Photosynthetic Light Requirements and Effects of Low Irradiance and Daylength on *Phalaenopsis amabilis*

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**ABSTRACT.** *Phalaenopsis* has become one of the most important potted plants around the world. Thus, we used a key commercial *Phalaenopsis amabilis* cultivar, TS97, as a model to determine the light requirements for maximal carbon fixation and photosystem II (PSII) efficiency in its leaves and to investigate the effects of low irradiance and daylength on photosynthesis and flower development. In mature ‘TS97’ leaves, the daily total CO₂ uptake capacity and net acid fixation increased with increasing photosynthetic photon flux (PPF) and saturated at ≈200 μmol m⁻² s⁻¹, whereas the fluorescence ratio values were significantly reduced to 0.68 to 0.75 above 325 μmol m⁻² s⁻¹ PPF, indicating photoinhibition of PSII. Positive assimilation of the nocturnal CO₂ uptake occurred at a very low PPF (less than 5 μmol m⁻² s⁻¹), suggesting highly efficient use of light energy by ‘TS97’ plants. Leaves developed under 30 μmol m⁻² s⁻¹ PPF exhibited lower light requirement of 125 μmol m⁻² s⁻¹ PPF to reach maximal CO₂ uptake, below which the daytime CO₂ uptake declined dramatically. Under a 12-hour daylength, exposing the leaves to a low PPF for 4 hours at any time during the day did not affect the photosynthetic capacity in ‘TS97’ leaves, suggesting that 8 hours of optimal irradiance is required for high-level photosynthesis, whereas the 12-hour daylength resulted in a higher CO₂ uptake rate and the daily total CO₂ uptake than the 8-hour daylength. Moreover, the 12-hour daylength promoted earlier flower formation and higher flower count compared with the 6- to 8-hour daylengths. Longer daylengths neither accelerated flowering formation nor enhanced total flower count. In conclusion, 8 hours of saturating PPF at 200 μmol m⁻² s⁻¹ and a 12-hour daylength are sufficient for maximizing photosynthesis and flower production in ‘TS97’ plants.

*Phalaenopsis* orchids have become one of the most valuable potted ornamental plants around the world. According to the U.S. Department Agriculture’s (USDA’s) Floriculture Crops Summary, potted orchids account for $191 million in wholesale revenue in 2011 (USDA, 2012) and the value is projected to increase by 15% annually in which 70% of these orchids are *Phalaenopsis* and imported from Taiwan. Both Taiwanese and U.S. growers have been focused on optimizing the orchid production to reduce the growing period and forcing time before and after overseas shipping, respectively. The emergence of an inflorescence and the quality of flowers are highly correlated with high levels of carbohydrates stored in orchid leaves (Hou et al., 2011; Konow and Wang, 2001; Lin and Hsu, 2004). The pre-shipment carbohydrate status is also critical for flowering forcing in *Phalaenopsis* orchids (Hou et al., 2011). Carbohydrates from photosynthesis account for 90% of the dry weight in most crops and light irradiance is often a key factor for maximal photosynthesis. In *Phalaenopsis*, low irradiance significantly slows the growth rate at all developmental stages and delays flower formation as well as reduces flower count (Lee, 2000; Lin and Lee, 1998; Wang, 1995). Thus, maximizing the photosynthetic carbon fixation with optimal light irradiance is important for best growth and flowering in *Phalaenopsis* orchids.

*Phalaenopsis amabilis* ‘TS97’ has many medium-sized flowers per inflorescence, a fast leaf production rate, and a short juvenile phase (Christenson, 2001). These physiological characteristics of traits have made ‘TS97’ one of the most important indigenous commercial cultivars in Taiwan and it represents a production model for most *Phalaenopsis* hybrids. Therefore, detailed studies on the photosynthetic performance of ‘TS97’ would provide a practical index for producing high-quality *Phalaenopsis*.

The TS97 cultivar, similar to other *Phalaenopsis* hybrids, performed the typical crassulacean acid metabolism (CAM) photosynthetic pathway, in which CO₂ is absorbed primarily during the night (Phase I) and fixed into malate or titratable acid. The stored acid is subsequently metabolized during the day through Calvin cycle to produce sugars (Phases II to III) (Cushman and Bohnert, 1999; Endo and Ikusima, 1989; Guo and Lee, 2006). A small amount of CO₂ uptake also occurs 8 h after plants are exposed to light (late afternoon, Phase IV) when CO₂ is fixed through the C₃ pathway accompanied by photorespiration (Cushman and Bohnert, 1999; Guo and Lee, 2006). Studies showed that the daily total CO₂ uptake, representing photosynthetic ability in a CAM plant, increased with maturability of leaves and reached the maximum at a leaf age of 80 d and then remained constant and declined after 240 d (Guo and...
The photosynthetic ability in a leaf is independent of plant age. The optimal temperature for photosynthetic fixation in ‘TS97’ leaves ranges from 29/25 to 32/28°C day/night temperature (Guo and Lee, 2006) that correlates well with the field practice for optimal vegetative growth in Phalaenopsis plants (Lee, 2000; Lin and Lee, 1988). Light has been shown to regulate CAM photosynthesis (Klugge and Ting, 1978); however, the impacts of light intensity and duration on photosynthetic ability in a Phalaenopsis leaf are not well established. A Phalaenopsis hybrid demonstrated that the net daily CO₂ assimilation will saturate at 130 μmol·m⁻²·s⁻¹ PPF (Ota et al., 1991), which is much lower than the level used in field production, normally ranging between 280 and 380 μmol·m⁻²·s⁻¹ PPF (Chen and Wang, 1996). Analysis of chlorophyll fluorescence in Phalaenopsis leaves suggested that photosynthesis is not saturated until above 300 μmol·m⁻²·s⁻¹ PPF (Lin and Hsu, 2004; Lootens and Heursel, 1998). However, chlorophyll fluorescence only represents the maximum photochemical efficiency in the chloroplast and does not linearly correlate with total carbon fixation (Maxwell and Johnson, 2000). On the other hand, during orchid production in Taiwan, short-term, low-light conditions often occur as a result of afternoon showers in the summers, which in turn complicate the greenhouse management for maximal light irradiance. In addition, during the winter production season, Phalaenopsis plants often develop leaves under relatively low-light irradiance. It is still not known how the short-term or long-term low-light exposure affect CAM fixation ability in Phalaenopsis leaves. Thus, the light requirement for maximal photosynthetic ability in ‘TS97’ requires further examination.

Daylength also affects photosynthetic capacities in CAM plants. In general, short daylengths promote nocturnal stomata opening and increase total nighttime CO₂ fixation as observed in Kalanchoe blossfeldiana (Queiroz, 1974). In contrast, long daylengths promote daytime CO₂ uptake and inhibit nighttime CO₂ fixation resulting in limited growth, as observed in Ananas comosus and Dendrobium ekapol (Nose et al., 1986; Sekizuka et al., 1995). However, in some obligate CAM plants, daylength only affects the rhythm of CO₂ uptake but does not affect total photosynthetic capacity (Brulft et al., 1982). Because the relationship between daylength and CAM photosynthesis varies among species, the effects of different daylengths on photosynthesis in Phalaenopsis leaves require further verification.

Therefore, in this study we determined the effect of light intensity on different aspects of photosynthetic efficiency, including diurnal CO₂ uptake rate, total CO₂ uptake capacity, malate fixation, and PSII quantum yield in ‘TS97’ leaves, and investigated the effects of low-light adaption, low-light exposure, and daylength on photosynthetic ability in leaves and on flower development.

**Materials and Methods**

**Plant materials.** Mature plants of the ‘TS97’ clone of *P. amabilis*, 14 months after deflasking, were purchased from Taiwan Sugar Corp. (Tainan, Taiwan). Plants were transplanted into 10.5-cm 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 grown in a greenhouse under natural daylength and light exposure for one to two months 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 PPF of 300 μmol·m⁻²·s⁻¹. Plants were watered as needed and fertilized weekly with 20N–8.6P–16.6K soluble fertilizer (Peters; Scotts, Marysville, OH) at a concentration of 1 g L⁻¹. To provide additional microelements, plants were also fertilized monthly with half-strength Johnson’s solution without the 20N–8.6P–16.6K fertilizer (Johnson et al., 1957). All the experiments were performed in controlled growth chambers and the default growth conditions were 200 μmol·m⁻²·s⁻¹ PPF and 30/25°C day/night temperature with a 12-h daylength (0700 to 1900 hr) unless otherwise noted.

**Measurement of diurnal net CO₂ uptake rate.** The net CO₂ uptake rate was measured using a portable photosynthesis system (LI-6200; LI-COR, Lincoln, NE) as described previously (Guo and Lee, 2006). In short, the second leaf from the apex was kept in a 250-mL transparent chamber for 20 to 30 s to equilibrate with the ambient microconditions. The light irradiance was provided directly from the growth chamber. Then the net CO₂ uptake was recorded every 4 s and the average from three consecutive recordings was used to calculate the net CO₂ uptake rate. The leaf area covered in the chamber was drawn on a paper and determined with an area meter (LI-3100; LI-COR). These measurements were repeated hourly or every 2 h during a 24-h period to establish a diurnal net CO₂ uptake curve. The net daytime, nighttime, and daily total CO₂ uptake were calculated by integrating the diurnal uptake curves (Nobel, 1991). Leaf and air temperature, PPF, and stomatal conductance of the analyzed leaves were also measured concomitantly.

**Measurement of leaf chlorophyll fluorescence.** The leaf chlorophyll fluorescence of the attached leaves that were used to measure diurnal net CO₂ uptake rates was measured by a portable fluorometer (PEA; Hansatech, Norfolk, U.K.). In all the experiments, the measurements were performed 1 h after the light period started (usually 0800 to 0830 hr). The chosen leaf spots were completely darkened with the leaf clips for 10 min to allow the relaxation of fluorescence quenching associated with thylakoid membrane energization (Maxwell and Johnson, 2000). Preliminary experiments showed that preincubation for 10 min in darkness was enough to obtain maximum fluorescence. Then the minimal fluorescence, Fₒ, was determined, whereas the maximal fluorescence, Fₘ, was also measured after a saturating pulse of red light. An increase in the Fₘ value represents a potential damage of the PSII reaction center that usually correlates with a decrease in the Fₘ value, indicating a reduction in maximal photochemical fluorescence quenching (Bolhar-Nordenkampf et al., 1989; Maxwell and Johnson, 2000). The variable fluorescence Fᵥ, where Fᵥ = Fₘ – Fₒ, and the fluorescence ratio, Fᵥ/Fₘ, were then calculated, representing current PSII photochemical efficiency. Therefore, a decline in Fᵥ/Fₘ is an indicator of photoinhibitory damage to PSII under environmental stresses (Bolhar-Nordenkampf et al., 1989; Maxwell and Johnson, 2000).

**Measurement of malate and titratable acidity.** The changes in malate and titratable acid concentration during the night are indicators of nocturnal carbon fixation in CAM plants (Osmond et al., 1991). To analyze the concentration of the accumulated acids, two leaf discs, ~0.1 g each in fresh weight (FW), were collected from the leaf 30 min before the light (pre-dawn) and the dark period (pre-dusk). Samples were frozen immediately in liquid N₂ and stored at –50°C for further analyses as described previously (Guo and Lee, 2006). In short,
the frozen tissues were ground in a prechilled mortar with 5 mL of distilled water. The crude extracts were boiled for 10 min, cooled, and centrifuged at 10,000 g, for 5 min. To analyze the malate concentration, 60 μL of the supernatant was added to the reaction mixture, which contained 0.9 μL of reaction buffer, 20 μL of malate–dehydrogenase (250 U/mL), and 20 μL of 30 mM nicotinamide adenine dinucleotide. The reaction mixtures were incubated at 30 °C for 1 h and then the absorbance at 340 nm was determined by an ultraviolet/visible light spectrophotometer (U-1800; Hitachi, Tokyo, Japan). The malate concentration was determined according to standard curves and expressed as micromoles per gram FW. To analyze the titratable acidity, 4 mL of the supernatant was titrated with 0.02 N NaOH (Merck, Whitehouse Station, NJ) to an end point of pH 8.3 to neutralize all the acidity. The amount of NaOH consumed was used to calculate the concentration of the titratable acid, expressed as micromoles H+ per gram FW.

**Effect of light intensity.** Mature plants were first normalized by exposure to the default growth conditions (see “Plant Materials”) in a controlled growth chamber for 2 d. Then plants were exposed to 30/25 °C day/night temperature and a 12-h daylength with one of the following levels of PPF: 0, 25, 90, 125, 220, 325, and 450 μmol·m⁻²·s⁻¹, which were averages measured on the second leaf from the apex (the newly matured leaf). After 5 d in the light treatment, the diurnal net CO₂ uptake rate of the second leaf was measured. Leaf chlorophyll fluorescence, diurnal changes of malate, and titratable acid level were also determined on the same leaves at the same time.

**Effect of low-light adaption.** To investigate how low-light adaption (LLA) would affect the CO₂ uptake ability in ‘TS97’ leaves, the diurnal net CO₂ uptake rate of LLA leaves was measured. When new emerging leaves had reached 3 to 4 cm in the greenhouse conditions (see “Plant Materials”), plants were transferred into a controlled growth chamber with 12 h of 30 μmol·m⁻²·s⁻¹ PPF and 30/25 °C day/night temperature for further development to maturity. After the leaves were fully mature under the low PPF (12 to 14 cm in length, after ≈70 d), the diurnal changes of net CO₂ uptake rate in response to 0, 22, 125, 207, and 307 μmol·m⁻²·s⁻¹ PPF were determined as described in the section of “Effect of Light Intensity.”

**Effect of low-light exposure.** To investigate how the photosynthetic ability was affected by low-light exposure (LLE) during the day, mature plants were transferred from the greenhouse condition to a controlled growth chamber with 30/25 °C day/night temperature and a 4-h low-light exposure (30 μmol·m⁻²·s⁻¹ PPF) during different time periods: in the morning [AL (0700 to 1100 HR)], at noon [ML (1100 to 1500 HR)], and in the afternoon [PL (1500 to 1900 HR)]. All the LLE treatments were under a 12-h daylength (0700 to 1900 HR) with 8 h of saturating light (200 μmol·m⁻²·s⁻¹ PPF). These plants were also compared with those grown under a 12-h saturating light condition (200 μmol·m⁻²·s⁻¹ PPF, HH). After 1 week of treatment, the diurnal CO₂ uptake curves, leaf chlorophyll fluorescence, and diurnal changes of malate and titratable acid were measured under the same treated conditions (AL, ML, PL, HH).

**Effect of daylength.** Plants were grown under a daylength of 8 h (1100 to 1900 HR), 12 h (0730 to 1930 HR), or 16 h (0330 to 1930 HR), respectively, with a day/night temperature of 30/25 °C. To prevent yellowing symptoms resulting from the heating effect of long saturating irradiance under a 16-h daylength and 30 °C temperature (observed previously), the 90% saturating PPF, 110 μmol·m⁻²·s⁻¹ (deduced from Fig. 1B), was used. After 1 week of treatment, the diurnal net CO₂ uptake rate of the second leaf was measured and the 24-h total carbon consumption was used to calculate the concentration of the titratable acid, expressed as micromoles H+ per gram FW.

**Fig. 1.** Effects of light intensity on (A) diurnal changes of net CO₂ uptake rate and (B) total CO₂ uptake during the day (○), during the night (●), and over a daily 24-h period (△), in *Phalaenopsis amabilis* ‘TS97’ leaves. The photosynthetic photon flux (PPF; μmol·m⁻²·s⁻¹) measured on the leaves were shown in the left corner (A). Levels of (C) malate and (D) titratable acidity at pre-dusk (○), pre-dawn (●), and net nocturnal accumulation (△) were also measured from the same leaves in A. Results are (A) means or (B–D) means ± se (n = 3); (●) means with the same letter are not significantly different at \( P < 0.05 \) by Duncan’s multiple range test. The underlined letters represent differences in the (B) nighttime uptake and the (C–D) pre-dawn acid concentration.
uptake was calculated. To study the effect of daylength on the flower formation, mature plants were grown under 6-, 8-, 10-, 12-, 14-, and 16-h daylength cycles with a cool day/night temperature of 23/18 °C and a PPF at 173 μmol·m⁻²·s⁻¹, which was the average from all the daylength treatments. In each treatment, the PPF on the top second leaves of five ‘TS97’ plants was measured. These growth conditions were applied through the entire period of the experiments to induce flower formation and allow flower anthesis (Lin, 1994). The date of flower stalk emergence (greater than 1 cm), visible flower bud, and anthesis was recorded. After four months of flowering forcing, the total flower count was recorded.

**Results**

**Effect of light intensity on normally grown leaves.** Under various PPF, ‘TS97’ leaves exhibited a typical CAM photosynthesis pattern (Fig. 1A). Most CO₂ uptake in ‘TS97’ leaves occurred mainly during the dark period (Phase I) and the nocturnal CO₂ uptake rate increased with light intensity. High PPF between ≈200 and 450 μmol·m⁻²·s⁻¹ resulted in the highest nocturnal CO₂ uptake rate (7 to 8 μmol·m⁻²·s⁻¹) and earlier onset of CO₂ uptake in the afternoon (Phase IV). For example, the treatment of 325 μmol·m⁻²·s⁻¹ PPF enabled CO₂ uptake 5 h before the dark period compared with 3 h under the 90 μmol·m⁻²·s⁻¹ PPF. When exposed to extremely low PPF (25 μmol·m⁻²·s⁻¹), both the daytime and nocturnal CO₂ uptake rates were significantly reduced, especially CO₂ uptake in the afternoon, which completely diminished. Moreover, a significant amount of CO₂ was released during the day when grown under 0 μmol·m⁻²·s⁻¹ PPF.

Because the daily total CO₂ uptake represents the efficiency of CAM photosynthesis, daytime, nighttime, and daily total CO₂ uptake were calculated from Figure 1A (Fig. 1B). A PPF at 125 μmol·m⁻²·s⁻¹ resulted in the maximal nighttime CO₂ uptake and 90% of the total daily CO₂ uptake. A PPF above ≈200 μmol·m⁻²·s⁻¹ enabled the maximal daytime and daytime total CO₂ uptake at ≈210 mmol·m⁻²·d⁻¹ and 30 mmol·m⁻² per 12 h, respectively. These data indicated that carbon fixation during the day (Phase IV) required higher saturating irradiance than that during the night. The ‘TS97’ leaves exhibited low-light requirement, 25 and ≈5 μmol·m⁻²·s⁻¹ PPF, for compensating respiratory carbon loss during the day and the night, respectively.

Because diurnal net accumulations of malate and titratable acid represent nocturnal carbon fixation capacity in CAM photosynthesis, we further determined levels of the acids before (pre-dusk) and after (pre-dawn) the dark period. The net accumulation of both malate and titratable acid increased with PPF (Fig. 1C–D) and can be divided into two stages. Under low PPF (90 μmol·m⁻²·s⁻¹ or less), the pre-dusk acid level significantly decreased with light intensities along with little change in the pre-dawn acid level. When exposed to high PPF (greater than 125 μmol·m⁻²·s⁻¹), the pre-dusk acid concentration greatly increased, whereas the pre-dusk acid level remained similar. At 325 μmol·m⁻²·s⁻¹ PPF, the capacity of nocturnal malate fixation decreased, whereas the level of malate increased again after exposure to 450 μmol·m⁻²·s⁻¹ PPF (Fig. 1C). The concentration of titratable acidity remains similar between 200 and 450 μmol·m⁻²·s⁻¹ PPF (Fig. 1D).

When grown at a PPF below 125 μmol·m⁻²·s⁻¹, no significant differences were observed in the values of Fv/Fm, Fo, and Fm in ‘TS97’ leaves (Fig. 2A–B). The Fv/Fm values ranged between 0.80 and 0.83, suggesting high PSII quantum efficiency. However, when exposed to a PPF higher than 325 μmol·m⁻²·s⁻¹, the Fo value increased along with a decrease in the Fm value, indicating potential damage in PSII reaction centers. A PPF at 325 and 450 μmol·m⁻²·s⁻¹ had reduced the Fv/Fm values to 0.68 to 0.74 lower than the optimal, demonstrating that PSII efficiency has been significantly reduced. The leaves appeared less green when plants were grown under 450 μmol·m⁻²·s⁻¹ PPF for 1 week (data not shown).

**Effect of low-light adaptation.** The LLA leaves exhibited similar CAM fixation patterns as those developed under saturating irradiance in the greenhouse (normal leaves) (Figs. 1A and 3A). However, when exposed to a saturating PPF (200 μmol·m⁻²·s⁻¹), the maximum nocturnal CO₂ uptake rate was only 4.2 μmol CO₂/m²/s in the LLA leaf (Fig. 3A). In particular, when exposed to a low PPF of 22 to 125 μmol·m⁻²·s⁻¹, the LLA leaves showed marked delays of daytime or nighttime CO₂ uptake compared with normal leaves (Figs. 1A and 3A). Moreover, darkness resulted in continuous net release of CO₂. Compared with the normal leaves, the daily total CO₂ uptake in the LLA leaves saturated at a lower PPF, 125 μmol·m⁻²·s⁻¹, and the maximal daily total carbon fixation was ≈150 mmol·m⁻²·d⁻¹ of CO₂. In contrast, the daytime total CO₂ uptake saturated at a higher PPF, greater than 300 μmol·m⁻²·s⁻¹ (Figs. 1B and 3B). In addition, the LLA leaves required a higher PPF than the normal leaves to enable positive carbon fixation during the day (≈80 μmol·m⁻²·s⁻¹ PPF) and the night (≈20 μmol·m⁻²·s⁻¹ PPF), suggesting that the efficiency to use the light energy had been reduced when leaves developed under a low PPF.
EFFECT OF LOW-LIGHT EXPOSURE. To investigate how LLE affects photosynthetic ability in Phalaenopsis, the ‘TS97’ leaves were analyzed after being subjected to 4 h of LLE (30 μmol·m⁻²·s⁻¹) during various times of the day, in the morning, at noon, and in the afternoon, and compared with those grown under 12 h of saturating light. Although the diurnal CO₂ uptake rhythms were unaffected (Fig. 4A), LLE during the day, regardless of its timing, delayed afternoon CO₂ uptake by 2 h (Phase IV) in ‘TS97’ leaves compared with the control condition (HH) (Fig. 4A). That in turn resulted in reduced levels of daytime total CO₂ uptake by almost 50% (Fig. 4B). Nevertheless, the nocturnal CO₂ uptake rate, nighttime total CO₂ uptake, and net malate accumulation in the LLE leaves were all similar to control leaves (HH) (Fig. 4B; Table 1). The daily total CO₂ uptake was slightly lower in the LLE leaves but not statistically different from the control leaves (HH). The Fv/Fm, Fm, and Fo values were also similar among treatments except that higher Fv/Fm ratios were observed in the PL-treated leaves. Interestingly, all of the LLE treatments significantly increased the concentration of net nocturnal titratable acidity compared with that in the control leaves (Table 1).

EFFECT OF DAYLENGTH. To understand how daylength affects CAM photosynthesis in Phalaenopsis, ‘TS97’ leaves were exposed to 8-, 12-, or 16-h daylength cycles for 1 week with 110 μmol·m⁻²·s⁻¹ PPF before measuring the diurnal CO₂ uptake rates. The ‘TS97’ leaves performed similar CAM rhythms, absorbing CO₂ mainly during the night, under all the photoperiods (Fig. 5A–C). Compared with that under the 12-h daylength, the daytime CO₂ uptake rates and capacities in leaves were significantly reduced under the 8-h daylength (Figs. 5A, 5B, and 5D). Moreover, the nighttime CO₂ uptake in ‘TS97’ leaves was diminished after 10 h in the dark period when exposed to a daylength of 8 h (Fig. 5A). As a result of a shorter period of darkness, the 16-h daylength cycle caused less nocturnal CO₂ uptake than the 12-h daylength cycle (Fig. 5B–D). Overall, the 12-h daylength resulted in the highest daily total CO₂ fixation and longer daylength did not further enhance the CAM photosynthetic fixation capacity (Fig. 5D).

To evaluate if the photosynthetic capacity also correlates with the following flower development, ‘TS97’ plants were induced to flower under various daylengths and 23/18 °C day/night temperature. The daylength had no effect on time of spiking (Fig. 6A). The effects of daylength on flower development became more obvious with increasing time in treatment. The short 6- to 8-h daylengths delayed the time to flower bud formation, 5 to 6 d later than the 12-h daylength (Fig. 6B). Similarly, short daylengths slowed anthesis, 6 to 10 d later than daylengths longer than 12 h (Fig. 6C). Eventually, the...
1900 HR) under a 12-h daylength; HH = control leaves grown under 12-h saturating light. PSII = photosystem II; FW = fresh weight.

Table 1. Effect of low-light exposure for 4 h on the values of F₀/Fₘ, initial and maximum chlorophyll fluorescence, F₀, and Fₘ in Phalaenopsis amabilis ‘TS97’ leaves.*

| Treatment | PSII efficiency | Malate | Titratable acidity |
|-----------|-----------------|--------|-------------------|
|           | F₀              | Fₘ     | Pre-dusk | Pre-dawn | Net | Pre-dusk | Pre-dawn | Net |
| HH        | 762 a           | 3207 a | 0.762 b   | 2.4 b     | 107.0 a | 104.6 a | 13.5 a  | 168.4 b | 148.9 b |
| AL        | 757 a           | 3148 a | 0.759 b   | 2.6 b     | 107.1 a | 104.5 a | 13.7 a  | 218.2 a | 204.5 a |
| ML        | 740 a           | 3244 a | 0.772 b   | 5.6 b     | 114.3 a | 108.8 a | 9.1 a   | 211.3 a | 202.1 a |
| PL        | 677 a           | 3298 a | 0.794 a   | 15.3 a    | 116.5 a | 100.2 a | 19.2 a  | 229.2 a | 210.0 a |

*The same leaves as those in Figure 4 were measured. Levels of malate and titratable acid at pre-dusk, pre-dawn, and net nocturnal accumulation are also shown. Results are means (n = 3).

Low-light treatment (30 mmol m⁻² s⁻¹) in the morning [AL (0700 to 1100 HR)], at noon [ML (1100 to 1500 HR)], and in the afternoon [PL (1500 to 1900 HR)] under a 12-h daylength; HH = control leaves grown under 12-h saturating light.

Mean separation (in columns) by Duncan’s multiple range test at P < 0.05. PSII = photosystem II; FW = fresh weight.

Discussion

Under various light conditions (Figs. 1A, 3A, 4A, and 5A–C), temperature, and developmental age (Guo and Lee, 2006), ‘TS97’ leaves exhibited the typical diurnal CO₂ uptake rhythm, indicating that P. amabilis is an obligate CAM plant. In a fast-growing ‘TS97’ plant, the daily total CO₂ uptake, an indicator of photosynthetic capacity, saturated at a higher PPF of ≈200 μmol m⁻² s⁻¹ (Fig. 1B) than a slow-growing Phalaenopsis hybrid, 130 μmol m⁻² s⁻¹ PPF (Ota et al., 1991). The same trend based on instant net CO₂ uptake rate has been reported in a Phalaenopsis hybrid (Hou et al., 2010). A PPF above 200 μmol m⁻² s⁻¹ promoted both daytime and nighttime CO₂ uptake rates (Figs. 1A and 3A) and resulted in low level of pre-dusk malate/titratable acid (Fig. 1C–D). These results are consistent with previous observations that high irradiance accelerates daytime malate decarboxylation and fixation to carbohydrate through Calvin cycle and thus enhances stomata opening in the afternoon (Phase IV) and the activity of nocturnal phosphoenolpyruvate carboxylase in CAM plants (Kluge and Ting, 1978; Lützge, 2008). That in turn explains that the CO₂ uptake rate and capacity, particularly the daytime total CO₂ uptake, in ‘TS97’ leaves were greatly reduced under low PPF (0 to 90 μmol m⁻² s⁻¹) (Figs. 1A–B and 3A–B). The relatively low-light requirement for activating positive carbon fixation in ‘TS97’ leaves (Fig. 1B–D) clearly shows the efficient use of light energy (Skillman and Winter, 1997). In general, the daytime CO₂ uptake required a higher PPF than the nighttime CO₂ uptake to compensate for the respiration-related carbon loss (Figs. 1B and 3B); that is probably because the CO₂ fixation during Phase IV is mainly through the C₂ pathway (Lützge, 2008). Previous reports have indicated that CAM plants can achieve a productivity close to C₃ or C₄ by maximizing carbon gain during Phase IV (Lützge, 2008). Taken together, saturating lighting in the afternoon may enhance photosynthetic capacity in Phalaenopsis that may partially contribute to better growth.

An increase of pre-dawn malate or titratable acid was usually accompanied by an initial decrease of pre-dusk acid level (Fig. 1C–D), indicating that the nighttime carbon fixation ability depends on whether the previously accumulated acid is completely metabolized during the day (Lützge, 2008). At least 90 μmol m⁻² s⁻¹ PPF was required to completely decarboxylate the accumulated malate during the day in ‘TS97’ leaves (Fig. 1B); that was consistent with the distinct daytime CO₂ uptake after exposure to 90 μmol m⁻² s⁻¹ PPF (Fig. 1A). Although a high PPF above ≈200 μmol m⁻² s⁻¹ did not decrease the total CO₂ uptake (Figs. 1B and 3B), the chlorophyll fluorescence ratio, F₀/Fₘ, had been reduced to 0.68 to 0.74 when leaves were exposed to 325 to 400 μmol m⁻² s⁻¹ PPF (Fig. 2A). The F₀/Fₘ value represents the maximal quantum yield of PSII, usually ranging between 0.75 and 0.85 for non-stressed plants (Bolhar-Nordenkampf et al., 1989). Thus, the low F₀/Fₘ value under a high PPF suggests the possibility of PSII photoinhibition that also correlates with the damages of PSII induced by an increase in F₀ values (Fig. 2B) (Maxwell and Johnson, 2000). Although a 10-min dark adaptation during our fluorescence measurements might not have been long enough to fully adapt the leaves that were exposed to a high PPF, the same result was also observed in a Phalaenopsis hybrid after exposure to 399 μmol m⁻² s⁻¹ PPF (Hou et al., 2010). Similarly, the net nocturnal malate fixation also decreased in leaves after exposure to 325 μmol m⁻² s⁻¹ PPF (Fig. 1C). Although the amount of acid in leaves remained at a high level under 450 μmol m⁻² s⁻¹ PPF, the accumulated acid was probably a protection mechanism to reduce photoinhibition (Osmond, 1982; Scheibe et al., 2005). Based on all the results regarding carbon fixation capacity and PSII efficiency, the optimal PPF for the maximal photosynthesis in Phalaenopsis leaves appears to be ≈200 μmol m⁻² s⁻¹ PPF.

When the ‘TS97’ leaves developed under low light (LLA), the daily total CO₂ uptake saturated at a low PPF, 125 μmol m⁻² s⁻¹. The same trend is also observed in LLA leaves of tropical CAM plants (Adams and Osmond, 1988). Our results also imply an enhanced sensitivity of LLA leaves to high-light photoinhibition in ‘TS97’ plants as observed in shade-adapted tropical CAM plants (Adams and Osmond, 1988). That also correlates with the observation that PSII photoinhibition occurs when Phalaenopsis leaves are directly exposed to 200 μmol m⁻² s⁻¹ PPF without acclimation after an extended time in darkness (Hou et al., 2010). Nevertheless, the maximum daily total CO₂ in a LLA leaf was only slightly lower than the normal leaf that developed under saturating irradiance (Figs. 1B and 3B), demonstrating great flexibility of carbon fixation to low-light acclimation in ‘TS97’ leaves. That is
consistent with the tolerance of PSII photochemical efficiency and leaf growth to long-term darkness observed in 'TS97' plants (Lin and Hsu, 2004) and another CAM species (Skillman and Winter, 1997). In addition, our results suggest that the photosynthetic capacity in mature 'TS97' leaves is mainly regulated by current light environments and less by the developmental factors such as plant age and leaf position, as observed previously (Guo and Lee, 2006). That also correlates with studies that, despite low-light stress during the vegetative stage, saturating irradiance supplemented during the reproductive stage is sufficient for high-quality flower formation in 'TS97' plants (Lin, 1994).

In addition, a 4 h of LLE at any time during the day under a 12-h daylength with 8 h of 200 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) PPF did not affect
total CO2 uptake, PSII efficiency, or nocturnal malate fixation (Fig. 4; Table 1), indicating that 8 h of a saturating PPF at 200 μmol·m−2·s−1 is sufficient to decarboxylate the accumulated malate to promote nocturnal carbon fixation in ‘TS97’ leaves. In CAM species, a short daylength would result in incomplete decarboxylation of the accumulated malate that inhibits stomata opening in Phase IV and thus lowers the nighttime CO2 fixation (Lüttege, 2008). The same results were observed in ‘TS97’ leaves in which the 8-h daylength nearly diminished the daytime CO2 uptake and caused a lower nocturnal CO2 uptake rate compared with those under the 12-h daylength (Fig. 5A–B). Although the 16-h daylength did not affect daytime CO2 uptake in ‘TS97’ leaves, it resulted in 5 h fewer of CO2 absorption and 25% less nocturnal total CO2 uptake than the 12-h daylength (Fig. 5). The trends of photosynthetic capacity as a result of various daylengths were consistent with later flowering performance (Fig. 6). In general, the 12-h daylength resulted in earlier flower development and higher flower count than 6- to 8-h daylengths. The results from this work are in conflict with a previous report that a short daylength of 8 h could promote flower formation and result in reduced CO2 fixation in Phalaenopsis orchid (Yoneda et al., 1991). It is possible that P. amabilis orchid is originated from the region close to latitude 23° N, where there is little daylength change during the year. Similarly, longer daylength than 12 h did not enhance flowering performance, indicating that artificial lighting of 12 h during flower-forcing in the field would be sufficient for maximizing flower production.

In conclusion, the light requirement of maximal photosynthetic ability in P. amabilis ‘TS97’ leaves is ≈200 μmol·m−2·s−1 PPF and 125 μmol·m−2·s−1 PPF for low-light-adapted leaves. A daylength of 12 h maximizes the photosynthetic CO2 fixation and flower development. To reduce the cost of artificial lighting, 8 h of saturating PPF that is followed by 4 h of low light is sufficient for optimal photosynthesis. A constant high PPF above 320 μmol·m−2·s−1 under a 12-h daylength would cause photo-inhibition and result in reduced CO2 fixation in Phalaenopsis leaves.

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