Photosynthetic Characteristics of Four Wild *Dendrobium* Species in China

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Abstract. Photosynthetic physiology of *Dendrobium nobile*, *Dendrobium pendulum*, *Dendrobium chrysotoxum*, and *Dendrobium densiflorum* was studied. A bimodal diurnal variation of the net photosynthetic rate (P_n) was observed in the four *Dendrobium* species with the first peak [5.09 to 6.06 $\mu\text{mol}(\text{CO}_2)$ per $\text{m}^{-2}\cdot\text{s}^{-1}$] $\approx$ 1100 HR and the second peak [3.83 to 4.58 $\mu\text{mol}(\text{CO}_2)$ per $\text{m}^{-2}\cdot\text{s}^{-1}$] at 1500 HR. No CO_2 fixation was observed at night. For all four *Dendrobium* species, the light compensation point (LCP) was 5 to 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, light saturation point (LSP) ranged from 800 to 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, apparent quantum yield (AQY) was 0.02, and CO_2 compensation points (CCP) and saturation point (CSP) were 60 to 85 $\mu\text{mol}\cdot\text{mol}^{-1}$ and 800 to 1000 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively. Carboxylation efficiency (CE) values ranged from 0.011 to 0.020. The optimum temperature for photosynthesis was between 26 and 30 °C. The measurement of P_n seasonal variation indicated that July to August had the higher P_n for *Dendrobium* species. Additionally, the chlorophyll *a/b* (Chl *a/b*) ratios of the leaves were 2.77 to 2.89. Measurement of key enzymes in the photosynthetic pathway indicated relatively high Ribulose-1,5-bisphosphate carboxylase (RuBPCase) and glycolate oxidase (GO) activities but very low phosphoenolpyruvate carboxylase (PEPCase) activities. It suggested that these four *Dendrobium* species are typical semishade C₃ plants.

The genus *Dendrobium* is one of the largest genera in Orchidaceae; there are $\approx$ 1500 species around the world. In China there are 74 species and two varieties (belonging to nine sections) and mainly distributed in the mountain ranges of southern and western China (Tsi et al., 1999). Most *Dendrobium* orchids are endangered species and are overexploited as

a result of their ornamental and medicinal values, e.g., *Dendrobium nobile*, *D. pendulum*, *D. chrysotoxum*, and *D. densiflorum* (Chen and Tsi, 1997). These four species all blossom in spring and can be cultivated as ornamental potted plants or used for extraction of polysaccharides and alkaloids from the stems. At present, *Dendrobium* is listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora. There have been considerable efforts in large-scale commercial cultivation of medicinal *Dendrobium* orchids. However, research on the physiology of wild *Dendrobium* species has been scanty, and only a few wild *Dendrobium* species was investigated (Chou et al., 2001; Su and Zhang, 2003a, 2003b; Zhu et al., 2013a, 2013b).

Generally, orchids can be divided into thick-leaved orchids and thin-leaved orchids according to their leaf thickness. The thick-leaved orchids belong to CAM plants, e.g.,

Phalaenopsis (Endo and Ikusima, 1989), *Dendrobium Phalaenopsis* (He and Woon, 2008), and *Cattleya* (Stancato et al., 2002); and the thin-leaved orchids are C₃ plants, e.g., *Dendrobiums* (Zhu et al., 2013a, 2013b). There are fairly extensive studies on the photosynthetic physiology of thin-leaved Oriental *Cymbidium* (Pan et al., 1997; Pan and Ye, 2006). The photosynthetic characteristics of thin-leaved *Oncidium* have also been shown to be a C₃ orchid (He et al., 2011; Li et al., 2002). No C₄ plant was found in Orchidaceae. In our previous studies, photosynthetic characteristics of wild *Dendrobium* (*D. williamsoi*, *D. longicornu*, *D. chrysanthum*, and *D. dixanthum*) in China were reported to be semishade C₃ orchids. There is no conclusive evidence to indicate that the photosynthetic pathway of wild *Dendrobium* species endemic to China belong to the *nobile* type that blossom in spring, and they are different from the CAM orchid *Dendrobium*, which blossom in fall (Khoo et al., 1997). More works are needed to understand the ecophysiological and photosynthetic characteristics of the wild Chinese *Dendrobium* orchids.

The objective of the present investigation is to carry out a systemic study of the photosynthetic characteristics of these four well-known wild *Dendrobium* species (*D. nobile*, *D. pendulum*, *D. chrysotoxum*, and *D. densiflorum*) in China. This study provides useful information for the conservation and rational use of these four endangered orchids.

Materials and Methods

Plant materials. Four wild Chinese *Dendrobium* species (*D. nobile* and *D. pendulum* in Sect. *Eugenanthe*; *D. chrysotoxum* and *D. densiflorum* in Sect. *Callista*) were collected from Yunnan Province in China and cultivated in pots using sawdust as a substrate in a greenhouse kept with 60% to 70% of shade, which provided a maximum mid-day light radiation intensity was $\approx$ 1250 $\pm$ 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The shaded greenhouse was 28 $\pm$ 2 °C in the day and 25 $\pm$ 3 °C at night, and relative humidity (RH) was between 65% and 80%. Plants were fertilized with one-third Hoagland nutrient solution weekly. The fourth mature leaf from the apex was selected for testing of photosynthetic characteristics in all experiments. All determinations were replicated three times.

Leaf structure. The anatomical structure of the leaves was examined under an optical microscope (DM 6000B; Leica, Germany) and a scanning electron microscope (S-3500N; Hitachi, Japan). Preparation of the sample for the scanning electron microscope was as described in the Cytology Laboratory, Institute of Botany, the Chinese Academy of Sciences (1974).

Measurement of photosynthetic characteristics. Various parameters including P_n , stomatal conductance, intercellular CO_2 concentration, transpiration rate (T_r), photosynthetically active radiation (*PAR*), and air temperature were measured simultaneously by an LI-6400 portable photosynthetic system (LI-COR).

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Light-response curve. Measurement was conducted between 0930 HR and 1130 HR. Using the automatic measurement function of the light response curve of the LI-6400 photosynthesis system, the built-in red and blue light sources (6400-02B) were set at a series of *PAR* gradients within the range of 0 to 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the leaf P_n corresponding to each gradient was measured. The corresponding curve was plotted with the paired values of P_n and *PAR*, and the LCP, LSP, and related parameters were obtained. Linear regression was performed on the paired values of *PAR* and P_n below 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the initial slope of the response curve $P_n - PAR$ was the AQY of photosynthesis. For photosynthesis measurement, the CO_2 concentration was set at $380 \pm 10 \mu\text{mol}\cdot\text{mol}^{-1}$, leaf

chamber temperature at $25 \pm 0.5 \text{ }^\circ\text{C}$, and RH at $70 \pm 15\%$.

CO_2 -response curve. The CO_2 concentration was regulated using the injection function (6400-01) of the LI-6400 portable photosynthesis system. The *PAR* was maintained at $800 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature at $25 \pm 0.5 \text{ }^\circ\text{C}$, and RH at $70 \pm 15\%$. The CO_2 concentration gradients were set within the range of 0 to 1500 $\mu\text{mol}\cdot\text{mol}^{-1}$ and the P_n corresponding to each gradient was measured. The corresponding curve was plotted with the paired values of P_n and *PAR*, and the CCP, CSP, and related parameters were acquired. Linear regression was performed on the paired values of CO_2 concentration and P_n below 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the initial slope was the CE of RuBPCase. The

P_n at the CO_2 light saturation point was the regenerating rate of RuBP.

Temperature response curve. The P_n value of each temperature gradient within the range of 18 to 34 $^\circ\text{C}$ was measured from low to high temperature using the leaf chamber temperature adjustment function (6400-13) of the LI-6400 portable photosynthesis system. During the measurements, *PAR* was set at $800 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, CO_2 concentration at $380 \pm 10 \mu\text{mol}\cdot\text{mol}^{-1}$, and RH at $70 \pm 15\%$.

Measurement of P_n diurnal variation and seasonal variation. For measurement of diurnal variation, the P_n and the related parameters were measured from 0700 HR to 1900 HR during 3 sunny days in the middle of May. The P_n seasonal variations were measured under natural conditions at 1000 HR to 1100 HR on

Fig. 1. Electron microscope images of the abaxial surface (A–D) and cross-section (E–H) of leaves of the four *Dendrobium* species. (A, E) *Dendrobium nobile*; (B, F) *Dendrobium pendulum*; (C, G) *Dendrobium chrysotoxum*; (D, H) *Dendrobium densiflorum*.

3 sunny days in the middle of each month from January to October.

Measurement of chlorophyll content. Chlorophyll measurement was done according to methods described by Wintermans and De Mots (1965).

Enzyme extraction and activity determination. Leaves of each *Dendrobium* species were illuminated for 2 h before enzyme extract. The sliced leaf tissues (0.2 g) were grounded in 1.5 mL of pre-cooled 100 mM Tris-HCL buffer solution (containing 10 mM $MgCl_2$, 5 mM mercaptoethanol, 1 mM EDTA, 12.5% glycerol, and 1% PVP, pH7.4) with N_2 in a semimicro Waring blender for 3 min. The homogenates were filtered through four layers cheesecloth and then centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was used for testing enzyme activity of RuBPCase (EC 4.1.1.39), PEPCase (EC 4.1.1.31), and GO (EC 1.1.3.1) (Zhang, 1990).

Determination of protein content. The protein content in the enzyme extracts was determined by Coomassie brilliant blue staining, and the standard curve was plotted using bovine serum albumin.

Fig. 2. Light intensity (A), CO_2 concentration (B), and temperature (C) response curves of the leaves of the four *Dendrobium* species.

Results

Anatomical structure of *Dendrobium* species leaves. *Dendrobium* showed bifacial anatomy with thin leaves $\approx 800 \pm 50 \mu m$ (Fig. 1E–H), suggesting these species were thin-leaved orchids compared with thick-leaved CAM orchids such as *Phalaenopsis amabilis*, which have a leaf thickness of $\approx 3000 \mu m$ (Endo and Ikusima, 1989). The adaxial epidermis cells of the leaves were covered by cuticle with no stomata. These cells were larger and square in shape and formed a single layer of cells $\approx 20 \mu m$ thick, which arranged in an orderly rectangular pattern. The abaxial epidermis cells were irregular in shape and sizes with stomatal intensities ranging from 110 to 130 mm^{-2} . The stomata were elliptical and the guard cell length and width were $\approx 30 \mu m$ and $20 \mu m$, respectively. All stomata were slightly sunken into the leaf epidermis and were covered by a waxy stomatal cover (Fig. 1A–D). The mesophyll tissue was well developed and differentiated into palisade and spongy tissues (Fig. 1E–H). The palisade tissue was composed of one to two layers of orderly arranged cylindrical cells with thickness of $60 \mu m$. The cells in the outer layer were densely arranged, deeply stained, and idioblast-shaped, whereas the cells in the inner layer were lightly stained and contained abundant chloroplasts. The spongy tissue cells were loosely arranged with a layer thickness of $40 \mu m$ and also contained many chloroplasts. The vascular bundles were arranged in a ring and the parenchyma cells in the bundle sheath were relatively small and distinctively arranged, containing no chloroplasts. No Kranz leaf anatomy was observed. Therefore, the anatomical structure of these four *Dendrobium* species indicated they were typical semishade C_3 plants.

Light intensity, CO_2 , and temperature response curves of the *Dendrobium* leaves. The four species exhibited similar P_n – PAR curves (Fig. 2A). Within 0 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, P_n increased linearly, but the increased rates became slow when PAR ranged from 300 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$; the P_n reached its peak at PAR of 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and began to decrease with the increase of PAR . The maximum of P_n , LCP, and LSP for the four *Dendrobium* species was 5 to 6 $\mu mol (CO_2)$ per $m^{-2} \cdot s^{-1}$, 5 to 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 800 to 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively (Fig. 2A; Table 1). Maximum photosynthetic rate reflected leaf photosynthetic efficiency, and *D. chrysotoxum* had the higher photosynthetic efficiency [$5.89 \mu mol (CO_2)$ per $m^{-2} \cdot s^{-1}$] and the highest demand for light, whereas

D. nobile had the lowest photosynthetic potential [$4.38 \mu mol (CO_2)$ per $m^{-2} \cdot s^{-1}$]. Generally, the four *Dendrobium* species have relatively low LSPs and LCPs, which reflect their shade habit. In addition, AQY is a key index for the light use efficiency of leaves that reflects the ability of leaves to use dim light. The AQYs of the four *Dendrobium* species were ≈ 0.02 and no significant differences were observed among the four *Dendrobium* species, which indicated that they had relatively low light use efficiency but relatively high requirements for the light quantum for photosynthesis.

The photosynthetic rates of the four *Dendrobium* species showed almost identical responses to CO_2 concentration (Fig. 2B; Table 1) and their CCPs were between 60 and 90 $\mu mol \cdot mol^{-1}$. For CO_2 in the range of 0 to 700 $\mu mol \cdot mol^{-1}$, the photosynthetic rates of the four species increased rapidly with increasing CO_2 concentration. The P_n peaked at ≈ 9 to 10 $\mu mol (CO_2)$ per $m^{-2} \cdot s^{-1}$ in CO_2 concentrations of 800 $\mu mol \cdot mol^{-1}$, indicating that the CO_2 saturation points of the *Dendrobium* species were in the range of 800 to 1000 $\mu mol \cdot mol^{-1}$. The CEs of the four *Dendrobium* species were ≈ 0.011 to 0.020, showing their ability to low use of CO_2 . High CO_2 concentration significantly increased the photosynthetic rates of these species. Temperature was the main influencing factor for the photosynthetic rate of plants. The P_n for the four *Dendrobium* species increased linearly with a rise in temperature between 18 and 26 °C and peaked at 28 to 30 °C. The maximum P_n of the four *Dendrobium* species were between 4.89 and 5.31 $\mu mol (CO_2)$ per $m^{-2} \cdot s^{-1}$ (Fig. 2C). The P_n decreased when temperature was over 30 °C, indicating that the optimum temperatures for *Dendrobium* photosynthesis were in the range of 26 to 30 °C.

P_n diurnal and annual variation of the four *Dendrobium* species. The diurnal variation in the photosynthetic rates of the four *Dendrobium* species showed the same bimodal curve (Fig. 3). There was an rapid increase of P_n from 700 h, reaching a peak of 5.09 to 6.06 $\mu mol (CO_2)$ per $m^{-2} \cdot s^{-1} \approx 1100$ HR but then decreased thereafter, and dropped to a minimum at 1300 HR to 1400 HR (Fig. 3A). From 1100 HR to 1300 HR, the environmental PAR and temperature rose continuously and exceeded the optimum conditions for *Dendrobium* photosynthesis. The high PAR and temperature exerted stress on the plant resulting in an increase in stomatal resistance, a decrease in CO_2 absorption, and an increased of Tr (Fig. 3B–D), which, in turn, caused “midday depression.” The second P_n

Table 1. Characteristics of light and CO_2 responses of the leaves of the four *Dendrobium* species.

Species	Characteristics of light response			Characteristics of CO_2 response		
	LCP ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	LSP ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	AQY	CCP ($\mu mol \cdot mol^{-1}$)	CSP ($\mu mol \cdot mol^{-1}$)	CE
<i>Dendrobium nobile</i>	5.39 ± 0.21	900 ± 50	0.021 ± 0.003	79.66 ± 5.51	800 ± 50	0.020 ± 0.003
<i>Dendrobium pendulum</i>	7.28 ± 0.17	800 ± 50	0.020 ± 0.001	66.27 ± 6.12	900 ± 50	0.011 ± 0.002
<i>Dendrobium chrysotoxum</i>	5.01 ± 0.34	950 ± 50	0.021 ± 0.003	77.54 ± 4.83	850 ± 50	0.016 ± 0.003
<i>Dendrobium densiflorum</i>	6.14 ± 0.22	850 ± 50	0.021 ± 0.003	84.46 ± 5.48	950 ± 50	0.012 ± 0.001

LCP = light compensation point; LSP = light saturation point; AQY = apparent quantum yield; CCP = CO_2 compensation point; CSP = CO_2 saturation point; CE = carboxylation efficiencies.

peak [3.83 to 4.58 $\mu\text{mol}(\text{CO}_2)$ per $\text{m}^{-2}\cdot\text{s}^{-1}$] began at 1500 HR to 1600 HR, after which P_n rapidly decreased. Furthermore, it showed that the four *Dendrobium* species showed no CO_2 absorption at night at our preliminary experiment (unpublished data). Therefore, these four species were C3 rather than CAM plants.

The P_n of the four *Dendrobium* species had relatively similar seasonal variation patterns (Fig. 4) with the highest value in June [the peak values of *D. nobile*, *D. pendulum*, *D. chrysotoxum*, and *D. densiflorum* were 5.87, 5.51, 6.27, and 6.22 $\mu\text{mol}(\text{CO}_2)$ per $\text{m}^{-2}\cdot\text{s}^{-1}$, respectively]. Measurement of seasonal photosynthesis was carried out from January to October and the photosynthesis rates of these *Dendrobium* species were higher during May to August, which may be correlated with temperature, light intensity, and leaf maturity.

The P_n during March and April was relatively low, which may be the result of the low temperature or immature leaves during this period.

Variation of chlorophyll content and activities of key photosynthetic enzymes. The Chl *a/b* ratios of the four *Dendrobium* species were varied from 2.77 to 2.89 (Table 2). Measurement of enzyme activities (Table 3) indicated that PEPCase activity in the four *Dendrobium* species were very low, all within a range of 1.2 to 1.3 $\text{nmol}(\text{CO}_2)/\text{mg}(\text{protein})/\text{min}$.

Discussion

The photosynthesis of tropical orchids has been studied and documented (Hew and Yong, 1997). Generally thin-leaved orchids are C₃

plants, whereas thick-leaved orchids are CAM plant. To date, no C₄ orchid has been reported (Pan et al., 1997; Pan and Ye, 2006; Ye et al., 1993). The four *Dendrobium* species have bifacial leaf structure and are thin-leaved orchids. No Kranz structure that displayed the characteristics of C₃ plants in the vascular bundle sheath cells was observed (Fig. 1E–H). Furthermore, the photosynthetic characteristics, especially the high CCP and CSP (Table 1) and low PEPCase activity (Table 3), demonstrated that these studied species were C₃ plants and not C₄ or CAM orchids. Our results differ from that of Su and Zhang (2003a, 2003b) who stated that *D. nobile* and *D. officinale* have a facultative CAM photosynthetic pathway (transformation of photosynthesis pathway in the CAM pathway and C₃ pathway) but were similar to our previous results that the four *Dendrobium* species (*D. williamsonii*, *D. longicornu*, *D. chrysanthum*, and *D. dioxanthum*) were C₃ plants (Zhu et al., 2013a, 2013b). The P_n diurnal variation of the leaves of the four *Dendrobium* species showed bimodal curves with a noticeable “midday depression” (Fig. 3A). That phenomenon is a known light defense mechanism that protects the plant’s photosynthetic apparatus from damage resulting from high PAR and temperature (Edwards and Walker, 1985). The LSPs of the four *Dendrobium* species were 40% to 50% (Table 1) of maximum light intensity (1800 to 2200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), indicating that the *Dendrobium* species exhibited characteristics of shade plants. The optimum temperature of photosynthesis for these *Dendrobium* plants ranged from 26 to 30 °C (Fig. 2C), indicating they should be suitable for growing in the subtropical and tropical area. These results are consistent with the optimum temperature range reported for the photosynthesis of *D. chrysanthum* and *D. dioxanthum* (26 to 30 °C; Zhu et al., 2013b). The CO_2 response curves showed that the photosynthetic rates improved with increasing CO_2 concentration, and the photosynthetic rates in CSPs were nearly twice (Fig. 2B; Table 1) as great as that of atmospheric CO_2 concentration (370 $\mu\text{mol}\cdot\text{mol}^{-1}$). Meanwhile, the values of Chl *a/b* ratio (2.77 to 2.89) of these plants (Table 2) also showed the characteristic of C₃ plants (Hew and Yong, 1997). Moreover, the P_n seasonal variation of the four *Dendrobium* species showed that the most vigorous growth was exhibited in May to August with the peak values of *D. nobile*, *D. pendulum*, *D. chrysotoxum*, and *D. densiflorum* being 5.80, 5.28, 6.14, and 6.06 $\mu\text{mol}(\text{CO}_2)$ per $\text{m}^{-2}\cdot\text{s}^{-1}$, respectively (Fig. 4). Finally, PEPCase is a key enzyme for C₄ plants, and its activity has been measured at over 100 $\text{nmol}(\text{CO}_2)/\text{mg}(\text{protein})/\text{min}$ in the leaves of C₄ sugarcane plant (Ye et al., 1993). In our present study, PEPCase is usually very low [1.2 to 1.3 $\text{nmol}(\text{CO}_2)/\text{mg}(\text{protein})/\text{min}$], similar to C₃ plants (Table 3). Those results are consistent with previous findings (Edwards and Walker, 1985) as well as our own previous research on *Cymbidium* species (Ye et al., 1993) and *Dedrobium* species (Zhu et al., 2013a, 2013b). Their relatively high

Fig. 3. Diurnal variation of net photosynthetic rate (P_n) (A), stomatal conductance (g_s) (B), transpiration rate (T_r) (C), and environmental photosynthetically active radiation (PAR) (D) of the leaves of the four *Dendrobium* species.

Fig. 4. Seasonal variation of net photosynthetic rate (P_n) of the four *Dendrobium* species.

Table 2. Variation of chlorophyll content in leaves of four *Dendrobium* species.

Species	chlorophyll content ($\text{mg}\cdot\text{g}^{-1}$ FW)			Chl <i>a/b</i>
	Chl <i>a</i>	Chl <i>b</i>	Total Chl	
<i>Dendrobium nobile</i>	1.28 ± 0.05	0.46 ± 0.02	1.75 ± 0.03	2.78
<i>Dendrobium pendulum</i>	1.27 ± 0.01	0.46 ± 0.02	1.73 ± 0.01	2.77
<i>Dendrobium chrysotoxum</i>	0.95 ± 0.01	0.33 ± 0.05	1.28 ± 0.03	2.89
<i>Dendrobium densiflorum</i>	1.32 ± 0.01	0.45 ± 0.01	1.75 ± 0.02	2.88

FW = fresh weight; Chl = chlorophyll.

Table 3. Variation of the activities of key photosynthetic enzymes in leaves of four *Dendrobium* species.

Species	PEPCase (nmol CO ₂ /mg protein/min)	RuBPCase (nmol CO ₂ /mg protein/min)	GO (μmol glyoxylate/mg protein/min)
<i>Dendrobium nobile</i>	1.3 ± 0.11	33.7 ± 0.33	15.6 ± 0.24
<i>Dendrobium pendulum</i>	1.3 ± 0.08	32.2 ± 0.29	13.8 ± 0.19
<i>Dendrobium chrysotoxum</i>	1.2 ± 0.17	34.3 ± 0.31	15.2 ± 0.17
<i>Dendrobium densiflorum</i>	1.2 ± 0.13	33.4 ± 0.36	14.2 ± 0.26

PEPCase = phosphoenolpyruvate carboxylase; RuBPCase = ribulose-1,5-biphosphate carboxylase; GO = glycolate oxidase.

RuBPCase and GO activities further supported that they were typical C₃ plants with high photorespiration rates, results also similar to our previous studies (Zhu et al., 2013a, 2013b).

This study revealed for the four *Dendrobium* species were C₃ plants, which would help in improving the cultivation techniques for their ex situ conservation as well as increase sustainable production. The correlation between the photosynthetic rate and environmental factors in the leaves of the four *Dendrobium* species indicated that CO₂ concentration, air temperature, and light intensity were very important for the growth of those plants. To clarify systematically the rule of *Dendrobium* blooming in spring photosynthesis pathway, it is necessary to continue to investigate the photosynthetic characteristics of more Chinese *Dendrobium* species that blossom in spring.

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