Concomitant CAM and C3 Photosynthetic Pathways in *Dendrobium officinale* Plants

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ABSTRACT. *Dendrobium officinale*, endemic to China, is a rare and endangered medicinal herb. As a result of its high economic value, slow growth, and diminishing wild population, protected cultivation is preferred. However, little information is available on its growing environment and photosynthetic characteristics. In this study, the photosynthetic patterns of *D. officinale* were investigated under various environmental conditions by measuring the net CO₂ exchange rates continuously for several days or weeks. Under non-stressed growth chamber conditions with 12-hour light and 12-hour dark periods, *D. officinale* had concomitance of C3 and crassulacean acid metabolism (CAM) photosynthesis patterns. Different degrees of CAM in *D. officinale*, expressed as the percentage of CO₂ exchanges in the dark period to the daily amount of CO₂ exchanges, were observed depending on environmental conditions. With decreasing substrate water content, a typical CAM pattern was found, and concomitance of C3 and CAM patterns was found again when plants were rewatered. The accumulation of leaf titratable acidity during a dark period increased as substrate dried out but decreased again as plants were rewatered. A shorter light–dark cycle (4-hour light and 4-hour dark periods) led to a C3 pattern alone. The substrate moisture and light–dark cycle were inducible factors for switching between C3 and CAM patterns in *D. officinale*. These results indicate that *D. officinale* is a facultative CAM plant and the C3 pathway can be induced by controlling the growing environment. Further studies are needed to identify the optimal environmental conditions to enhance the growth of *D. officinale*.

The photosynthetic pathways of C3, C4, and CAM are known to have different photosynthetic properties (Larcher, 1995; Nobel, 1991). Although the dry matter accumulation rate of CAM plants is lower than that of C3 and C4 plants, CAM plants have the ability to adapt to extreme environments such as drought, high temperature, and salt stress (Osmond, 1978; Rascher et al., 1998; Winter and Holtum, 2007). Leaf succulence of *Aeonium* plants is correlated with CO₂ assimilation patterns (Larcher, 1995), and the CO₂ assimilation pattern of some CAM plants can be strongly regulated by environmental conditions such as light intensity, temperature, soil water content, and photoperiod (Brulfert et al., 1988, 1996; de Mattos and Lüttge, 2001; Maxwell, 2002).

Many epiphytes found in tropical and subtropical forests grow on rocks or tree trunks where water deficit is frequent, and many of them are CAM plants (Silvera et al., 2005). Almost all *Dendrobium* species are epiphytes. Because of their high ornamental and medicinal values, many *Dendrobium* species are cultivated in the open field or greenhouses. Only a small percentage of *Dendrobium* species have been studied for their photosynthetic pathways. *Dendrobium taurinum* (Fu and Hew, 1982), *D. ekapol* [sic] (Sekizuka et al., 1992), *D. tortile*,

D. crumenatum (Sinclair, 1984), *D. primulinum* (Ren et al., 2010), and *D. beckeri* (Winter et al., 1983) have CAM pathways, whereas *D. chrysotoxum* has a C3 pathway (Ren et al., 2010) based on the diurnal changes of net CO₂ change rates, leaf titratable acidity, malic acid, leaf stomatal conductance, and the $\delta^{13}\text{C}$ value.

Dendrobium officinale, endemic to China, is a rare and endangered orchid with extremely slow growth (Su and Zhang, 2003). Its stems have many health benefits such as promotion of the immune system as well as antiaging and anticancer properties (Li et al., 2011). The photosynthesis of *D. officinale* is affected by temperature (Ai et al., 2010), light quality (Gao et al., 2012), light intensity (Bao et al., 2007), air humidity (Zhang et al., 2013), and other cultural conditions (Mao, 2008). Su and Zhang (2003) measured the daily changes of net CO₂ exchange rates of *D. officinale* under various conditions and determined it was a facultative CAM plant. That is, *D. officinale* had a CAM pattern on sunny days, a C3 pattern on rainy days, and an intermediate pattern between CAM and C3 on cloudy days. Nevertheless, the specific inducing environmental factors that cause the switching between C3 and the CAM pathway are still unknown.

Because stomata of a CAM plant are closed during the light period and the malic acid accumulated overnight is broken down to release CO₂ that is fixed by ribulose biphosphate carboxylase/oxygenase (Rubisco) during the light period behind closed stomata, the actual photosynthesis cannot be determined by measuring the gas exchange rates only during a light period or by instantaneous gas exchange rates. The CAM

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contribution to the whole-day carbon assimilation can be determined from the continuous measurement of net CO₂ exchange rates of CAM plants for a whole day; thus, the photosynthetic pattern can be accurately determined. The continuous measurement of photosynthesis had been applied for determining the photosynthetic pathway of some obligate and facultative CAM plants such as *Clusia rosea*, *C. cylindrica*, and *Mesembryanthemum crystallinum* (Winter et al., 2009; Winter and Holtum, 2007).

The objectives of this study were to determine the photosynthetic pathway of *D. officinale* and identify the inducing factors for switching between C3 and the CAM pathway. The effects of drought stress and various light–dark cycles on CO₂ assimilation patterns of *D. officinale* and nocturnal accumulation of leaf titratable acidity were also investigated.

Materials and Methods

PLANT MATERIALS AND CULTURE CONDITIONS. Tissue-cultured *D. officinale* plants were grown in an unheated greenhouse for commercial production in Jinhua city, Zhejiang province (lat. 29°0'14" N, long. 119°60'32" E) for 3 years before being transported to China Agricultural University in Beijing (lat. 40°0'25" N, long. 116°21'27" E) on 29 Apr. 2012. The Jinhua greenhouse annual average air temperature was 17.5 °C, the lowest air temperature was –2.8 °C, and the highest air temperature was 38.9 °C. The 3-year-old plants were grown in 0.4-L round plastic pots (two plants per pot) filled with a substrate mix of composted small pine bark (3 to 8 mm), medium pine bark (5 to 10 mm), pine bark powder, perlite, and composted sawdust in a volume ratio of 1:1:1:1:0.5. Thirty-five pots were kept in a greenhouse with natural light and 25 were kept in a walk-in growth chamber under artificial light. These plants were used for CO₂ exchange rate measurement. Plants in both greenhouse and growth chambers were watered with nutrient solution once every 2 to 3 d as needed. The nutrient solution contained (in mg·L^{–1}) 205 Ca(NO₃)₂·4H₂O, 60 MgSO₄·7H₂O, 136 KH₂PO₄,

80 NH₄NO₃, 3.6 MnSO₄·H₂O, 2.7 H₃BO₃, 13.4 FeSO₄·7H₂O, 0.1 CuSO₄·5H₂O, 0.4 ZnSO₄·7H₂O, and 0.1 (NH₄)₆Mo₇O₂₄·4H₂O.

MEASUREMENT SYSTEM OF NET CO₂ EXCHANGE RATE. When measuring net CO₂ exchange rate of a single leaf using a fluorescence cuvette (6400-40; LI-COR, Lincoln, NE) of the portable photosynthesis system (LI-6400; LI-COR) in the growth chamber, low net CO₂ exchange rates [between –2 and 4 μmol·m^{–2}·s^{–1} every 3 min (data not shown)] with large fluctuations were observed. Because of the large variation, it was not possible to judge whether the net CO₂ exchange rates were positive or negative. By using a conifer cuvette (6400-05; LI-COR) of the same portable photosynthesis system with multiple leaves enclosed, the net CO₂ exchange rates had smaller fluctuations with low rates of less than 2 μmol·m^{–2}·s^{–1}. These results indicate that increasing leaf area or biomass within the cuvette can improve the measurement accuracy for species with low photosynthetic rates such as *D. officinale*. However, from the non-continuously measured net CO₂ exchange rates of *D. officinale* by the portable photosynthesis system, the photosynthesis pathway of CAM plants cannot be accurately determined.

To more accurately determine the photosynthetic pathway of *D. officinale*, a continuous photosynthesis measurement system was constructed. The shoots were enclosed in the cuvettes, whereas the container (roots and substrate) was excluded. The system consisted of two gas-tight rectangle acrylic cuvettes, an infrared (IR) CO₂ analyzer (GXH-3052; Beijing Beifen-Ruili Analytical Instrument Co., Beijing, China), two air pumps (FML201.5; Chengdu Qihai E&M Manufacturing Co., Chengdu, China), three mass airflow meters (AWM5102; Honeywell, Morristown, NJ), two connected to the two cuvettes and one to the CO₂ analyzer, two cuvette temperature control modules, and an embedded computer (Fig. 1). Each cuvette (25 × 15 × 6 cm) was equipped with a small fan for mixing air, and the airflow from the cuvette inlet to the outlet. The differential CO₂ concentrations of the inlet and outlet were automatically

Fig. 1. The schematic diagram of the gas circuit in the continuous photosynthesis measurement system. Arabic numbers indicate the sequence switch of solenoid valve. IRGR = infrared CO₂ analyzer.

determined by the IR CO₂ analyzer every 105 s for one cuvette. Carbon dioxide assimilation of each cuvette was measured every 7 min by switching the four solenoid valves. Photosynthetic photon flux (*PPF*) at the upper outer surface of the cuvette was measured by a quantum sensor (LI-190SA; LI-COR).

The net CO₂ exchange rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for the plant inside the cuvette is calculated as follows: Net CO₂ exchange rate = $k \times (C_1 - C_2) \times F/LA$ where, k is the conversion coefficient ($\text{mol}\cdot\text{L}^{-1}$); C_1 and C_2 are the CO₂ concentrations at the inlet and outlet of the cuvette, respectively ($\mu\text{mol}\cdot\text{mol}^{-1}$); F is airflow rate ($\text{L}\cdot\text{min}^{-1}$); and LA is leaf area (m^2). LA was determined according to Yang et al. (2002).

The zero calibration of the IR CO₂ analyzer was conducted by pumping CO₂-free air (passing the air through soda lime) into the IR CO₂ analyzer. The CO₂ exchange rates of the empty chambers were measured before and after each measurement. The noise level, which is the difference in CO₂ concentrations between inlet and outlet of the empty cuvette, was $\pm 3.3 \mu\text{mol}\cdot\text{mol}^{-1}$.

MEASUREMENT OF NET CO₂ EXCHANGE RATES UNDER VARIOUS ENVIRONMENTAL CONDITIONS. Net CO₂ exchange rates were continuously measured using the mentioned system for 2 d on 23 and 24 Apr. 2012 for plants in the Jinhua commercial greenhouse, 30 and 31 May 2012 for plants in the Beijing research greenhouse, and 2 and 3 Sept. 2012 for plants in the growth chamber. The continuous measurement system allowed monitoring of photosynthetic rates in two cuvettes simultaneously that were averaged for analysis and illustration in figures.

For the walk-in growth chamber at China Agricultural University, the average air temperature was (mean $\pm$ SD) 25 ± 1.0 °C, relative humidity (RH) at $65\% \pm 5\%$, and a 12-h photoperiod (24-h light–dark cycle). CO₂ concentration was controlled at $500 \pm 50 \mu\text{mol}\cdot\text{mol}^{-1}$ and *PPF* at canopy level was maintained at $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, provided by fluorescent lamps.

EFFECTS OF DROUGHT AND REWATERING ON NET CO₂ EXCHANGE RATE. Two uniform plants of *D. officinale* grown in the growth chambers were selected for measurement. The selected plants were well watered with nutrient solution to container capacity on 5 July 2012. After drainage was completed, shoots were inserted in the cuvettes to initiate the net CO₂ exchange rate measurement on 6 July 2012 (Day 1). Irrigation was withheld for the first 12 d. At the end of the light period on Day 12, plants were rewatered. The net CO₂ exchange rates of the two shoots were measured continuously in a growth chamber until 24 July for a total of 18 d.

EFFECT OF DIFFERENT LIGHT AND DARK CYCLES ON NET CO₂ EXCHANGE RATE. Net CO₂ exchange rates of *D. officinale* increased rapidly after the onset of light, reached the peak in 1 to 2 h, and then gradually decreased. This phenomenon may indicate that much of the net CO₂ fixation may occur early in the light period. The original light–dark cycle was 24 h·d⁻¹ with 12 h light and 12 h dark on 27 to 29 Aug. 2012. By shortening the light and dark period cycle, the net CO₂ exchanges of *D. officinale* in a 24-h period may be increased and the CO₂ assimilation pattern may be changed. Net CO₂ exchange rates of *D. officinale* at shorter cycles (12-h and 8-h cycles with equal length for light and dark periods, that is, 6 h light and 6 h dark and 4 h light and 4 h dark, respectively) were measured on 26 to 28 Sept. 2012 and on 20 to 22 Oct. 2012 in the growth chamber. The 1-d time course of net CO₂ exchange for *D. officinale* plants under 8-, 12-, and 24-h cycles were presented.

Before the initiation of net CO₂ exchange measurement for a different light and dark cycle, plants were grown under the new cycle regimen for 3 d for acclimatization. Plants were grown in the growth chamber and watered every 3 d. The average air temperature in the growth chamber was maintained at 25 ± 1.0 °C and RH at $65\% \pm 5\%$. CO₂ concentration was controlled at $500 \pm 50 \mu\text{mol}\cdot\text{mol}^{-1}$ and *PPF* at canopy level was $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by fluorescent lamps.

LEAF SAMPLES FOR TITRATABLE ACIDITY ANALYSIS. In addition to the plants that were measured for net CO₂ exchange rate, 12 pots (24 plants) grown in the growth chamber were selected for relative water content (described subsequently) and another 12 pots for titratable acidity analysis of leaves. Irrigation was withheld for six pots designated as drought treatment, whereas another six pots were well watered and designated as controls. At 2030 HR (30 min after the onset of dark period) on Day 1 (6 July), Day 11, and Day 17 and at 0730 HR (30 min before the onset of light period) on Day 2, Day 12, and Day 18, six leaves (the fourth to sixth leaves of the shoot from the top to bottom) per treatment were sampled from both drought and control (well-watered) plants.

For the different light and dark cycles, leaf samples were taken from the 8-h cycle (4-h light and 4-h dark periods) for titratable acidity analysis. Leaves were sampled at 0400, 0800, 1200, and 2000 HR on 20 Oct. 2012.

DETERMINATION OF TITRATABLE ACIDITY. Leaf samples were cut into small segments and a portion was weighed (0.6 g/sample) and ground in 10 mL distilled water in a mortar. The resultant slurry was transferred to a 100-mL flask that was placed in a boiling water bath for 30 min to extract the acid. While in the boiling water bath, the sample was mixed several times. The titratable acidity was determined by measuring the volume of 10 mM NaOH required to neutralize each sample to pH 7.0. The titratable acidity in the dark period was determined as the difference between titratable acidities of two consecutive sampling times.

RELATIVE WATER CONTENT OF LEAVES IN THE DROUGHT TREATMENT. Leaves were sampled on Day 1 (6 July), Day 12 (before rewatering), and Day 18 to quantify leaf water status in the drought treatment. The relative water content (RWC) of plant leaves was calculated as: $\text{RWC} (\%) = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100$ where FW is leaf fresh weight, TW is the turgid weight after the leaves were immersed in water for 24 h, and DW is the dry weight after oven-drying at 85 °C for 72 h. The fifth leaf from the shoot tip was sampled for RWC measurement and five leaves were used for each treatment.

STATISTICAL ANALYSIS. A one-way analysis of variance was performed to test the significance of drought and well-watered plants on titratable acidity and RWC. All data analyses were run in Excel 2007 (Microsoft Corp., Redmond, WA) and SPSS 18.0 (IBM Corp., Armonk, NY). Unless otherwise noted, *P* values less than 0.05 were considered statistically significant.

Results and Discussion

CO₂ ASSIMILATION PATTERNS UNDER VARIOUS ENVIRONMENTAL CONDITIONS. The Jinhua commercial greenhouse environment was maintained at an average air temperature of (mean $\pm$ SD) 30.2 ± 5.9 °C and RH of $51\% \pm 12\%$ during the day and 20.7 ± 4.3 °C and $62\% \pm 7\%$ RH at night. The Beijing research greenhouse environment was maintained at an average air temperature of 28.1 ± 4.0 °C and RH of $28\% \pm 12\%$ during the

day and 25.3 ± 2.0 °C and $32\% \pm 12\%$ RH at night. The maximum *PPF* on the measurement days in the Jinhua and Beijing greenhouses were 168 and $426 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. For the growth chamber, *PPF* was $163.5 \pm 1.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and daily average air temperature was 26.2 ± 1.2 °C.

In the growth chamber with a relatively constant environment, the net CO_2 exchange rate of *D. officinale* reached maximums of 3.4 and $3.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 1 h after the transition from dark to light period on 2 and 3 Sept., respectively, then decreased slightly and maintained a positive net CO_2 exchange

Fig. 2. Time course of net CO_2 exchange rates of *Dendrobium officinale* in the growth chamber on 2 and 3 Sept. 2012 (A), in the Jinhua commercial greenhouse and Beijing research greenhouse on 23 and 24 Apr. 2012 and on 30 and 31 May 2012 (C), and time course of measured photosynthetic photon flux (PPF) and temperatures inside cuvette in the Jinhua and Beijing greenhouses (B). For the growth chamber, *PPF* was $163.5 \pm 1.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and daily average air temperature was 26.2 ± 1.2 °C. Dark bars in the x-axis (A) indicate the dark period in the growth chamber. The dotted lines on top and bottom of the solid line indicate SD (A, n = 2).

rate throughout the light period (Fig. 2A). Positive, low net CO₂ exchange rates were detected during the dark period. The daily net CO₂ exchange amounts were 110 and 116 mmol·m⁻²·d⁻¹, and dark net CO₂ exchange percentages to the daily amount of net CO₂ exchanges were 6% and 5% on 2 and 3 Sept., respectively. The percentage of CO₂ exchanges in the dark period to the daily amount of CO₂ exchanges reflected the degree of CAM in *D. officinale*. The results of net CO₂ exchange rates

indicated a low degree of CAM in *D. officinale* under relatively stress-free growth chamber conditions.

Net CO₂ exchange rates of *D. officinale* grown in the Beijing research greenhouse were measured on a sunny day (30 May 2012) and a cloudy day (31 May 2012). On the sunny day, *PPF* reached a maximum of 426 μmol·m⁻²·s⁻¹ and a maximum temperature inside the cuvette of 40.3 °C. On the cloudy day, *PPF* reached a maximum of 282 μmol·m⁻²·s⁻¹ and a maximum

Table 1. Relative water content (RWC) of *Dendrobium officinale* leaves and leaf titratable acidity (TA) accumulated in the dark period under drought-treated and well-watered conditions on Day 1 (6 July 2012), Day 12, and Day 18.^z

Treatment	Day 1		Day 12		Day 18	
	RWC (%)	TA (μmol·g ⁻¹ FW)	RWC (%)	TA (μmol·g ⁻¹ FW)	RWC (%)	TA (μmol·g ⁻¹ FW)
Drought-treated	93.4	160.1	91.8	196.9	95.9	155.2
Well-watered	94.9	156.1	96.5	125.3	95.5	156.6
ANOVA	NS ^y	NS	*	*	NS	NS

^zThe plants were well watered on Day 1 and irrigation was withheld for 12 d thereafter. At the end of Day 12, plants were rewatered.

^yNS and * indicate nonsignificant and significant at *P* ≤ 0.05.

FW = fresh weight; ANOVA = analysis of variance.

Fig. 3. Time course of net CO₂ exchange rates of *Dendrobium officinale* in the growth chamber measured continuously by the continuous photosynthesis measurement system for 18 d. The plants were well watered on Day 1 (6 July 2012) and irrigation was withheld for 12 d. At the end of Day 12, the plants were rewatered. Arrow indicates rewatered day, and dark bars in the x-axis indicate the dark period. The x-axis of the lower graph is the continuation of the upper graph, which means the two graphs' x-axes should be connected. The dotted lines on top and bottom of the solid line indicate sd (n = 2).

cuvette temperature of 37.2 °C (Fig. 2B). The maximum net CO₂ exchange rate of *D. officinale* in the Beijing greenhouse was only 0.9 μmol·m⁻²·s⁻¹, which occurred at ≈0.5 h after sunrise on both days and then decreased gradually to zero at ≈3 h after sunrise (Fig. 2C). The net CO₂ exchange rate was negative in the middle of the day. However, net CO₂ exchange rate was positive throughout the night. Low net CO₂ exchange rates were possibly the result of high light intensity and high temperatures inside the cuvette. High light and high temperature often cause photoinhibition in *D. officinale* (Ai et al., 2010; Su and Zhang, 2003), which explains the low net CO₂ exchange rates observed. The daily net CO₂ exchange amounts were 16 and 21 mmol·m⁻²·d⁻¹ and dark net CO₂ exchange percentages were 70% and 66% on 30 and 31 May 2012, respectively, indicating a high degree of CAM.

Net CO₂ exchange rates of *D. officinale* plants grown in the Jinhua commercial greenhouse were measured on a sunny day (23 Apr. 2012) and a rainy day (24 Apr. 2012). A shade cloth was used in the greenhouse from 0900 to 1600 HR on sunny days. The maximum PPF reached 168 and 85 μmol·m⁻²·s⁻¹ on 23 and 24 Apr., respectively (Fig. 2B). The maximum temperature inside the cuvette reached 35.1 and 27.3 °C on 23 and 24 Apr., respectively (Fig. 2B). The net CO₂ exchange rate reached a maximum of 4.0 and 1.6 μmol·m⁻²·s⁻¹ at 2 h after sunrise on both days (Fig. 2C). A higher degree of CAM pattern of *D. officinale* was observed on the sunny day, whereas the duration of positive CO₂ exchange rates during the daytime was longer on the rainy day. The daily net CO₂ exchange amounts were 53 and 44 mmol·m⁻²·d⁻¹ on 23 and 24 Apr., and the dark net CO₂ exchange percentages were 31% and 29%, respectively. These results indicated an intermediate degree of CAM in *D. officinale* under the Jinhua greenhouse conditions compared with the plants in the Beijing greenhouse and growth chamber conditions. These differences in net CO₂ exchange rates during the day and night periods were the result of the differences in temperatures and light intensities.

CO₂ ASSIMILATION PATTERN UNDER DROUGHT AND AFTER REHYDRATION. The relative water content of *D. officinale* leaves

without irrigation for 12 d was 4.7% less than that of well-watered plants on the same day (Table 1) and the net CO₂ exchange rate decreased as the substrate water content decreased over time (Fig. 3). The daily net CO₂ exchange amount on Day 12 was 55% compared with that on Day 1 (Fig. 4). When the plants were rewatered on Day 12, net CO₂ exchange rate increased again instantly. On Day 18, 6 d after rewatering, the daily net CO₂ exchange amount increased by 51% compared with that of Day 12. The percentage of CO₂ exchanges observed during the dark period increased as drought stress increased or as RWC decreased, but decreased again as plants were rewatered (Fig. 4). The percentage of net CO₂ exchange during dark period to the daily amount of CO₂ exchanges on Day 12 was 51%, 189% higher than Day 1. On Day 12, at RWC of 91.8%, the CO₂ assimilation pattern exhibited the typical four phases of a CAM pattern (Fig. 3). After being rewatered, the percentage of net CO₂ exchange during the dark period to the daily amount of CO₂ exchanges again decreased gradually. By Day 18, this percentage decreased by 76% compared with that of Day 12 (Fig. 4). A similar response was reported for *Kalanchoë porphyrocalyx* and *Kalanchoë mimiata* (Brulfert et al., 1996). Drought stress increased CO₂ assimilation in the dark period, representing a short-term response mechanism to compensate for reduced diurnal supply of carbon and maintain a positive carbon balance (de Mattos and Lüttge, 2001; Dodd et al., 2002).

The accumulation of titratable acidity during the dark period for drought-stressed and well-watered *D. officinale* plants did not differ on Day 1 or Day 18. However, drought-stressed plants accumulated 57% more titratable acidity than well-watered plants on Day 12 (Table 1). These results indicate that the titratable acidity accumulation during the dark period increased as substrate water content decreased and decreased again as substrate water content increased after being rewatered. *Talinum triangulare* had a dark titratable acidity accumulation of 6 μmol·g⁻¹ FW when it was in the C3 assimilation pattern, whereas its dark titratable acidity accumulation increased to 100 μmol·g⁻¹ FW when the plant switched to CAM (Herrera

Fig. 4. Daily (total) net CO₂ exchange amount of *Dendrobium officinale* (square) and the daily net CO₂ exchange amount during the dark periods accumulated over 24 h expressed as the proportion of the total daily amount in percentage (triangle, accumulated in 24 h). The plants were well watered on Day 1 (6 July 2012) and irrigation was withheld for 12 d. At the end of the Day 12, the plants were rewatered. Arrow indicates rewatered day. Vertical bars represent SD (n = 2).

et al., 1991). Similarly, *Clusia minor* leaves had more titratable acid accumulation under dry than under wet conditions (Borland et al., 1992, 1998). It is obvious that drought stress induces CAM in facultative CAM species. In *D. officinale*, the gradual decrease in the substrate moisture content provided gradual drought stress, which led to the plant CO₂ assimilation pattern to switch from dominant C3 to CAM. After rewatering, the CO₂ assimilation pattern reversed from CAM to dominant C3.

CO₂ ASSIMILATION PATTERN UNDER DIFFERENT LIGHT-DARK CYCLES. The net CO₂ exchange rate of *D. officinale* increased rapidly after the onset of the light period and decreased again in 4 to 6 h (Figs. 2A and 2C). Therefore, shortening the light-dark cycles from 24 h to 8 h or 12 h may increase the daily net CO₂ exchange rate. When light and dark periods were shortened, net CO₂ exchange rates in the dark period changed from positive to negative (Fig. 5A–C). At the 8-h cycle, there was no positive

Fig. 5. Time course of net CO₂ exchange rates of *Dendrobium officinale* in 8-h (A, 4 h light and 4 h dark), 12-h (B, 6 h light and 6 h dark), and 24-h (C, 12 h light and 12 h dark) cycles. Dark bars in the x-axis indicate the dark period. The dotted lines on top and bottom of the solid line indicate SD (n = 2).

net CO₂ exchange rate in the dark period, and plants exhibited a C3 CO₂ assimilation pattern (Fig. 5A).

The average leaves titratable acidity of *D. officinale* plants grown under a 8-h cycle at 0400, 0800, 1200, 1600, and 2000 HR was $167.4 \pm 5.2 \mu\text{mol} \cdot \text{g}^{-1} \text{FW}$ with small variations among sampling times (data not shown), which indicated that *D. officinale* may have only a C3 pathway under an 8-h cycle.

The effect of light and dark period cycles and length of photoperiod on CO₂ assimilation pattern varied with species and other factors. For example, the tropical species *K. blossfeldiana* exhibited only a CAM pattern during short days (Brulfert et al., 1988). For the subtropical *Portulacaria afra*, long days are more inductive of a CAM pattern in well-watered plants, whereas drought-stressed plants have a typical CAM pattern regardless of photoperiod (Guralnick et al., 1984). More studies are needed for *D. officinale* to identify the optimal light and dark period cycle and length of the photoperiod along with other environmental conditions, which lead to the switching between C3 and CAM pathways.

Su and Zhang (2003) measured the net CO₂ exchange rates of *D. officinale* plants every 2 h using the portable photosynthesis system (LI-6400; LI-COR). They reported that *D. officinale* had a typical CAM on sunny days, C3 on rainy days, and an intermediate pathway between C3 and CAM on cloudy days. In our study, a higher degree of CAM was also observed on a sunny day, whereas the CO₂ assimilation pattern on the rainy day was a low-degree CAM but was somewhat different from that on a sunny day (Fig. 2C). Our results indicated that the CO₂ assimilation pattern of *D. officinale* was affected by the environmental conditions such as temperature and light intensity. In other words, the degree of CAM in *D. officinale* changed and adapted to changing environmental conditions. The intermediate pathway between C3 and CAM reported by Su and Zhang (2003) might indicate the different degrees of CAM as found in our studies. As a result of the unique CO₂ assimilation pattern in *D. officinale*, it is inappropriate to compare with other studies without knowing the exact environmental conditions.

In the growth chamber, the majority of CO₂ exchange was observed in the light period but there was still a small positive amount of CO₂ exchange in the dark period. The CO₂ assimilation pattern of *D. officinale* exhibited concomitant CAM and C3 photosynthetic pathways when grown in the growth chamber. In addition, the accumulation in leaf titratable acidity for CAM plants was observed in plants under well-watered growth chamber conditions (Table 1). In facultative species, CAM may be induced by environmental factors such as high PPF (Maxwell, 2002), temperature (Haag-Kerwer et al., 1992), and the relative air humidity (Schmitt et al., 1988). The dark net CO₂ exchange percentages of the 2 d in the Beijing greenhouse were 10.7 and 12.2 times that in the growth chamber. The degree of CAM in *D. officinale* was different when placed in the Beijing greenhouse with high temperatures and high light intensity conditions and in the relatively stress-free growth chamber. Plants under the high temperatures and high light intensity conditions (greenhouse in this study) had stronger CAM compared with the plants grown in the relatively non-stressed growth chamber conditions.

Changes in CO₂ assimilation pattern and leaf titratable acidity in *D. officinale* plants were similar to those of well-watered facultative CAM plant *K. porphyrocalyx* and *K. mimia* (Brulfert et al., 1996). The CO₂ assimilation pattern of well-watered

facultative CAM plants *T. triangulare* (Herrera et al., 1991), *Guzmania monostachia* (Maxwell et al., 1994), and *Tillandsia utriculata* (Stiles and Martin, 1996) exhibited a C3 pattern (no positive net CO₂ exchange rates in the dark period), different from *D. officinale* in the CO₂ assimilation pattern, but the accumulation of the leaf titratable acidity in the dark period was similar. The accumulation of titratable acidity during the dark period indicates the activity of phosphoenolpyruvate carboxylase (PEPC), whereas net CO₂ exchange rates during the dark period depend on the dark respiration rate and the PEPC CO₂ fixing rate. The positive net CO₂ exchange rates in *D. officinale* during the dark period indicated that dark respiration rate was smaller than the PEPC CO₂ fixation rate, whereas dark respiration rates for *T. triangulare*, *G. monostachia*, and *T. utriculata* were greater than their PEPC fixation rates.

The CO₂ assimilation pattern of *D. officinale* in the relatively stress-free condition was different from typical facultative CAM plants such as *M. crystallinum* (Eastmond and Ross, 1997; Winter and Holtum, 2007) and *C. minor* (Borland et al., 1993) because there were no titratable acidity accumulation in leaves during the dark period when *M. crystallinum* and *C. minor* were well watered. Because leaf titratable acidity accumulated in the dark period, it is difficult to confirm a C3 pathway alone for *D. officinale* under relatively stress-free and a 24-h light-dark cycle conditions as observed in well-watered facultative CAM plants *M. crystallinum* and *C. minor*. For *D. officinale*, C3 and CAM pathways might coexist under relatively stress-free conditions (growth chamber conditions in this study).

Conclusion

Based on the net CO₂ exchanges rates under various conditions and titratable acidity accumulation, *D. officinale* had concomitant CAM and C3 pathways when grown in the relatively non-stressed growth chamber conditions and have higher net CO₂ exchange rates compared with greenhouse conditions with suboptimal temperatures and light intensities. Drought stress and rewatering induced a switch of the CO₂ assimilation patterns from C3 dominant to the CAM pathway and from CAM-dominant to the C3 pathway. Growth of *D. officinale* may be promoted by controlling the growing environment to induce the C3 pathway to increase the daily net CO₂ assimilation amount.

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