Effect of Leaf and Plant Age, and Day/Night Temperature on Net CO₂ Uptake in *Phalaenopsis amabilis* var. *formosa*

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ABSTRACT. In this study, effects of leaf age (20 to 240 days), plant age (4, 8, and 14 months after deflasking), and various day/night temperature regimes (16 to 33 °C) on photosynthesis of *Phalaenopsis amabilis* L. Blume var. *formosa* Shimadzu (*Phal.* TS97) leaves were investigated. The diurnal net CO₂ uptake in *Phal.* TS97 leaves was measured and integrated to obtain total net CO₂ uptake, which represents photosynthetic efficiency in plants performing crassulacean acid metabolism (CAM). Under all conditions, *Phal.* TS97 leaves performed typical CAM photosynthesis and reached their highest net CO₂ uptake rate, $\approx 6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, after 3 to 4 hours in the dark under a 12-hour photoperiod. When grown under 30 °C day/25 °C night temperature, the total net CO₂ uptake of leaf increased with maturation and was highest at 80 days old, 20 days after full expansion. The CAM photosynthetic capacity of mature leaves remained high after maturation and began to decline at a leaf age of 240 days. The trend was consistent with malate fixation but the highest nocturnal malate concentration was observed in 100-day-old leaves. Young leaves or leaves from small juvenile plants had higher daytime CO₂ fixation compared to mature leaves or large plants, suggesting that *Phal.* TS97 leaves progressed from C₃-CAM to CAM during the course of maturation. The second newly matured leaf from the top had the highest net CO₂ fixation when the newest leaf was 8 cm in length. Although plant age did not influence total CO₂ uptake in the leaf, photosynthetic efficiency of leaves in small younger plants was more sensitive to high light intensity, $340 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux. The day/night temperature of 32/28 and 29/25 °C resulted in the highest total net CAM CO₂ fixation in vegetative *Phal.* TS97 plants than higher (33/29 °C) and lower temperatures (21/16 °C).

As one of the most important indigenous species and breeding parent in Taiwan, *Phalaenopsis amabilis* var. *formosa* (classified as *P. aphrodite* Reichb. f. ssp. *formosana* Miwa by Christenson, 2001) has been used extensively to study *Phalaenopsis* Blume cultivation in Taiwan. It was originally found in the mountains at Hengchun Peninsula and on Lanyu Island (the “orchid island”) in Taiwan. This species grew on high branches of tropical thickets at an altitude between sea level and 800 m. To date, *Phal.* TS97 seedlings have been produced by selfing selected plants and developed through embryo culture in vitro (Lee, 1990). Fourteen months after transferring out of flask culture (deflasking), the mature plants often produce two inflorescences, each with 20 to 26 flowers of 7 cm in diameter after exposure to 18 to 26 °C. The individual flower lasts more than 3 months and the whole inflorescence often stays in bloom for at least 5 months after the opening of the first flower (Chen, 2001). In commercial production, large plants are exported for sale 13 to 15 months after deflasking. *Phal.* TS97 plants were reported to flower 7 months after deflasking (Lee, 1990), indicating a relatively short juvenile phase compared with other large white-flowered *Phalaenopsis* hybrids (Lee, 1991; Wang, 1991). Since there is a linear relationship between flower number and plant age after maturation (Lin, 2002), this early

maturation characteristic provides *Phal.* TS97 a great advantage for flower production.

Despite the importance of photosynthetic assimilation during flower development (Lin and Lee, 1998), the photosynthetic characteristics and efficiency of *Phal.* TS97 were hardly addressed. The photosynthetic characteristics in tropical orchids can be roughly grouped into two classes by leaf thickness (Arditti, 1992; Hew and Yong, 1997). Within Orchidaceae, orchids of leaf thickness <1 mm typically perform C₃ fixation, while those with thicker leaves usually perform crassulacean acid metabolism (CAM) (McWilliams, 1970; Neales and Hew, 1975). *Phal.* TS97 has thick leaves with large vacuoles in the parenchyma cells (Lee and Lee, 1991) and is expected to exhibit CAM photosynthesis (Wang, 1991), as observed in several *Phalaenopsis* hybrids (Endo and Ikusima, 1989; McWilliams, 1970; Ota et al., 1991). *Phalaenopsis* is a monopodial orchid having opposite leaves borne on a stem with very short internodes, resulting in the upper leaves heavily shading the lower leaves. Ota et al. (1991) reported that leaf age or mutual shading did not affect net CO₂ uptake in *Phalaenopsis* hybrid leaves. However, the nighttime net CO₂ uptake rate in *Phal.* TS97 leaf was significantly reduced in the lower, older leaves (Lin, 1994). The influence of leaf age on photosynthesis in *Phal.* TS97 needs to be examined more closely.

Temperature is not only the key factor for flower development in *Phalaenopsis* but also affects leaf production, which is closely related to the photosynthetic capacity of the whole plant. In *Phalaenopsis* hybrids, Lootens and Heursel (1998) and Ota et al. (1991) reported that leaves have maximum net CO₂ uptake when grown at a daytime temperature of 20 to 25 °C and nighttime temperature of 15 °C. However, leaf production and floret formation in *Phal.* TS97 and other white-flowered hybrids were greatly inhibited when grown under a 20/15 °C day/night temperature

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regime (Lee and Lin, 1984, 1987; Lin and Lee, 1988). In addition, *Phal.* TS97 and other white-flowered hybrids had more vegetative growth when grown under 30/25 °C than at 25/20 or 20/15 °C (Lee and Lin, 1984, 1987; Wang, 2005; Yang, 1996). During the winter season in Taiwan and Japan, the commercial greenhouses must be heated to 28 °C to enhance leaf growth and to prevent the emergence of the flowering stem (spiking) (Lee, 1988). Thus, more research is required to determine the optimal temperature for photosynthesis in *Phal.* TS97. In this study, we investigated how net CO₂ uptake changed with leaf age and plant age. Effects of various day/night temperatures on net CO₂ uptake in *Phal.* TS97 leaves also were examined.

Materials and Methods

PLANT MATERIALS. Mature *Phal.* TS97 plants, 14 months after deflasking, were purchased from Taiwan Sugar Corp. (Tainan, Taiwan) in Aug. 1998. They were transplanted into 10.5-cm-diameter plastic pots with sphagnum moss as the medium. Generally, plants had five or six mature leaves, which were 10 to 13 cm in length and 5.5 to 6 cm in width. Plants were placed in a greenhouse in Taipei (lat. 25°02'N, long. 121°38'E) under natural photoperiod before being used in various experiments. The cooling and shading systems in the greenhouse were programmed to maintain an average day/night temperature of 30/25 °C and a maximum photosynthetic photon flux (*PPF*) of 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Plants were watered as needed and fertilized once weekly with Peters 20N–8.6P–16.6K soluble fertilizer (Scotts, Marysville, Ohio) at a concentration of 1 g·L⁻¹. To provide additional microelements, plants were also fertilized once monthly with half-strength Johnson's solution without the Peters fertilizer (Johnson et al., 1957). Juvenile, young, and mature *Phal.* TS97 plants (4, 8, and 14 months following deflasking, respectively) were also purchased.

DIURNAL NET CO₂ UPTAKE. To obtain the typical diurnal net CO₂ uptake pattern, six *Phal.* TS97 plants were placed in a growth chamber, which was set at 30/25 °C and 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPF* with a 12-h photoperiod and 70% to 80% relative humidity. After 1 week, the diurnal net CO₂ uptake rate of the second leaf from the top (the newly matured leaf) was measured hourly for 25 h.

LEAF AGE. Six 19-month-old plants (from the time of deflasking) with six healthy leaves were selected from the greenhouse. Leaves were numbered basipetally: L1 (the new leaf, 8 to 10 cm), L2 (just matured), L3, L4, L5, and L6 (bottom old leaf). The approximate leaf ages were: L1 = 30 d old; L2 = 70 d old; L3 = 110 d old. The L4, L5, and L6 leaves were produced before purchased and the plants had flowered before experiment. During flower development, the leaf production in *Phal.* TS97 was almost arrested. Therefore, the leaf ages of these old leaves were not able to establish. Since the L6 leaf was produced no later than 4 months after deflasking, the L6 leaf was more than 360 d old. *Phalaenopsis* is a monopodial orchid having opposite leaves borne on a stem with very short internodes, resulting in the upper leaves heavily shading the lower leaves. To exclude the shading effect on photosynthesis (without shading), the upper leaves above the analyzed one were tied upward. This allowed for full light exposure to the lower leaves being measured (L3, L4, L5, and L6). These plants were grown in a growth chamber under 30/25 °C and 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPF* for 1 week, then two leaf positions in one plant were measured hourly for net CO₂ uptake. A total of six leaf positions and 12 leaves from six plants were measured at the same time.

To estimate the effect of mutual shading on photosynthesis (with shading), another two plants were selected, in which all leaves of six leaf positions in two plants were naturally positioned. These plants were also grown in a growth chamber under 30/25 °C and 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPF* for 1 week, then 12 leaves from six leaf positions on two plants were measured hourly for net CO₂ uptake.

NET CO₂ UPTAKE DURING LEAF DEVELOPMENT. To monitor changes in net photosynthetic capacity during leaf development, twenty plants, each with three mature leaves and one emerging new leaf, were chosen. They were placed in a growth room under 30/25 °C and 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPF* with a 12-h photoperiod. The growth of new leaves was monitored weekly and the new leaves were marked "day 1" when they reached 1 cm in length. These leaves grew 1 to 1.5 cm per week and were fully expanded to 11 to 13 cm in length after 60 d (Guo, 1999). Due to the difficulty of working with the small newly emerged leaves, the diurnal net CO₂ uptake rate was first measured after the leaves had reached 7 to 8 cm (≈ 45 d). These leaves were monitored weekly before the leaves were fully expanded. After the leaves reached 60 d, measurements were repeated monthly until the leaves were 240 d. Four or five leaves from individual plants were measured each time and the corresponding leaf age was calculated. Leaf samples (1 cm diameter) were also harvested for malate analysis.

Since a new leaf was produced approximately every 40 d, an additional four leaves were produced during the experimental period. The leaves being measured became the fifth after 240 d (counting basipetally). To allow for full exposure of light on the monitored leaf, the second newly developed leaves were removed during the experiment. Cutting new leaves off would affect sink–source relationship, which could enhance the photosynthesis efficiency in the remaining leaves (Candolfi-Vasconcelos and Koblet 1991; Petrie et al., 2000). However, our preliminary experiment suggested that the CO₂ uptake of *Phal.* TS97 leaves was not significantly affected after this treatment.

PLANT AGE. Juvenile, young and mature *Phal.* TS97 plants (4, 8, and 14 months after deflasking, respectively), three of each size, were acclimatized in a growth chamber for 1 or 2 d under 30/25 °C, and 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPF* with a 12-h photoperiod. This step would ensure that the photosynthesis capacities in these plants were at the same stages before they were placed under various light treatments. Then plants were exposed to one of four levels of *PPF*: 0, 100, 200, and 340 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which were averages measured on the second basipetal leaf (the newly matured leaf). After treating the plants at a desired light level for 2 d, the diurnal net CO₂ uptake rate of the second leaf was measured hourly for each plant.

TEMPERATURE RESPONSE. To determine how air temperatures would alter net CO₂ uptake, three plants were placed in each of five naturally lighted phytotrons with day/night temperature settings at either 35/30, 30/25, 25/20, 20/15, or 15/13 °C. Plants were grown under these conditions for 1 week (29 Apr.–11 May 1998) and the *PPF* on the leaves was maintained at ≈ 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. To prevent heat injury, leaves were misted at noon during sunny days and no signs of sunburn were observed during the experiment. The diurnal net CO₂ uptake rate of the second acropetal leaf was determined at 2-h intervals. During measurements, leaf temperatures were similar to the actual ambient temperatures, which, however, were higher than the settings by 3 to 4 °C. The average day/night leaf temperatures recorded were as following: 33/29, 32/28, 29/25, 25/20, and 21/16 °C.

MEASUREMENT OF NET CO₂ UPTAKE. The leaf net CO₂ uptake rate was measured using a LI-COR 6200 portable photosynthesis system (LI-COR, Lincoln, Nebr.) (Nobel and Israel, 1994). The standard 250-mL assimilation chamber was extended with a cylindrical adaptor having two layers of foam rubber gasket attached to its distal end. The initial air temperature, water vapor concentration, PPF, and CO₂ level in the chamber were similar to the ambient conditions. The infrared gas analyzer was calibrated using a CO₂ gas standard (600 $\mu\text{mol}\cdot\text{mol}^{-1}$). The chamber was firmly placed at the middle of the leaves. During measurement, the leaf was kept in the chamber for 20 to 30 s to equilibrate with the ambient micro-conditions. Then the net CO₂ uptake was recorded every 4 s and the average from three consecutive recordings was used to calculate net CO₂ uptake rate per leaf area. The leaf area covered in the chamber was marked and drew on a paper. The shape was cut out and the area was determined with an area meter (LI-3100; LI-COR). The same analysis was repeated either hourly or every 2 h during a 24-h period to establish a diurnal net CO₂ uptake curve. Since the total net CO₂ uptake represents the efficiency of CAM photosynthesis (Nobel, 1985), the day, night and daily total net CO₂ uptake were calculated by integrating the uptake curve (Nobel, 1991). Leaf, air temperature, PPF, and stomatal conductance were also measured concomitantly.

MALATE LEVEL MEASUREMENT. In CAM plants, the CO₂ absorbed during the night was mainly fixed into malate, which is an indicator of CAM fixation capacity (Osmond et al., 1991). To analyze leaf malate concentration, three leaf discs, ≈ 0.1 g fresh weight (FW), were collected half an hour before the light (pre-dawn) and dark period (pre-dusk). Samples were frozen immediately in liquid N₂ and stored at -50°C until analysis. The frozen tissues were ground in a prechilled mortar with a pestle and 5 mL of distilled water was added. The crude extract was transferred to a test tube and boiled for 10 min, cooled to room temperature, and clarified by centrifugation at $10,000 g_n$ for 5 min. An aliquot of 60 μL of the supernatant was added to the reaction mixture, which contained 0.9 mL of reaction buffer, 20 μL of malate-dehydrogenase (250 unit/mL), and 20 μL of 30 mM nicotinamide adenine dinucleotide (β -NAD) for a final volume of 1 mL (adapted from Osmond et al., 1991). The reaction buffer included 7.5 g of glycine, 5.2 g of ammonium sulfide, and 0.2 g of ethylenediaminetetraacetic acid (EDTA) in 100 mL of deionized water (pH = 9.5). The reaction mixtures were incubated at 30°C for 1 h and then the absorbance was determined by an ultraviolet or visible light (UV/Vis) spectrophotometer (U-1800; Hitachi, Tokyo) at 340 nm. Malate concentration was calculated according to standard curves and expressed as $\mu\text{mol}\cdot\text{g}^{-1}$ FW.

Results and Discussion

DIURNAL NET CO₂ UPTAKE PATTERN. The typical diurnal net CO₂ uptake pattern of *Phal. TS97* leaf (Fig. 1) exhibits four characteristic phases of CO₂ exchange observed in other CAM species (reviewed by Winter and Smith, 1996). Following illumination, no net CO₂ uptake was observed during the early part of the day (Phase II to Phase III). During this period, malate formed at night is decarboxylated to release CO₂, which is fixed to sugars through the Calvin cycle. The net CO₂ uptake resumed after exposing to light for 8 h and lasted for 4 h before the dark period began (Phase IV). The net CO₂ uptake rate increased with time during Phase IV, when CO₂ is fixed through the C₃ pathway accompanied by photorespiration. The majority of CO₂ uptake happened during the night, Phase I. After 3 to 4 h in the dark

Fig. 1. Net CO₂ uptake rate (O) and stomatal conductance (▲) in *Phalaenopsis amabilis* var. *formosa* leaves over a 24-h period. Plants were grown under 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF and 30/25 $^\circ\text{C}$ day/night air temperature for 1 week, then the second leaf, counting from the top, of each plant was measured. Results are means $\pm$ SE of six leaves; ■ = darkness.

(Phase I), the nighttime net CO₂ uptake rate reached its peak value of 6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, then declined gradually toward the end of the dark period. During Phase I, atmospheric CO₂ is fixed into malate. After 3 h in the dark, the stomatal conductance remained relatively constant for several hours despite the gradual decline of net CO₂ uptake rate. The difference between net CO₂ uptake rate and stomatal conductance suggests that the nocturnal net CO₂ uptake rate in *Phal. TS97* leaf is mainly controlled by enzyme activity or malate concentration but not stomatal conductance (Kluge and Ting, 1978).

LEAF AGE. To investigate the effect of leaf age on photosynthesis in *Phal. TS97* leaf, the diurnal net CO₂ uptake patterns from six leaf positions were measured. The bottom leaf (L6, more than 360 d) was the oldest leaf and the top leaf (L1, ≈ 30 d) was the youngest. All leaves were fully exposed to 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF and performed CAM net CO₂ uptake (Fig. 2). The maximum nocturnal net CO₂ uptake rate decreased with leaf age (L2 > L3 > L4 > L5 > L6). The newly matured L2 leaf had the highest nocturnal net CO₂ uptake rate of 5.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and the oldest leaf, L6, had the lowest rate of 2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ while the highest daytime net CO₂ uptake rate (3 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was observed in the youngest leaf (L1). When total net CO₂ uptake was calculated, L2 (≈ 70 d) had the highest nocturnal and daily total net CO₂ uptake, followed by L1, L3, and L4 (Fig. 3A). When compared to daily total net CO₂ uptake of L2 leaf, the photosynthesis in the bottom L5 and L6 old leaves had decreased by 36% and 57%, respectively. In contrast, the young L1 leaf had the highest proportion of daytime total net CO₂ uptake. There was no significant difference in daytime total net CO₂ uptake between mature (L2, L3, L4) and old leaves (L5, L6) (Fig. 3A).

To estimate how mutual shading from upper leaves affects the photosynthesis in the lower leaves (L4, L5, L6) in *Phal. TS97*, leaves from six leaf positions on similar plants were also measured when the leaves were naturally positioned (Fig. 3B). As in the previous experiment, the newly matured leaf had the highest total net CO₂ uptake that decreased with increasing leaf age (Fig. 3B). Under shaded conditions from the upper leaves, the PPF on the shaded L5 and L6 leaves was only 20 to 50

Fig. 2. Effect of leaf age on diurnal net CO₂ uptake in *Phalaenopsis amabilis* var. *formosa* over a 24-h period. Plants were grown under 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF and 30/25 °C day/night air temperature for 1 week then leaves from six positions were measured. All the leaves were allowed to full exposure of light. L1, L2, L3, L4, L5, and L6 represented leaf positions counting from the top. Results were means of two leaves; ■ = darkness.

Fig. 3. Effect of leaf age on total net CO₂ uptake rate in *Phalaenopsis amabilis* var. *formosa* leaves grown (A) without or (B) with mutual shading condition. Day, night and daily total net CO₂ uptake were obtained by integrating net CO₂ uptake curves in Fig. 2. L1, L2, L3, L4, L5, and L6 represented leaf positions counting from the top. Results are means $\pm$ SE of two leaves. The percentage (Δ) of the maximum daily total net CO₂ uptake was calculated relative to the L2 leaf. Means with the same letter are not significantly different at $P < 0.05$ by Duncan's multiple range test.

$\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when plants were grown under 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Despite low light intensity, total net CO₂ uptake in the shaded L4, L5, L6 decreased by only 8% to 13% compared to those without mutual shading (Fig. 3 A and B). However, shading did increase variation in photosynthetic capacity in the bottom L5 and L6 leaves (Fig. 3B). This may be due to the varied light intensity available on the leaf surface. Since all leaves analyzed had similar total chlorophyll concentrations (Guo, 1999), these results suggested that reduced photosynthesis in bottom old leaves of *Phal.* TS97 likely was mainly due to advanced leaf age.

To monitor how photosynthetic capacity changes during leaf development, net CO₂ uptake and malate accumulation were monitored after new leaves emerged. The nocturnal and daily total net CO₂ uptake increased with leaf age and was highest at 80 d, 20 d after the leaves had fully expanded (Fig. 4 A and B). The total net CO₂ uptake in leaves remained at a high value after maturation until 240 d (Fig. 4A). In young leaves (50 to 70 d), significant higher daytime net CO₂ uptake was observed when compared to other stages of leaves (Fig. 4C). At 240 d, little daytime net CO₂ uptake was measured.

Similar trends were also observed in diurnal malate concentration (Fig. 5). As early as 20 d old (5 cm in length), the *Phal.* TS97 leaves had developed CAM metabolism with nocturnal net accumulation of malate (Fig. 5 A and B). The nocturnal net malate accumulation increased with leaf age from 20 to 100 d and then remained relatively constant until 240 d (Fig. 5A). In contrast, much higher malate accumulation before the dark period was seen in the young leaves (Fig. 5C). The pre-dusk malate accumulation in a 20-d-old leaf were 80% higher than a 40-d-old leaf. This resulted in little nocturnal net malate fixation in young 20-d-old leaves (Fig. 5A). Low pre-dusk malate concentration in leaves was observed after 50 d of growth (Fig. 5C).

Taken together (Figs. 2–5), these results demonstrated that the net CO₂ uptake capacity increased with increasing leaf age and it was not completely developed until 20 d after full expansion of leaves (80 d) in *Phal.* TS97 when grown under 30/25 °C and 150–200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF. The nocturnal malate fixation in *Phal.* TS97 leaves required an additional 40 d of growth to reach the highest capacity. Then, the CAM capacity of *Phal.* TS97 leaf remains high at least until 240 d and then declined with leaf aging (L5 and L6 in Fig. 3).

These results are consistent with earlier observations that nocturnal net CO₂ uptake rate of *Phal.* TS97 leaves decreased when leaves began to age or senesce (Lin, 1994; Wang, 1991). Similarly, in another CAM orchid, *Aranda* (an intergeneric hybrid between *Arachis* Blume and *Vanda* R. Br.), nighttime net CO₂ fixation reached its maximum after leaves had matured and decreased significantly in older leaves (Hew and Yong, 1997). However, Ota et al. (1991) proposed that leaf position or age did not affect photosynthetic efficiency in a *Phalaenopsis* hybrid, that had only four leaves 2 years after deflasking. This suggests that the plants may not have grown well. Those leaves may have developed over a long period, similar to the stage of the L3 or L4 leaves in this current study (Fig. 3). Therefore, the discrepancy between these studies may be attributed to the wide range of leaf age examined.

Significantly higher daytime net CO₂ uptake in young leaves was also observed in another *Phalaenopsis* hybrid (Ota et al., 1991). During phase IV, CO₂ is mainly fixed through the C₃ pathway (Winter and Smith, 1996). A higher proportion of daytime net CO₂ uptake indicates that new leaves of CAM plants tend to perform more C₃ fixation than CAM metabolism (Ting et al.,

Fig. 4. Total net CO_2 uptake (A) over a 24-h period (daily, Δ), (B) during the night ($\bullet$), and (C) during the day ($\circ$) in developing leaves of *Phalaenopsis amabilis* var. *formosa*. Plants were grown under $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF and $30/25^\circ\text{C}$ day/night temperature. The diurnal net CO_2 uptake patterns at the indicated leaf ages were similar to Fig. 1 and the total net CO_2 uptake was calculated. To prevent mutual shading on the lower analyzed leaves, the upper leaves were cut off when the analyzed leaves were 110 d old (the indicated arrow). Each symbol represents one measurement from one leaf of indicated age.

1996). In the tropical *Peperomia scandens* Ruiz and Pav. (Holthe et al., 1987) and *Clusia uvitana* Pitt. (Zotz and Winter, 1996), young developing leaves predominantly perform C_3 fixation and gradually shift to C_3 -CAM and obligate CAM during leaf maturation. Thus, leaves of *Phal.* TS97 may also gradually develop CAM metabolism during leaf maturation and the leaves may mainly utilize the C_3 pathway when younger than 20 d. High pre-dusk malate concentration in 20-d-old *Phal.* TS97 leaves also indicates less efficient decarboxylation of malate during phase III in the new leaves. The malate accumulated in turn inhibits nocturnal phosphoenolpyruvate carboxylase (PEPC) activity and results in less nighttime CO_2 uptake (Baker et al., 1997; Kluge and Ting, 1978).

In *Phal.* TS97 plants each with six or seven leaves, the newly matured leaf (second from the top) had the highest CAM capacity and could be best used to study effects of environmental factors on photosynthetic capacity. With high photosynthetic efficiency, the upper second and third leaves would potentially be the major sources of carbohydrates for flower development (Lin, 1994). In fact, in *Phal.* TS97, the axillary bud is not mature enough to be induced when two more mature leaves have been

Fig. 5. Malate concentration: (A) change during the night (Δ -malate, Δ), (B) before the light period (pre-dawn, $\bullet$), and (C) before the dark period (pre-dusk, $\circ$) in developing *Phalaenopsis amabilis* var. *formosa* leaves. Plants were the same as those in Fig. 4. Each symbol represents one measurement from one leaf of indicated age.

developed above the node of that bud (Lin, 2002). These results further highlight the importance of the first three mature leaves from the apex and explain why flower formation in *Phal.* TS97 plants is typically located at the third or fourth leaf node from the apex (Lin, 2002).

PLANTAGE. The CAM capacity of the second leaf from juvenile (4 months), young (8 months), and mature (14 months) plants was also investigated. As early as 4 months after deflasking, *Phal.* TS97 mature leaves performed typical CAM metabolism similar to that in Fig. 1 (data not shown). This is consistent with the observation that young seedlings of *Phal.* TS97 have developed CAM metabolism during in vitro culture, when leaves are 6 to 8 cm in length (Chen and Lee, 2002).

Leaves of all three plant ages had similar light response curves (Fig. 6). For all plant ages, $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF resulted in the highest daily total CO_2 uptake (Fig. 6A). This is similar to previous studies that the light saturation point for *Phalaenopsis* hybrids is $130 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF (Lin, 1994; Ota et al., 1991). However, a PPF of $340 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ slightly reduced daily and nighttime total net CO_2 uptake in juvenile plants compared to young and mature plants (Fig. 6A and B). This suggests that juvenile plants (4 months old) were more sensitive to high light exposure. In fact, a PPF in the range of 100 to $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ is generally used to grow juvenile plants in Taiwan (Lee, 1988). Interestingly, when

Fig. 6. Effect of plant age on light response curve of (A) daily, (B) night, and (C) day total net CO₂ uptake in *Phalaenopsis amabilis* var. *formosa* leaves from juvenile (●, 4 months old), young (△, 8 months old) and mature (■, 14 months old) plants. The diurnal net CO₂ uptake curves of the second leaves from the top were similar to Fig. 1 and were used to calculate total net CO₂ uptake. Results are means $\pm$ SE of three leaves.

grown under 100 to 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF, mature leaves from small juvenile plants tend to have higher daytime total net CO₂ uptake than medium and large plants (Fig. 6C). This suggests that younger *Phalaenopsis* plants may have a greater proportion of C₃ fixation than older plants, as observed in several CAM species (Cushman et al., 1990; Hew and Khoo, 1980).

TEMPERATURE. Under various temperature regimes, *Phal.* TS97 leaves exhibited typical CAM photosynthesis (Fig. 7). The 32/28 and 29/25 °C day/night temperatures resulted in the highest nighttime net CO₂ uptake rate. Exposure to 21/16 °C markedly reduced the nocturnal net CO₂ uptake rate. In contrast, 25/20 °C resulted in the highest daytime net CO₂ uptake rate (Fig. 7). Interestingly, the uptake curve under low day/night temperatures (25/20 and 21/16 °C) was more dome-shaped than under higher temperatures (33/29, 32/28, and 29/25 °C).

When total net CO₂ uptake was calculated, temperatures of 32/28 and 29/25 °C resulted in the maximum daily and nighttime total net CO₂ uptake (Fig. 8). Although 25/20 °C did not affect daily total net CO₂ uptake, this temperature regime significantly reduced nighttime net CO₂ uptake. Both high 33/29 °C and low 21/16 °C temperatures significantly reduced total daily and nighttime net CO₂ uptake. These results correspond to their growth under these temperature regimes. The *Phal.* TS97 plants and other *Phalaenopsis* hybrids had the highest leaf production rate when grown under 30/25 °C and the growth was greatly slowed under 20/15 °C (Lee and Lin, 1984, 1987; Lin, 1994; Lin and Lee, 1988; Wang, 2005). The 33/29 and 21/16 °C caused yellowing and chilling symptoms in leaves, respectively (Guo, 1999). The

Fig. 7. Effect of day/night air temperature on diurnal net CO₂ uptake rate in *Phalaenopsis amabilis* var. *formosa* leaves over a 24-h period. Plants were grown at indicated phytotron under natural lighting for 2 weeks then the second acropetal leaves were measured. The average day/night leaf temperatures measured were shown. Results were means of three leaves from three plants measured on 11–12 May 1998; ■ = darkness.

Fig. 8. Effect of day/night temperature on day (□), night (■) and daily total net CO₂ (Δ) uptake in *Phalaenopsis amabilis* var. *formosa* leaves. The average day/night leaf temperatures measured were shown. Data were obtained by integrating diurnal uptake in Fig. 7. Results are means $\pm$ SE of three leaves. Means with the same letter are not significantly different at $P < 0.05$ by Duncan's multiple range test.

30/25 °C day/night temperature regime has been widely used for *Phalaenopsis* production in Taiwan (Hung, 1998; Lee, 1988).

Previous studies reported that the optimal temperature for photosynthesis in other *Phalaenopsis* hybrids was ≈ 20 °C and lower nocturnal temperature of 15 °C resulted in higher net CO₂ uptake (Looten and Heursel, 1998; Ota et al., 1991). It was speculated that high night temperature of 25 °C would increase respiration and decrease daily net CO₂ uptake (Looten and Heursel, 1998; Ota et al., 1991). This report is in conflict with our current study and other reports (Lin, 1994; Wang, 2005), in which warm 32/28, 30/25, and 29/25 °C day/night temperature are optimal for leaf growth. Since the optimal temperature for CO₂ uptake in CAM

plants may adapt to the ambient growth temperature (Nobel and Hartsock, 1984), the highest photosynthetic rates usually occur at temperatures similar to those of the natural habitat of a species. In southern Taiwan, where the majority of *Phalaenopsis* orchid growers are located, the daytime maximum temperature is usually between 30 and 33 °C while the nighttime minimum temperature is 25 to 27 °C in the summer. It is possible that the temperature optima of CAM fixation in *Phal.* TS97 produced in Taiwan has adapted to a higher temperature range. Based on this, growth of exported orchids from Taiwan may be sensitive to cold temperature when forced to flower at temperate locations.

Although potted, blooming *Phalaenopsis* has been produced and commercialized in larger numbers for several years, the photosynthetic properties of *Phalaenopsis* orchids are little understood. In this current study, we have attempted to show that one of the most important breeding parents, *Phal.* TS97, exhibits typical CAM metabolism. Leaf age and day/night temperature significantly affect its CAM fixation. Although these experiments were not repeated, the absolute values of net CO₂ uptake should be taken with caution. However, these results are consistent with previous studies on growth and flower development in *Phal.* TS97. This information will be helpful for further improvement in *Phalaenopsis* cultivation. The influence of other environmental factors, such as light, daylength, on *Phalaenopsis* photosynthesis should be addressed in the future.

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