A photosynthesis continuous monitoring system for CAM plants

Yongsan Cheng, Dongxian He*

(*Key Laboratory of Agricultural Engineering in Structure and Environment in Ministry of Agriculture and Rural Affairs, China Agricultural University, Beijing 100083, China*)

Abstract: Diurnal CO_2 exchanges in crassulacean acid metabolism (CAM) plants are significantly different from those in C3 and C4 plants. The instantaneous short-time CO_2 exchange of a single leaf measured by commercial portable photosynthesis measuring systems with a small leaf chamber cannot reflect the plant photosynthetic capacity for CAM plants because of the CO_2 fixation property. Therefore, a photosynthesis continuous monitoring system with two canopy cuvettes was developed for measuring diurnal net CO_2 exchange rates for CAM plants. To evaluate stability and applicability of the photosynthesis continuous monitoring system, continuous measurement of net CO_2 exchange rates of plants with different photosynthetic pathways were conducted. An obligate CAM plant (*Kalanchoe daigremontiana*), four facultative CAM plants (*Dendrobium officinale, D. chrysotoxum*, *D. nobile*, and *D. primulinum*), a C3 plant (Strawberry, *Fragaria ananassa*), and a C4 plant (Corn, *Zea mays*) were selected as model plants. *K. daigremontiana* had a significant CO_2 absorption during the dark period and its net CO_2 exchange rates fluctuated around 0 μmol/(m^2·s) during the photoperiod in a growth chamber. Net CO_2 exchange rates of *F. ananassa* and *Z. mays* in a greenhouse gradually increased after sunrise, reaching a maximum at about 12:00, and then gradually decreased to negative values during the night time. It is interesting to observe that *D. officinale* in the greenhouse and growth chamber absorbed CO_2 during both day and night times. The photosynthetic pathways of *D. chrysotoxum*, *D. nobile*, and *D. primulinum* were also well distinguished by this photosynthesis continuous monitoring system. The results showed that the photosynthesis continuous monitoring system is capable for quantitative evaluation of diurnal net CO_2 exchange characteristics not only in the CAM plants but also in small size C3 and C4 plants with low net photosynthetic rates for long-time and high-accuracy measurements.

Keywords: diurnal net CO_2 exchanges, infrared CO_2 analyzer, mass airflow meter, net photosynthetic rate, photosynthetic pathway

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1 Introduction

Crassulacean acid metabolism (CAM) is one of the three photosynthetic pathways of vascular plants and special photosynthetic metabolic adaption to environmental stress. This is modified on the basis of C3 pathway, which increases the CO_2 concentration around Rubisco, thereby inhibiting photorespiration. CAM pathway involves a temporal separation of CO_2 fixation from atmosphere predominantly at night time by opening stomata and subsequently assimilating this CO_2 to carbohydrate during day time. During the night time, ambient CO_2 is fixed into malic acid via phosphoenolpyruvate carboxylase (PEPC) and oxaloacetic acid, which accumulates in the mesophyll cells containing chloroplasts. In the following photoperiod, the CO_2 released is subsequently fixed by Rubisco in the Calvin cycle. Decarboxylation of malic acid produces a high intercellular CO_2 concentration, which causes stomatal closure during the day time[1]. The photosynthetic pathways of C3 and C4 plants are known to have different photosynthetic properties from CAM plants[2-4]. C3 and C4 plants show CO_2 fixation during the day time and CO_2 release at night time. Net CO_2 exchange rates of CAM plants in day time are minus or low because of closed stomata or non-uniform stomata closure. The diurnal net CO_2 exchange pattern for CAM plants is more complicated than those of C3 and C4 plants. The photosynthetic characteristics of CAM plants are very difficult to be accurately reflected by the use of small chambers and discontinuous measurements. Continuously measuring the diurnal net CO_2 exchange rates of the whole plant can distinguish the different photosynthetic pathway[5].

Extent of CAM pathway relative to regular C3 pathway is considerably variable. Depending on plant species, ontogeny, and environment, the contribution of nocturnal CO_2 fixed to daily carbon gain can range from nearly 0% to 100%[6]. In its weakest form, the average CO_2 fixation rate may be less than0.1 μmol/(m^2·s), which is challenging to distinguish using conventional commercial portable photosynthesis measuring system[7]. With more biomass or larger leaf area in the leaf chamber, accuracy of photosynthesis measurement at low net photosynthetic rates can be improved[8-14].

Commercial portable photosynthesis measuring systems are developed for measuring short-time net CO_2 exchange rate based on photosynthetic properties of C3 and C4 plants[15-17]. Thus, the photosynthetic capacity of CAM plants is hard to be accurately assessed by net CO_2 exchange rate of a leaf using this kind of instruments. Therefore, a continuous monitoring system with a bigger leaf chamber for photosynthesis determination was developed in this study to continuously measure diurnal net CO_2 exchange rates for CAM plants.

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Biographies: Yongsan Cheng, PhD candidate, research interests: plant environmental physiology, Email: 348522460@qq.com.

***Corresponding author**: **Dongxian He**, PhD, Professor, research interests: plant environmental physiology and bio-environment engineering. Key Lab. Agricultural Engineering in Structure and Environment in Ministry of Agriculture and Rural Affairs, China Agricultural University, Beijing 100083, China. Tel/Fax: +86-10-62737550, Email: hedx@cau.edu.cn.

Dendrobium is the second largest genera of Orchidaceae plants with 1500 to 1600 native species, mainly distributed in tropical Asia and Pacific islands. There are about 76 species in China and one-sixth of them are used medicinally with high economic value[18]. Several *Dendrobium* species as important ornamental plants and medicinal plants were used for photosynthesis research and industrial application[19-23]. Many *Dendrobium* species have evolved into CAM photosynthetic pathways in adapting to harsh epiphytic environment[24]. Su and Zhang measured the diurnal net CO_2 exchange rates of *D. officinale* under various weather conditions and found that it had a CAM photosynthetic pattern on sunny days, a C3 photosynthetic pattern on rainy days, and an intermediate photosynthetic pattern between CAM and C3 on cloudy days[21]. *D. officinale* was identified as an obvious facultative CAM plant by continuously measuring the diurnal net CO_2 exchange rate during water stress and different light/dark cycles[25]. *D. chrysotoxum* and *D. nobile* showed C3 photosynthetic pathway under well-watered conditions and switched from C3 pathway to CAM photosynthesis under drought stress; thus, these two species were facultative CAM plants. Yang et al. reported that diurnal net CO_2 exchange rate in *D. primulinum* had four distinct phases in the 24-h CAM photosynthetic cycle[28]. However, most of the above results were based on portable photosynthesis measuring instruments. Therefore it requires further confirmation with a novel photosynthesis continuous monitoring system.

2 Materials and methods

2.1 System description

A photosynthesis continuous monitoring system consists of a host computer box, two cuboid acrylic canopy cuvettes, four gas circuit pipes, two communication lines connected to two photosynthetic photon flux density (PPFD) sensors and two communication lines connected to two temperature control modules (Figure 1) . It was developed to measure diurnal net CO_2 exchange rates for CAM plants. The plant shoots are enclosed in the cuvettes while the roots and substrate are excluded. The host computer box consists of an infrared CO_2 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 International, Morristown, NJ, USA), one temperature control module for cuvette temperature control, and an embedded computer. Two of the mass airflow meters are connected to the two cuvettes and the other one is connected to the CO_2 analyzer. Each cuvette with 250 mm × 150 mm × 60 mm is equipped with a small fan for mixing air inside. The airflow is controlled by an air pump forcing the airflow from inlet to outlet of the cuvette. A quantum sensor (LI-190SA, LI-COR Inc., Lincoln, NE, USA) is fitted at the upside of each cuvette for measuring the PPFD. The temperature inside the cuvette is controlled by the temperature control module based on either atmospheric temperature tracking mode or fixed temperature control mode.

The net CO_2 exchange rate, μmol/(m^2·s), for plants inside the cuvette is calculated as follows:

$$\text{Net } CO_2 \text{ exchange rate}=k\times(C_{\text{inlet}}-C_{\text{outlet}})\times F/LA$$

where, k is the conversion coefficient, mol/L; C_{inlet} and C_{outlet} are CO_2 concentrations at inlet and outlet of the cuvette, respectively, μmol/mol; F is airflow rate, 1.0 L/min; and LA is leaf area, m^2. The LA was determined according to Yang et al.[26]

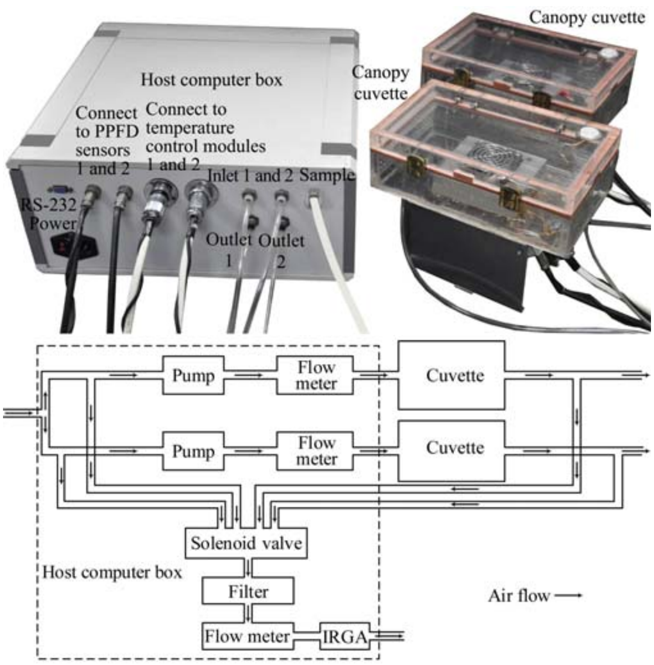


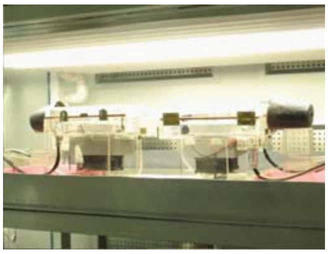
Figure 1 System composition and schematic diagram of gas circuit in the photosynthesis continuous monitoring system

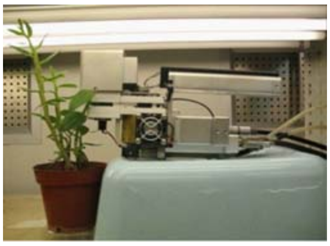
2.2 Plant cultivation and net CO_2 exchange rate measurements of plants with different photosynthetic pathways

To evaluate the stability and applicability of the photosynthesis continuous monitoring system, net CO_2 exchange rates of four species with different photosynthetic pathways were continuously measured in two different greenhouses and a growth chamber for two days (Figure 2). An obligate CAM plant (*Kalanchoe daigremontiana*), a facultative CAM plant (*D. officinale*), a C3 plant (Strawberry, *Fragaria ananassa*), and a C4 plant (Corn, *Zea mays*) were selected for evaluating the system stability. *F. ananassa*, *Z. mays*, and *K. daigremontiana* plants were cultivated in a greenhouse located at College of Water Resources and Civil Engineering, China Agricultural University, Beijing, China. The range of maximum PPFD of each day during the measurement was 600-620 μmol/(m^2·s). The range of temperature, relative humidity, and CO_2 concentration in the greenhouse were 14°C-29°C, 24%-53%, and 310-650 μmol/mol, respectively. The plants were grown in 0.4-L round plastic pots (two plants per pot) filled with a substrate mixture of composted small pine bark (3-8 mm), medium pine bark (5-10 mm), pine bark powder, perlite, and composted sawdust in a volume ratio of 1:1:1:1:0.5. The *D. officinale* were cultured under artificial lighting with 12 h/d photoperiod and 160 μmol/(m^2·s) PPFD provided by fluorescent lamps in a walk-in growth chamber at the same building. Inside the growth chamber, the average air temperature was controlled at 25.0°C±1.0°C, relative humidity was controlled at 65%±5%, and CO_2 concentration was controlled at 500±50 μmol/mol. The plants in both greenhouse and growth chamber were watered with nutrient solution once every 2 d or 3 d as the plant needed.

When *F. ananassa*, *Z. mays*, and *K. daigremontiana* plants had 8, 4 and 6 leaves, respectively, their net CO_2 exchange rates were measured using the photosynthesis continuous monitoring system in the greenhouse. When the *D. officinale* plants had 12 leaves, their net CO_2 exchange rate was measured using a fluorescence leaf chamber (6400-40, LI-COR Inc., Lincoln, NE, USA) and a conifer leaf chamber (6400-05, LI-COR Inc., Lincoln, NE, USA) controlled by a portable photosynthesis measuring system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA) in the growth chamber. The measurement of net CO_2 exchange rate for *D. officinale* was also conducted by the photosynthesis continuous monitoring system in the growth chamber and in an unheated commercial greenhouse located in Jinhua City, Zhejiang Province, China. The annual average air temperature in Jinhua greenhouse was 17.5°C, the lowest and highest air temperatures were –2.8°C and 38.9°C, respectively. The range of maximum PPFD of each day during measurement was 100-360 μmol/(m^2·s) (Figure 2). The range of temperature, relative humidity, and CO_2 concentration in the greenhouse during measurement were 7°C-22°C, 30%-66%, and 316-476 μmol/mol, respectively.


a. Photosynthesis continuous monitoring system in the greenhouse


b. Photosynthesis continuous monitoring system in the growth chamber


c. Portable photosynthesis measuring system with a fluorescence leaf chamber in the growth chamber

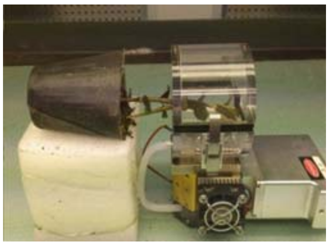

d. Portable photosynthesis measuring system with a conifer leaf chamber in the growth chamber

Figure 2 Photosynthesis measurements of *Dendrobium officinale*

2.3 Continuous measurements of net CO_2 exchange rates for photosynthetic pathway determination of *Dendrobium* plants

D. chrysotoxum, *D. nobile*, and *D. primulinum* plants from Puer City, Yunnan Province, China were cultured in the same growth chamber for three months. The cultured environment conditions and irrigation management were the same as those mentioned above for *D. officinale*. When they have 10 leaves, the diurnal net CO_2 exchange rates of these three *Dendrobium* species were measured by the photosynthesis continuous monitoring system in the growth chamber for 3 days. The diurnal net CO_2 exchange rates of *K. daigremontiana* with 6 leaves as an obligate CAM plant were also simultaneously measured by the same system as a reference. The photosynthetic pathways of *Dendrobium* plants were determined by dark net CO_2 exchange percentage which was calculated by the formula[25]:

$$\text{Dark net } CO_2 \text{ exchange percentage} = \frac{\text{Dark net } CO_2 \text{ exchange amount}}{\text{Daily net } CO_2 \text{ exchange amount}} \times 100\% \qquad (1)$$

where, dark net CO_2 exchange amount was defined as the integrated net CO_2 exchange amount of the dark period, and daily net CO_2 exchange amount was defined as the integrated CO_2 exchange amount of 24 h.

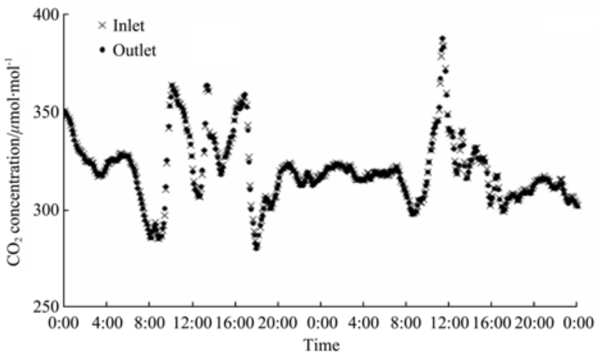
3 Results and discussion

3.1 Performance tests of the photosynthesis continuous monitoring system

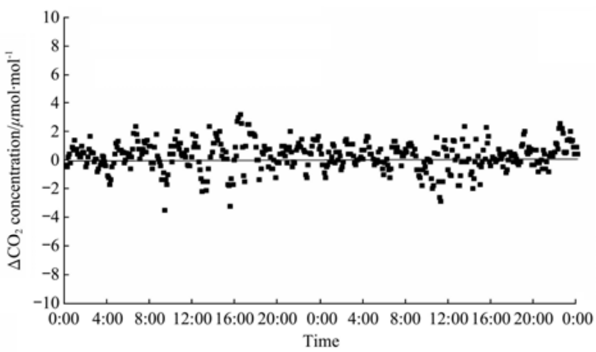
Net CO_2 exchange rates of the empty cuvettes were measured before and after each photosynthesis measurement. The CO_2 concentrations in the inlet and the outlet were close to each other (Figure 3a). The noise level which is the difference in CO_2 concentrations between inlet and outlet of the empty cuvette, was about ±3.3 μmol/mol (Figure 3b). Therefore, the photosynthetic capacity of plants could be truly reflected by the photosynthesis continuous monitoring system only if the CO_2 concentration difference between the inlet and outlet of the cuvette was greater than 3.3 μmol/mol. The cuvette temperature was controlled at 25.2°C±0.3°C by using the fixed temperature control mode (Figure 4a) with a target of 25.0°C or well controlled by temperature tracing mode (Figure 4b). The fixed temperature control mode was of particular importance for studying the endogenous rhythms of CAM plants under constant environmental conditions[27]. The temperature tracing mode was particularly important for studying the response and adaptation of CAM plants to changes in the surrounding environment[28]. Therefore, the photosynthesis continuous monitoring system could be used to measure diurnal CO_2 exchange rates for CAM plants owning to the high detection accuracy and independence of its systematic error.

3.2 Continuous measurements of net CO_2 exchange rate of plants with different photosynthetic pathways

The *K. daigremontiana* plants had a large amount of net CO_2 absorption during the dark period with a peak value at about 04:00 and fluctuated around 0 μmol/(m^2·s) during the photoperiod (Figure 5). Net CO_2 exchange rates of *F. ananassa* and *Z. mays* plants gradually increased after sunrise and reached a maximum at about 12:00 which were 6.2 μmol/(m^2·s) and 11.2 μmol/(m^2·s), respectively, and then gradually decreased to a negative value in the night time. These results showed that the photosynthesis continuous monitoring system was suitable not only for quantitative evaluation of the diurnal net CO_2 exchange characteristics for CAM plants, but also for C3 and C4 plants for a long-time measurement because of the diurnal automatic monitoring and higher measurement accuracy.

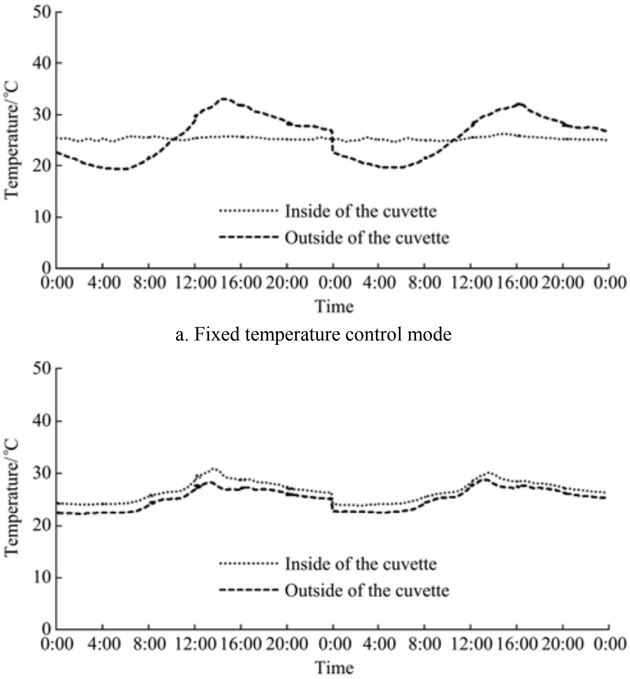


a. CO_2 concentration in the inlet and outlet of cuvette



b. ΔCO_2 concentration in the inlet and outlet of cuvette

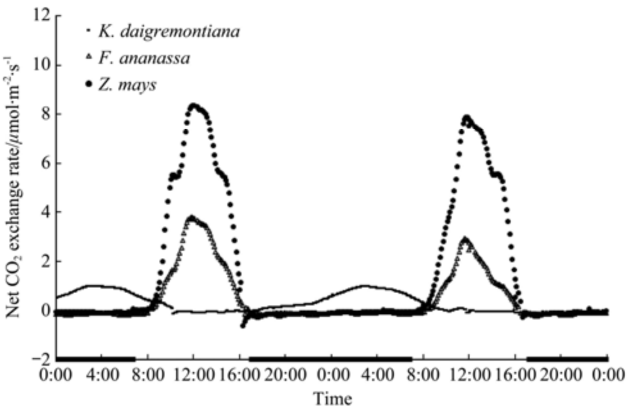
Figure 3 Time course of CO_2 concentration and ΔCO_2 concentration in the inlet and outlet of cuvette (the photosynthesis continuous monitoring system was supplied by compressed air for 2 d)



a. Fixed temperature control mode

b. Atmospheric temperature tracking mode

Figure 4 Time course of temperature in outside and inside the cuvette of the photosynthesis continuous monitoring system by using fixed temperature control mode with a target at 25.0°C and atmospheric temperature tracking mode

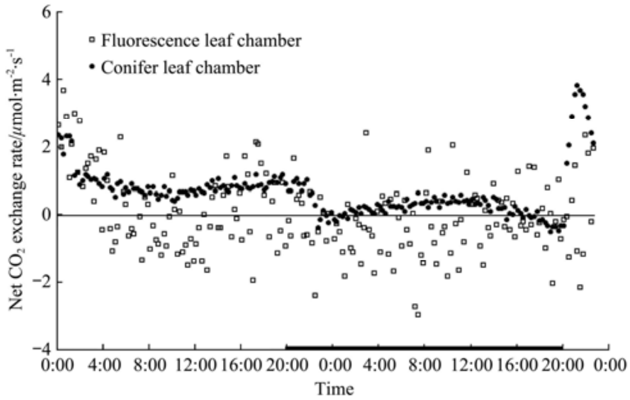


Note: The atmospheric temperature tracking mode was used. Dark bar in the *x*-axis indicates the dark period in the greenhouse.

Figure 5 Time course of net CO_2 exchange rates of *K. daigremontiana* as an obligate CAM plant, *F. ananassa* as a C3 plant, and *Z. mays* as a C4 plant measured continuously by the photosynthesis continuous monitoring system in Beijing greenhouse for 2 d

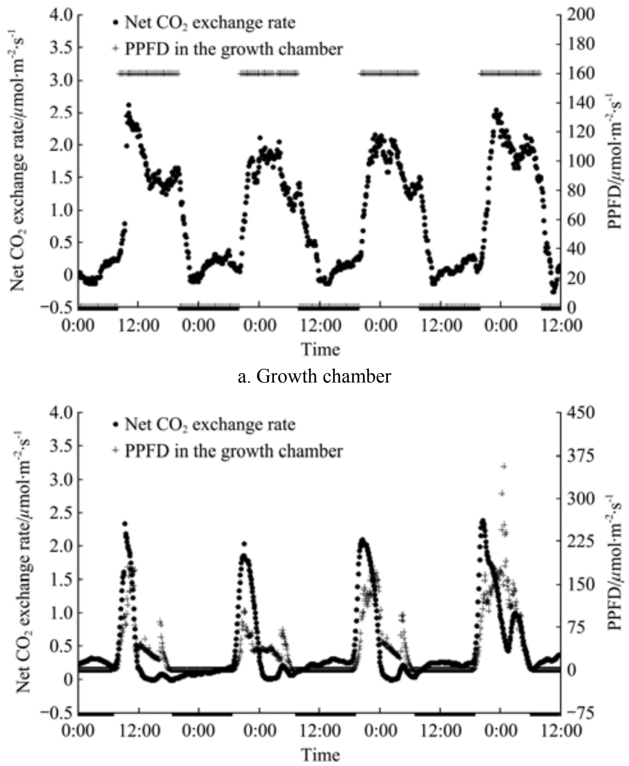
3.3 Continuous net CO_2 exchange rate measurements of *D. officinale* as a facultative CAM plant

When measured every 3 min by fluorescence leaf chamber of a portable photosynthesis measuring system (LI-6400XT), net CO_2 exchange rates of a single leaf between –2.0 μmol/(m^2·s) to 4.0 μmol/(m^2·s) with large fluctuations were observed for *D. officinale* as a facultative CAM plant in the growth chamber (Figure 6). It was not possible to judge whether the net CO_2 exchange rates were positive or negative due to the large variations. By using a conifer leaf chamber controlled by the same portable photosynthesis measuring system with multiple leaves enclosed, the net CO_2 exchange rates had smaller fluctuations with low value less than 2.0 μmol/(m^2·s). However, with the photosynthesis continuous monitoring system in the growth chamber, the net CO_2 exchange rates of the whole plant had a peak value at 2.5 μmol/(m^2·s) and decreased to stable value at 1.5 μmol/(m^2·s) (Figure 7a). The diurnal net CO_2 exchange rates with fluctuating PPFD could also be well measured in the greenhouse. The net CO_2 exchange rate of *D. officinale* firstly had a sharp increase with the PPFD in the morning, then decreased to almost 0 μmol/(m^2·s) after 12:00, and had a small peak in the afternoon (Figure 7b). The net CO_2 exchange rates of *D. officinale* in the growth chamber and greenhouse were positive by this photosynthesis continuous monitoring system (Figure 7). These results indicated that increasing leaf area or biomass within the cuvette could improve the measurement accuracy for species with low net CO_2 exchange



Note: Dark bar in the *x*-axis indicates the dark period in the growth chamber.

Figure 6 Time course of net CO_2 exchange rate of *D. officinale* measured by the portable photosynthesis measuring system (LI-6400XT) with a conifer chamber for multiple leaves and a fluorescence chamber for a single leaf



a. Growth chamber

b. Greenhouse

Note: The atmospheric temperature tracking mode was used. Dark bar in the *x*-axis indicates the dark period in the growth chamber and greenhouse respectively.

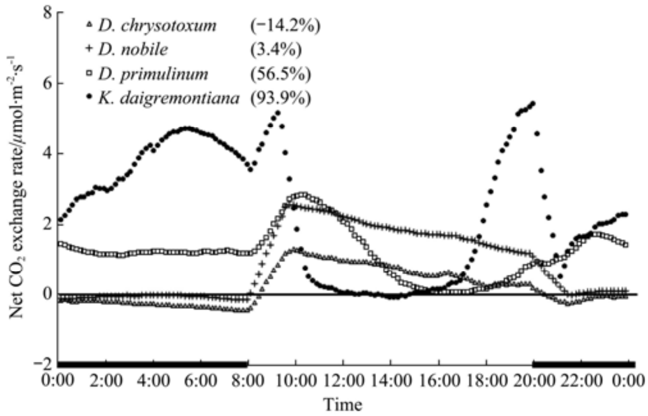
Figure 7 Time course of net CO_2 exchange rates of *D. officinale* measured continuously by the photosynthesis continuous monitoring system in the growth chamber and greenhouse for 4 d rates such as *D. officinale*. From the non-continuously measured net CO_2 exchange rates of *D. officinale* by the portable photosynthesis measuring system, the photosynthetic pathway of CAM plants could not be accurately determined because of the small leaf chamber of the instrument and the stomata opening property of the CAM plants[18].

Based on continuous measurement of diurnal net CO_2 exchange rates, the photosynthetic characteristics of CAM plants in CO_2 absorption during the night time could be obviously reflected (Figure 7). The photosynthesis continuous monitoring system not only solved the problem of low net CO_2 exchange rates of *D. officinale* plants with a relatively sharp instrumental noise, but also reduced sampling error. Moreover, the commercial portable photosynthetic measuring system generally measures a small portion of a leaf, which cannot represent the photosynthetic characteristics of the whole plant.

3.4 Continuous net CO_2 exchange rate measurements for photosynthetic pathways determination of other *Dendrobium* plants

Diurnal net CO_2 exchange rates of *D. chrysotoxum* were similar to those of C3 plants, which featured negative values in the dark period. The net CO_2 exchange rate of *D. nobile* during the dark period was around 0 μmol/(m^2·s), and that in photoperiod was positive (Figure 8). Net CO_2 exchange rate of *D. primulinum* showed obvious four-phase changes in photosynthetic characteristics in typical CAM plants, namely, the net CO_2 uptake in the dark period (phase I), the rise at the beginning of the photoperiod (phase II), almost zero in the middle of the photoperiod (phase III), and the rise in the late photoperiod (phase IV). The dark net CO_2 exchange percentages were more50% for *D. primulinum*, negative value for *D. chrysotoxum*, and close to 0% for *D. nobile*, respectively. The net CO_2 exchange rates of *K. daigremontiana* as the obligate CAM plant were around 0 μmol/(m^2·s) most of the time in the photoperiod and had positive values in the dark period, while the phase Ⅳ was not obvious. The dark net CO_2 exchange percentage of *K. daigremontiana* reached more than 90%.

Based on the proportion of daily net CO_2 fixation occupied by dark net CO_2 fixation, different extent of CAM plants could be distinguished easily[22,30]. This indicated that the photosynthetic pathway of *D. chrysotoxum* should match C3 property in this environment and the *D. nobile* was mainly in C3 property with a weak CAM feature. However, *D. chrysotosum* could not be considered as a C3 plant, because many facultative CAM plants usually showed C3 pathway under well irrigated conditions, and only under drought stress could they show CAM pathway[31-33]. More accurately, *D. nobile* at this time was closer to the "Nearly-C3" or "CAM cycling" type[34]. The photosynthetic pathway of *D. primulinum* was mainly in CAM property with some C3 feature (Figure 8). These results were in consistence with findings of Ren's team by measuring the activity of phosphoenolpyruvate carboxylase (PEPC)[35]. *K. daigremontiana* exhibited a full CAM pathway. Compared to the results of three *Dendrobium* plants obtained by portable photosynthetic systems[29], this continuous photosynthetic system could more accurately evaluate the photosynthetic pathway of *Dendrobium* plants. Therefore, *Dendrobium* plants with different extents of CAM property could be well distinguished by this photosynthesis continuous monitoring system. The dark net CO_2 exchange percentage can be used to evaluate quantitatively the CAM photosynthetic property and distinguish easily the obligate or facultative CAM plants.



Note: The atmospheric temperature tracking mode was used. Dark bar in the *x*-axis indicates the dark period in the growth chamber. The numbers in the legend are the averages of dark net CO_2 exchange percentage of 3 days for these four species.

Figure 8 Diurnal net CO_2 exchange rates of *D. chrysotoxum*, *D. nobile*, *D. primulinum*, *and K. daigremontiana* measured by the photosynthesis continuous monitoring system in the growth chamber

4 Conclusions

The diurnal net CO_2 exchange rates measurement is suitable not only for CAM plant, but also for small size C3 and C4 plants with low photosynthetic rates due to the diurnal automatic monitoring and higher accuracy by using the photosynthesis continuous monitoring system. The system is also feasible to evaluate quantitatively CAM photosynthetic property and to distinguish easily the obligate or facultative CAM plants by using the dark net CO_2 exchange percentage.

Acknowledgements

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