Full Length Research Paper

CO₂ uptake patterns in *Phalaenopsis amabilis*

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A whole-plant open-system chamber was developed to measure CO₂ exchange in *Phalaenopsis amabilis*. The consistent and continuous curves of CO₂ gas exchange rhythms at various conditions, sampled at 1-min interval, were used to evaluate the factors affecting on the CO₂ exchange pattern. The influencing factors included the day/night temperature, sample size, light period, light intensity and water stress. The adequate growth conditions of *P. amabilis* were 26 to 30° C day temperature, 20 to 22° C night temperature, 14 to 16 h light and 180 to 215 µmol m⁻² s⁻¹ of light intensity. Water stress had the most significant effect on the CO₂ exchange pattern. The gas exchange measurement system developed in this study is a useful and reliable measurement system for studying the physiological characteristics of *Phalaenopsis* varieties.

Key words: CO₂ pattern, open-system, *Phalaenopsis*, CAM plant, water stress, light rhythms.

INTRODUCTION

Because of the richness of cultivars and favorable climate in Taiwan, *Phalaenopsis* is the most important plant of the country’s flower industry. The greatest concentration of species and hybrids is Philippine Islands. As a member of the Orchidaceae, *Phalaenopsis* has the characteristics of rapid growth and short juvenile periods, easy-to-control spiking and flowering, stylish and exotic shape, various sizes of blossoms and long-term flowers. The global production has been increasing over the years. However, many culture problems still existed, such as what is the adequate climate for the culture and flowering of these orchids?

From the study of temperature effect on the growing and flowering of *Phalaenopsis*, the different optimum temperatures were recommended by researchers (Sakanishi et al., 1980; Ota et al., 1991; Ichihashi et al., 2008; Lootens and Heursel, 1998). These inconsistent results indicate the importance of the physiological monitoring of *Phalaenopsis*. Study of the CO₂ uptake pattern provides an opportunity to observe the factors affecting the CO₂ uptake rhythms in the vegetative phase.

CO₂ uptake patterns have been studied by many researchers. Kano and Naitoh (2001) measured the CO₂ uptake of *Phalaenopsis* leaves by placing each leaf into an assimilation box with the environment controlled by a walking chamber. A sample container was developed by Endo and Ikasima (1989) to measure the diurnal rhythm of photosynthesis for the leaf and root of *Phalaenopsis*. A novel delicate assimilation cuvette was used to record the daily course of CO₂ exchange in the florets, flower stalk and leaves of *Phalaenopsis* (Endo and Ikasima, 1992); the authors found that the stalk had the carbon fixation ability and a weak CAM characteristic. Goh et al. (1984) observed CO₂ uptake of the young and nature leaves of an orchid hybrid *Arachnis maggie* Oei. Samples were placed in a cylindrical leaf chamber and the CO₂ exchange was measured with an infra-red gas analyzer. Lootens and Heursel (1998) studied the factors influencing the photosynthesis in *Phalaenopsis* hybrids by the leaf gas-exchange system. Samples were placed in curettes and the CO₂ exchange was detected with an IRGA infrared gas analyzer.

Two methods are used to measure the CO₂ uptake pattern in plants. The first is to place parts of the plant (usually leaves) into a leaf chamber or cuvette and detect the CO₂ change inside closure. This technique has been adopted to study the photosynthetic characteristics of...
Plant materials

Phalaenopsis amabilis was studied by using 14 months large plants with five and half leaves and grown in 12 cm pots. The medium plants were 9 months old with four and half leaves and grown in 9 cm pots and small plants were 5 months with three and half leaves old and grown in 7.5 cm pots. The plastic pots were filled with sphagnum moss. These plants of different sizes were only used to test the effect of plant size. The other experiments involved large samples. All plants were placed in benches in a greenhouse with a photosynthetic photon flux (PDF) ranging from 230 (Jan.) to 275 (July) μmol m$^{-2}$ s$^{-1}$. The temperature of greenhouse ranged from 20 to 30°C. The plants were fertilized with 20N-20P-20K soluble fertilizer (Hyponex Crop., Marysville, OH). The moisture content of the medium was measured by a WET meter (Delta-T, Delta Co., UK). When the moisture content was below 15% (wet basic), plants were irrigated with deionized water.

CO$_2$ exchange measurement

A sample chamber was designed according to the criteria of Alterio et al. (2006). The size of this chamber was 40 x 50 x 56 cm. Two holes for the air inlet and outlet were prepared. Two whole-plant orchid pots were easily placed inside the chamber. The inlet and outlet sensing containers connected to the air entry and exit tubes. The CO$_2$, temperature and flow rate meters were placed in the sensing container. The schematic of this measurement system was presented in Figure 1. The CO$_2$ exchange measurement system was placed in a growth chamber to control the ambient air temperature, RH and the photon flux density (PFD, 300 to 850 nm). The accuracy of the controlling performance for the growth chamber was ±0.2°C and ±4.0% RH. The PFD could be adjusted from 60 to 220 μmol m$^{-2}$ s$^{-1}$. The relative humidity of growth chamber was maintained at 70 to 90%.

Before the data recording, samples were placed in the sample chamber maintained at the same setting environments for two days. The total recording periods were two days. Because a day was the period unit, there were two replicates for each treatment. Two plants were placed in a chamber each time and two repetitions for each treatment. The CO$_2$ uptake rate was calculated as the change of CO$_2$ concentrations divided to the total leaf area at each time unit.

Measurement sensors

All the measurement sensors and their specifications were listed in Table 1. All sensors were connected to a data logger (Delta-T device LTP, UK) to continuously record measurement signals at 1 min intervals.

Leaf area measurement

The leaf area of Phalaenopsis of this study was measured by a nondestructive technique (Chen and Lin, 2004). After measuring the length and the maximum width of the leaf, then the leaf area was calculated by the allometric relation (Area = 0.7221 x leaf length x maximum leaf width).

Experimental design

The experiment design of this study was listed in Table 2.

RESULTS

Daily rhythms CO$_2$ fixation

The typical repetitions of the diurnal CO$_2$ pattern of P. amabilis at two different day and night temperatures are
indicated that the detection device developed in this study could be adopted for measuring the CO\(_2\) uptake pattern. Data were sampled every minute. The data are presented in 5 min interval in following figures. The continuous data distribution provided useful information for further analysis.

The daily course of fixation and release of CO\(_2\) at 24/20°C and 28/23°C are presented in Figure 3. The CO\(_2\) uptake pattern was similar to that of a CAM plants. There are four phases for this CAM species. In phase I, the dark period, the maximal CO\(_2\) uptake was 6.1 µmol m\(^{-2}\) s\(^{-1}\) at 20°C (Figure 3a) and 7.3 µmol m\(^{-2}\) s\(^{-1}\) at 23°C (Figure 3b). The CO\(_2\) uptake pattern at 23°C showed a sharp decrease and then increased. Phase II was the beginning of the light period. The consumption of CO\(_2\) by photosynthesis was less than that of the production by photo-respiration, so the CO\(_2\) uptake was decreased. At

![Diagram of the measurement system](image)

**Figure 1.** The schematic of the measurement system. F, air flow rate meter; T, temperature meter; C, CO\(_2\) meter; P, photon flux density meter; RH, relative humidity meter.

**Table 1.** Specification of the measurement sensors.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CO(_2) concentration</th>
<th>Photon flux density meter</th>
<th>Temperature</th>
<th>Relative humidity</th>
<th>Air flow rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensing element</td>
<td>Infrared-ray source</td>
<td>Quantum sensor</td>
<td>PT-100</td>
<td>THP-B7J sensor</td>
<td>Top-Trak 8255 sensor</td>
</tr>
<tr>
<td>Measuring range</td>
<td>0-2000 ppm</td>
<td>0-1000 µmol m(^{-2}) s(^{-1})</td>
<td>0-100°C</td>
<td>0-100%RH</td>
<td>0-1.0 ms(^{-1})</td>
</tr>
<tr>
<td>Accuracy</td>
<td>± 20 ppm</td>
<td>± 3%</td>
<td>± 0.15°C</td>
<td>± 0.7%</td>
<td>± 1.5%</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Vasala GMP 20 transmitter (Vaisala, Finland)</td>
<td>LI - 190 SA quantum sensor (Li-cor Co, NE USA)</td>
<td>Shinyei Kaisha co. Tokyo Japan</td>
<td>Shinyei Kaisha co. Tokyo Japan</td>
<td>Sierra Instrument USA</td>
</tr>
</tbody>
</table>
Table 2. Experimental design of CO$_2$ uptake patterns in Phalaenopsis amabilis.

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>Day/night Temperature (°C)</th>
<th>Sample size</th>
<th>Light Period (h)</th>
<th>Light intensity (µmolm$^{-2}$s$^{-1}$)</th>
<th>Drought Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day/night temperature</td>
<td>Eight treatment: 20/18, 22/19, 24/20, 26/21, 28/22, 23/24, 32/24, and 34/25</td>
<td>Large size</td>
<td>14</td>
<td>180</td>
<td>None</td>
</tr>
<tr>
<td>Sample size</td>
<td>Four treatments: 20/18, 22/19, 28/22, and 30/23</td>
<td>Large, Medium, Small</td>
<td>14</td>
<td>180</td>
<td>None</td>
</tr>
<tr>
<td>Light period</td>
<td>28/22</td>
<td>Large</td>
<td>6, 10, 12, 14, 16, and 18</td>
<td>180</td>
<td>None</td>
</tr>
<tr>
<td>Light intensity</td>
<td>28/22</td>
<td>Large</td>
<td>14</td>
<td>60, 100, 140, 180, and 220</td>
<td>None</td>
</tr>
<tr>
<td>Drought treatment</td>
<td>28/22</td>
<td>Large</td>
<td>14</td>
<td>180</td>
<td>Drought treatment for 22 days.</td>
</tr>
</tbody>
</table>

the phase III, the CO$_2$ uptake reached the equilibration. However, phase III period was 2 h at 24°C (Figure 3a) and 40 min at 28°C (Figure 3b). At the phase IV, the stomata reopened and C$_3$ photosynthesis occurred. The CO$_2$ uptake was gradually increased.

**Effect of plant size**

The CO$_2$ uptake patterns of the three different plant sizes are presented in Figures 4a and b. The quantifications of CO$_2$ uptake was checked by integrating the total CO$_2$ uptake values and found they were not affected significantly by the plant size. In the dark period of 20°C, the maximal CO$_2$ uptake was 6.2 µmol m$^{-2}$ s$^{-1}$ for large plants, 6.52 µmol m$^{-2}$ s$^{-1}$ for medium plants and 6.85 µmol m$^{-2}$ s$^{-1}$ for small plants, respectively. The same trend occurred under could 22/19, 28/22 and 30/23°C conditions.

**Effect of day and night temperature**

The effect of day temperature on the CO$_2$ uptake pattern is shown in Figure 5. The day temperature had no clear effect on the phase II and III. The phase II and III periods were nearly 6 and 2 h. However, the CO$_2$ uptake patterns at phase IV were affected by the day temperature. The maximum CO$_2$ uptake was found at three day temperatures, 26, 28 and 30°C. The lowest CO$_2$ uptake was found at the lower temperature from 18 to 22°C.

The effect of night temperature in the phase I is presented in Figure 6. The night temperature had a significant effect on the CO$_2$ uptake pattern in the early 4.5 h. At the lower temperature, from 17 to 20°C, distribution of the uptake was smooth. However, the pattern was decreased sharply and then increased gradually when the night temperature was higher than 21°C. When temperature was higher than 24°C, a release of CO$_2$ pattern was observed.

**Effect of light period**

The effects of light period on the CO$_2$ uptake pattern are shown in Figure 7. When the light period was maintained for 6 h, the phase IV pattern changed completely. A CO$_2$ release was found. If the plants received 10 h light irradiation, the CO$_2$ uptake decreased sharply then increased at phase IV. When light period longer than 12 h, the CO$_2$ uptake patterns in the light periods of 14 and 18 h were similar. The maximum CO$_2$ uptake was found in the light periods of 14 h.

The CO$_2$ uptake pattern of 10 days’ continuous dark treatment is shown in Figure 8. In this condition of no light energy, the CO$_2$ uptake pattern was continuous. Four phases still existed in the dark period. The CO$_2$ of the
Figure 2. The repetitions of the diurnal CO$_2$ pattern of Phalaenopsis amabilis at 22/19 and 24/20 °C day/night temperature. The relative humidity and the light intensity of the growth chamber were maintained at 70-90% and 180 μmol m$^{-2}$ s$^{-1}$. The light period was the first 14 h.

ambient air was still absorbed and released by the plant in the dark environment.
The CO$_2$ uptake pattern in the continuous light environment is presented in Figure 9. No fixed CO$_2$ could be found. The released of CO$_2$ increased in the first day and had the same pattern after 5 days’ light period.
Effect of light intensity

The CO$_2$ uptake pattern of samples at five different light intensities is shown in Figure 10. The light intensity had an effect on the CO$_2$ uptake pattern. However, the data distribution at 180 and 215 µmol m$^{-2}$ s$^{-1}$ was similar. With the light intensity lower than 140 µmol m$^{-2}$ s$^{-1}$, the CO$_2$ uptake in phase III was negative.
Figure 4. The diurnal CO$_2$ pattern of three sample sizes of *Phalaenopsis amabilis* plants. The relative humidity and light intensity of the growth chamber were maintained at 70-90% and 180 µmol m$^{-2}$ s$^{-1}$. The light period was 14 h: (a) at 20/18 and 22/19°C day/night temperature; (b) at 28/23 and 30/24°C day/night temperature.

**Effect of drought treatment**

The effects of drought treatment on the CO$_2$ uptake pattern are shown in Figure 11a and b. After 6 days of no watering, the CO$_2$ uptake values become positive at the phase III. In this stage, the stomata of the leaf were open, and gas exchange was obvious. After ten days’ drought, phase IV could not found. With water stress for 21 days, the CO$_2$ pattern changed. The CO$_2$ pattern indicated that the stomata were closed gradually.
Figure 5. The diurnal CO$_2$ pattern of phase II, III, and IV of *Phalaenopsis amabilis* at night day/night temperatures. The relative humidity and light intensity of the growth chamber were maintained at 70-90% and 180 µmol m$^{-2}$ s$^{-1}$. The light period was 14 h.

Figure 6. The diurnal CO$_2$ pattern of phase I of *Phalaenopsis amabilis* at different day/night temperatures. The relative humidity and light intensity of the growth chamber were maintained at 70-90% and 180 µmol m$^{-2}$ s$^{-1}$. The light period was 14 h.

DISCUSSION

The CO$_2$ uptake pattern of *P. amabilis*

The CO$_2$ uptake pattern in *P. amabilis* of this study indicated that it had some difference with the typical CO$_2$ uptake patterns of CAM species (Osmond, 1978; Luttge, 2002). In term of the diurnal course in a CAM plant, the CO$_2$ uptake increased sharply and then decreased to zero with a shorter time in phase II and III lasted for
Figure 7. The diurnal CO$_2$ pattern of three light periods (6/18, 10/14, 12/12, 14/10h and 18/6h) of *Phalaenopsis amabilis* at 28/22°C night/day temperatures. The relative humidity and light intensity of the growth chamber were maintained at 70-90% and 180 µmol m$^{-2}$ s$^{-1}$. In the 6-18h light period, the CO$_2$ uptake becomes negative.

Figure 8. The diurnal CO$_2$ pattern of 10 days’ continuous dark treatment of *Phalaenopsis amabilis* at 28/22°C night/day/night temperatures. The relative humidity and light intensity of the growth chamber were maintained at 70-90%.
Figure 9. The diurnal CO\textsubscript{2} pattern of 10 days' continuous light treatment of *Phalaenopsis amabilis* at 28/22°C night day/night temperatures. The relative humidity and light intensity of the growth chamber were maintained at 70-90\%. In the dark period, the CO\textsubscript{2} uptake becomes negative.

Figure 10. The diurnal CO\textsubscript{2} pattern of five light intensities of *Phalaenopsis amabilis* at 28/22°C night day/night temperatures. The relative humidity and light intensity of the growth chamber were maintained at 70-90\% and 180 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1}. The light period was 14 h. If the light intensity was too low, the CO\textsubscript{2} uptake becomes negative.

several hours. However, the CO\textsubscript{2} uptake pattern of *P. amabilis* in phase II in this study revealed that the CO\textsubscript{2} uptake curves decreased slowly and the period of phase III was pretty short. In some case, this period of phase III
Effect of plant size

The results of this study indicated that the small plants have similar CO₂ uptake ability to the medium and large plants. Goh et al. (1984) showed the mature leaves of a monopodial orchid hybrid, *Arachnis Maggie Oei*, have the higher net CO₂ uptake than that of young plants during the day period. However, Ota et al. (1991) found no difference in CAM photosynthesis by leaf age. Lin and Hsu (2005), using the Chlorophyll fluorescence technique, found no significant different in the maximal

was less than 1 h in this study. More CO₂ was absorbed in phase IV in *P. amabilis* than in other CAM species. These results were similar to those of Lootens and Heursel (1998) and Endo and Ikusima (1989).

In the study of Ichihashi et al. (2008), the interval of CO₂ uptake measurement was wide, 5 or 10 min. So the characteristics of CO₂ rhythms were not easy to observe. In this study, a 1-min data collection interval was used which resulted in a consistent and continuous data distribution that was helpful and reliable in observing the CO₂ uptake pattern and influencing factors.
quantum efficiency, photochemical quenching or non-photochemical quenching between the upper and lower leaves of *P. amabilis*.

**Effect of day and night temperature**

From the CO₂ uptake pattern, *P. amabilis* showed maximal CO₂ fixation rate in the day temperatures ranging from 28 to 30°C and night temperatures ranging from 20 to 22°C. These results are inconsistent with those in the literatures. For the "Wataboushi" variety, the optimum day and night temperature were 25/15°C (Ota et al., 1991). For two varieties '70' and 'L', the optimal temperature was 20/15°C (Lootens and Heursel, 1998). Kaziwara et al. (1992) found that *Phalaenopsis* grew better under 30/25 than 25/20°C. Ichihashi et al. (2008) proposed that the variety White Dream 'MM74' had the highest CO₂ absorption at 20°C in phases III and IV. Kubota and Yoneda (1990) suggested that the *Phalaenopsis* varieties they tested had the better growth conditions under a constant temperature of 30°C than under 20°C. The culture guides of Floricultura (2007) and Anthura (2004, 2005) recommended a day/night temperature growth temperature of 28/26°C. In addition, Blanchard et al. (2005) suggested high temperature range of 28 to 32°C for the vegetative stage. These inconsistent results indicated the diverse of the *Phalaenopsis* plants used for research.

**Effect of light period**

The results of this study indicated that the optimal light period for CO₂ uptake ranged from 14 to 16 h. The culture guide for Floricultural b.v. (2007) recommended a light period for CO₂ absorbed CO₂ progressed after 10 days in dark. The metabolism of *Luttge, 2003; Rascher et al, 2001*, the rhythm of net CO₂ uptake was still existed. The time-series of rhythmic gas exchange under continuous light condition was studied in *Kalanchoe daigremontiana* leaves (Wyka and Lutte, 2003; Rascher et al, 2001), the rhythm of net CO₂ exchange was still existed with 7 days' continuous light.

In the continuous dark or light conditions, the rhythm of CO₂ uptake was still existed. The time-series of rhythmic gas exchange under continuous light condition was studied in *Kalanchoe daigremontiana* leaves (Wyka and Lutte, 2003; Rascher et al, 2001), the rhythm of net CO₂ exchange was still existed with 7 days' continuous light.

The results of this study revealed that the optimal light intensity was ranged from 80 to 215 µmol m⁻² s⁻¹ for the large *P. amabilis* plants. Lower light intensity, range 60 to 100 µmol m⁻² s⁻¹ (nearly 3340-5560 lux in daylight), could reduce the CO₂ uptake rate. Inconsistent results on light intensity are found in the literature. Ota et al. (1991) found that the 130 µmol m⁻² s⁻¹ was the saturation point for the "Wataboushi" variety. The saturating photosynthetic photo flux was 180 µmol m⁻² s⁻¹ for the 'L' and '70' varieties at 20°C (Lootens and Heursel, 1998). Konow and Wang (2001) studied the effect of irradiance levels on the growth of a hybrid *Phalaenopsis* (the TSC 22 clone of Arien Kuala). High levels of greenhouse irradiance would result in larger plants because of the increase in photosynthesis leading to increased growth rate. The irradiance in the high light level ranged from 150 to 330 µmol m⁻² s⁻¹ for 18 months. Lin and Hsu (2004) suggested that the limit irradiance level for *P. amabilis* was 200 µmol m⁻² s⁻¹ to prevent photo-inhibition on the surface of upper leaves. Blanchard et al. (2005) recommended a light irradiance ranging from 100 to 300 µmol m⁻² s⁻¹ for the vegetative growth stage. However, the culture guides for Anthura (2004, 2005) and Floricultura (2005) recommended 5000 to 8000 and 4000 to 6000 lux, respectively.

These inconsistent results for light irradiance could be explained by the difference in varieties, plant size and temperature conditions. The interaction of these factors on the light saturation point requires further study.

**Effect of water stress**

The severe water stress on the CO₂ rhythms for the 'Wataboushi' variety revealed that the CO₂ uptake was depressed in ten days drought treatment (Ota et al., 1991). In our study, the depression of CO₂ uptake began at day 14 of drought treatment. The CO₂ uptake in phase I was decreased at day 10. With 16-dat drought, the phase II was changed. At day 21, the C₃ pattern was found in the phases II, III and IV.

In our study, the ambient RH was maintained at a ranged from 70 to 90%. If the RH was set lower, the stomatal behavior may be different. Further study is needed to be executed to consider the interaction between aerial environment and drought stress.

**Further study of the CO₂ uptake pattern**

The sampling interval of this study, 1 min, produced consistent and continuous curves of CO₂ gas exchange rhythms under various conditions, which was useful to evaluate the effect of factors such as day and night temperature, light intensity and period. The gas exchange measurement of the whole plant was an effective way to study the gas exchange pattern. The photosynthesis function of the reproductive organs of *Phalaenopsis*, such as flower stalk, florets, and epiphytic roots could be studied with the measurement system developed in this study.
Further research is needed on the interaction effect of the aerial environment and the effect of physiological characteristics of different Phalaenopsis varieties. The first method is to develop and validate a CO₂ fixation CAM model to quantify these factors. The second method is to calculate the integrated net CO₂ uptake for four CAM phases using statistical technique. The results of the both methods will be compared and discussed in further study.

Conclusions

A whole-plant open-system chamber was developed to measure CO₂ exchange in Phalaenopsis amabilis. The consistent and continuous curves of CO₂ gas exchange rhythms at various conditions, sampled at 1-min interval, were used to evaluate the factors affecting on the CO₂ exchange pattern. The influencing factors included the day/night temperature, light period and light intensity. The results indicated that the adequate growth conditions of P. amabilis were 26 to 30°C day temperature, 19 to 21°C night temperature, 14 to16 h light and 180 to 215 µmol m⁻²s⁻¹ of light intensity. The gas exchange measurement system developed in this study is a useful measurement system for studying the physiological characteristics of Phalaenopsis varieties.

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