3.3 Effects of short light/dark cycles on biomass and soluble polysaccharide accumulations of *D. officinale*

The increases of total fresh weight, total dry weight, stem fresh weight, stem dry weight, leaf fresh weight, leaf dry weight, and root fresh weight of *D. officinale* treated with short light/dark cycles of 4 h/4 h and 2 h/2 h were significantly higher than those treated with 12 h/12 h light/dark cycle. The increase of root dry weight in 2 h/2 h shorter light/dark cycle was significantly higher than that in 12 h/12 h and 4 h/4 h light/dark cycles (Figure 4). The effects of short light/dark cycles of 4 h/4 h and 2 h/2 h on the long-term biomass accumulation of *D. officinale* were consistent with their effects on the short-term daily net CO₂ exchange amount of *D. officinale*.

Note: Different growth parameters were recorded at day 1 and day 150, respectively. Different letters indicate statistically significant differences by Duncan’s multiple range test (*p* < 0.05).

**Figure 4** Biomass accumulation of *D. officinale* under different short light/dark cycles

Compared with 12 h/12 h light/dark cycle, the soluble polysaccharides content of *D. officinale* treated with 4 h/4 h short light/dark cycle decreased significantly. When the light/dark cycle was shortened to 2 h/2 h, the soluble polysaccharides content increased to the level of 12 h/12 h light/dark cycle (Figure 5). The soluble polysaccharides yield of *D. officinale* treated with 2 h/2 h shorter light/dark cycle was significantly higher than that treated with 12 h/12 h and 4 h/4 h light/dark cycles. Studies had shown that light intensity and light quality affected the accumulation of soluble polysaccharides in *D. officinale*, while the accumulation of soluble polysaccharides was positively correlated with environmental stresses[^27,28]. The soluble polysaccharides content of *D. officinale* treated with 2 h/2 h shorter light/dark cycle was significantly higher than that treated with 4 h/4 h and 12 h/12 h short light/dark cycles, which might be related to the responses of plants to a sudden induction of light environment stress. Soluble polysaccharides play a role by plants in protecting plants from abiotic stresses[^29]. The accumulation of anthocyanins also proved that the shorter light/dark cycle of 2 h/2 h might cause some photopreception of *D. officinale* against a sudden change of light. The results of Lin and Lai[^28] also showed that the soluble sugar polysaccharide content in *D. officinale* was positively correlated with...
with the activity of PEPC, while the expression of CAM photosynthetic pathway was closely correlated with the activity of PEPC\(^{[10]}\). It explained why the soluble polysaccharides content in \textit{D. officinale} treated with 4 h/4 h short light/dark cycle was significantly lower than that treated with 12 h/12 h light/dark cycle. While it could not explain why the \textit{D. officinale} treated with a 2 h/2 h light/dark cycle had higher soluble polysaccharides content than that treated with 12 h/12 h light/dark cycle.

4 Conclusions

Based on photosynthetic electron transfer characteristics, photosynthetic pathways, biomass and soluble polysaccharides accumulation of \textit{D. officinale} plants grown aeroponically under three light/dark cycles for 150 d, we concluded that photosynthetic efficiency of \textit{D. officinale} under 4 h/4 h and 2 h/2 h short light/dark cycles was better than that under 12 h/12 h light/dark cycle because of the photosynthetic pathway switching from CAM to C3. The results also demonstrated that under 4 h/4 h and 2 h/2 h short light/dark cycles, \textit{D. officinale} plants had great increases in daily net CO\(_2\) exchange amount, and fresh/dry weights compared with those in 12 h/12 h light/dark cycle. The soluble polysaccharides yield of \textit{D. officinale} in the 2 h/2 h light/dark cycle was higher than that in 12 h/12 h light/dark cycle. Therefore, shorter light/dark cycles of 2 h/2 h is recommended for light environment adjustment of \textit{D. officinale} for achieving the growth and quality control by keep the higher soluble polysaccharides content and yield.

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