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Effect of low irradiance on the photosynthetic performance and spiking of *Phalaenopsis*

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Abstract

Lowering irradiance can delay the flower stalk, *i.e*., spike development, in order to schedule flowering time of *Phalaenopsis*; however, the effect on photosynthetic performance and spiking inhibition remains poorly understood. We compared light and shade treatments of *Phalaenopsis aphrodite* subsp. *formosana* in order to determine how limiting light affects day-night changes in the photosynthetic capacity of leaves and the carbon pool of leaves and stems resulting in delayed spiking. The low irradiance treatment [20 μmol(photon) m^{-2} s^{-1}] for six weeks did not affect potential functions of photosynthetic apparatus estimated by chlorophyll *a* fluorescence analysis, but it significantly reduced the net CO_2 uptake and O_2 evolution rates, carbohydrate and organic acid concentrations, and amplitudes of CAM activity in new and fully expanded leaves of *Phalaenopsis* and delayed the spiking compared with the control kept at 150 μmol(photon) m^{-2} s^{-1}. The shortened stem contained a remarkably high sucrose concentration, accounting for more than 80% of total soluble sugars for both treatments throughout the day. Moreover, the sucrose concentration was unaffected by the lowering of irradiance. The relationship between the sucrose content and spiking seemed to be loose; the major factor(s) for spiking in *Phalaenopsis* remained to be ascertained as the flower stalk bud is attached to the shortened stem.

Additional key words: CAM; carbohydrate; chlorophyll fluorescence; flower stalk; gas exchange; oxygen evolution.

Introduction

Phalaenopsis orchids are popular and valued potted ornamental plants worldwide for their beautiful long-lasting flowers with a variety of shapes, sizes, and colors (Endo and Ikusima 1992, Chugh *et al*. 2009). In order to meet market demands, flowering time must be precisely scheduled. One of key steps in regulating *Phalaenopsis* flowering is to control the emergence of the flower stalk or spiking.

Lowering of irradiance is a known strategy to delay spiking in *Phalaenopsis* (Kubota and Yoneda 1993, Wang 1995, Wang 1997, Hisamatsu *et al*. 2001, Liu *et al*. 2010, Wu *et al*. 2013). For example, Wang (1995) demonstrated that the spiking of *Phalaenopsis* cultured in a growth chamber was inhibited under irradiance of 8 μmol(photon) m^{-2} s^{-1} during a 12-h photoperiod or complete darkness for 6 weeks. Many studies have mentioned that the decrease in the sucrose concentration under lower irradiance may be an inhibitory signal for spiking, but their deduction was only based on the results from studies where new and fully expanded leaves were used (Konow and Wang 2001, Kataoka *et al*. 2004, Guo and Lee 2006, Tsai *et al*. 2008, Wu *et al*. 2013).

Phalaenopsis is a monopodial orchid with thick leaves alternating on each side of the plant and the bases

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Abbreviations: Chl – chlorophyll; CK – control; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v – variable fluorescence; F_v/F_m – maximum quantum efficiency of PSII photochemistry; FM – fresh mass; g_s – stomatal conductance; NPQ – nonphotochemical quenching; P_N – net CO_2 assimilation rate; SH – shade; Φ_{PSII} – actual photochemical efficiency of PSII.
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of leaves are connected to a shortened stem. One or two dormant spike buds emerge from the stem at the base of the third to fourth leaves that are numbered basipetally from the aerial portion of the plant (Sakanishi *et al*. 1980). Although the leaf is the site of light perception, it is reasonable to speculate that the signal in the stem of *Phalaenopsis* is involved more directly with spike development than with the leaf, because the spike bud is connected with the stem. To our knowledge, little is known about the relationships between carbohydrates, particularly, sucrose concentrations in the stem, and spiking inhibition of *Phalaenopsis* subjected to low irradiance.

A reduction in photosynthesis by lowering of irradiance is axiomatic. Photosynthetic capacity can be investigated by noninvasive methods, such as net CO_2 assimilation rate and chlorophyll (Chl) *a* fluorescence, and the invasive method, *e.g*., O_2 evolution rate. *Phalaenopsis* is an obligate CAM plant with a day-night fluctuation of photosynthetic characteristics: malic acid accumulation with open stomata during the night time and starch deposition with closed stomata during the day time, which implies that the maximum malate and starch contents should occur at dawn and dusk, respectively. On the contrary, the minimum malate and starch contents are exhibited at dusk and dawn, respectively (Endo and Ikusima 1989, Guo and Lee 2006, Chen *et al*. 2008, Pollet *et al*. 2011). Hence, we examined the diurnal changes in photosynthetic capacity of *Phalaenopsis* exposed to lowering [20 μmol(photon) m^{-2} s^{-1}] and control [150 μmol(photon) m^{-2} s^{-1}] irradiances, together with the day-night changes of the contents of photosynthesis-related metabolites in the new and fully expanded leaf and stem to clarify how limiting light affects the day-night variations in photosynthetic performance, and the cause-and-effect of photosynthates on the spike development of *Phalaenopsis.*

Materials and methods

Plant material and growth conditions: Mature *P. aphrodite* subsp. *formosana* plants were purchased from the Wusulin Farm of the Taiwan Sugar Corp., Tainan County, southern Taiwan (23°34'N, 120°38'E) and transplanted in transparent plastic pots (10.5 cm) filled with sphagnum moss in an environment-controlled greenhouse at 28.0 ± 1.2°C for four months in order to inhibit spiking. The plants with six leaves were then kept for six weeks in a growth chamber under a photoperiod with 14 h of light (06:00–20:00) and 10 h of dark (20:00–06:00) with 150 and 20 μmol(photon) m^{-2} s^{-1} of PAR supplied with cool-white fluorescence tubes (*F20T12*, *GE Inc*., Schenectady, NY, USA) at the tops of the plants for control (CK) or shade (SH) treatment, respectively. The day and night temperature for both treatments were 28.5 ± 0.4 and 20.5 ± 0.4°C, respectively. The plants were irrigated once a week, alternating between water and Peters fertilizer (1.0 g L^{-1}) (*Hyponex Corp*., Marysville, OH, USA) at about 1 mS cm^{-1}, as measured with a conductivity meter (*Model 3250*, *JENCO Inc*., CA, USA).

Spiking characteristics: Spiking was defined as the appearance of a flower stalk greater than 0.5 cm in length. We performed three batch experiments, with 12 individual plants each, to determine the spiking ratio for each treatment. The spike number represented the number of the flower stalks per spiking plant and the spike length was measured from the base of the flower stalk at the junction between the base of the leaves and shortened stem to the apex of the flower stalk (Liu *et al*. 2013).

Sampling procedure: The leaves were numbered basipetally and the second leaf (*ca*. 85 cm^2) was considered a newly expanded leaf and had approximately double the size of the first leaf. After noninvasive Chl *a* fluorescence and gas exchange analyses (*see* below), the second leaf from different plants was removed at four sampling points: midnight (00:00 h), dawn (06:00 h), midday (12:00 h), and dusk (18:00 h) [phase I, II, III, and IV, respectively, see Osmond (1978) for a definition of the four CAM-phases] for metabolite analysis. Leaf discs (*ca*. 0.28 cm^2) were cut from the middle and approximately 0.5 cm away from the main vein of the second leaf with a cork borer (Chen *et al*. 2008). After all leaves were completely removed, a knife was used to harvest the stem from the junction between the base of the flower stalk bud and the shortened stem, and the flower stalk was removed (Liu *et al*. 2013). The leaf discs and stems were weighed and frozen in liquid N_2 for analysis.

Metabolite analyses: To determine concentrations of organic acids, leaf discs were homogenized and extracted with distilled water at 100°C (Callaway *et al*. 1997). Organic acids were analyzed by anion exchange chromatography (*AS-11*, *Dionex Corporation*, Sunnyvale, CA, USA) using 5–100 mM NaOH for linear gradient elution and the eluted acids were quantified using a *Dionex* electrochemical detector (*ED50, Dionex Corporation*, Sunnyvale, CA, USA) (Chen *et al*. 2008). The contents of organic acids (malate and citrate) were calculated from the integrated peak area of the corresponding standards, total organic acid [μg g^{-1}] = (organic acid content from the corresponding standard [μg] × total extract volume [mL]/ analysis volume [mL])/fresh mass (FM) [g]. The amount of malic acid accumulated nocturnally can be estimated by its content at 06:00 minus that at 18:00 (Popp *et al*. 2003).

To determine soluble sugar concentrations, leaf discs and stems were separately homogenized and extracted with 80% (v/v) ethanol at 80°C (Chen *et al*. 2008). Sugars were separated on a *CarboPac PA10* column (*Dionex Corporation*, Sunnyvale, CA, USA) with 18 mM NaOH as an eluent and quantified by use of a *Dionex* pulsed

amperometric detector (Chen *et al*. 2008). The contents of soluble sugars (glucose, fructose and sucrose) were calculated from the integrated peak area of the corresponding standards, total soluble sugar [μg g^{-1}] = (soluble sugar content from the corresponding standard [μg] × total extract volume [mL]/analysis volume [mL])/fresh mass (FM) [g]. Sediment was digested with a mixture of pullulanase and amyloglucosidase and the released glucose was estimated by a glucose oxidase and peroxidase method to determine the starch content (Chen *et al*. 2008). The amount of starch deposited at day time can be estimated by its content at 18:00 h minus that at 06:00 h (Popp *et al*. 2003).

Chl *a* fluorescence measurements were performed using a pulse-amplitude modulation fluorometer (*PAM-210*, Walz, Effeltrich, Germany). The second leaf was acclimated to dark for at least 30 min. The minimal fluorescence (F_0) was determined under a radiation low enough [< 0.2 μmol(photon) m^{-2} s^{-1}] to induce any significant variable fluorescence. The maximal fluorescence value (F_m) was determined by a saturating pulse (SP) with 3,500 μmol(photon) m^{-2} s^{-1} on the dark-adapted leaf. Then, the leaf was continuously illuminated with an ambient actinic light of about 150 and 20 μmol(photon) m^{-2} s^{-1} for CK and SH treatment, respectively, to reach a stable fluorescence level (F_s). Thereafter, a second SP was imposed to determine the maximal fluorescence value on the light-adapted leaf (F_m'). In the present study, we adopted the maximum quantum yield (F_v/F_m) calculated as $1 - F_0/F_m$ to estimate the potential yield of PSII photochemical reactions (Roháček and Barták 1999, Baker 2008). The actual quantum yield of PSII (Φ_{PSII}) was calculated as $1 - F_s/F_m'$ in order to estimate the fraction of light absorbed by PSII antennae utilized in PSII photochemistry (Genty *et al*. 1989, Demmig-Adams *et al*. 1996). The actual nonphotochemical quenching (NPQ) was calculated by $F_m/F_m' - 1$ as the estimate of the dissipation of absorbed energy as heat in PSII antenna complexes in the light-adapted state (Bilger and Björkman 1990, Demmig-Adams *et al*. 1996).

Gas exchange: The stomatal conductance (g_s) and photosynthetic gas exchange of the second leaf was measured by use of an infrared gas analyzer system (*LI-6400*, *Li-Cor Inc*., Lincoln NE, USA). External air was scrubbed of CO_2 and mixed with a supply of pure CO_2 to create a standard concentration of 400 μmol mol^{-1}. Flow rate was 500 μmol s^{-1}. Measurements were made at PAR of 150 and 20 μmol(photon) m^{-2} s^{-1} for the CK and SH treatments, respectively; provided by a light emitting diode source (*6400-02*, *Li-Cor Inc*., Lincoln NE, USA), attached to the sensor head during day time, and the light source was turned off during night time.

O_2 evolution rate: The photosynthetic O_2 evolution rate of the second leaf was measured using an oxygen electrode (*Leaflab 1 System*, *Hansatech*, UK). The sample leaves were cut at three time points: at dawn (06:00 h), at midday (12:00 h), and at dusk (18:00 h). Each leaf was cut by a sharp razor and leaf discs (*ca*. 10 cm^2) were punched out at the middle and away from main veins of the leaf by a borer and immediately placed into the electrode chamber in a closed system of air with 1% (v/v) CO_2 supplied with 0.2 ml of 1 M sodium bicarbonate solution. The O_2 evolution rate was measured under air temperature of 28°C and irradiance at about 150 and 20 μmol(photon) m^{-2} s^{-1}, respectively.

Statistical analysis: Data are means ± SE. The data were subjected to one-way analysis variance (*ANOVA*) and the differences between means were measured at the 5% probability level using *Fisher*'s protected least significant difference (LSD) test (*CoHort Software*, Monterey, CA).

Results

Flower stalk development: When grown under SH conditions for six weeks, plants exhibited a spiking ratio of 18%, with the mean spike number of 1.0 per a spiking plant, and a mean spike length of 0.6 cm (Table 1). These results were all significantly lower than those for the CK treatment with almost all plants exhibiting spiking with the mean spike number of 1.7 per the spiking plant, and the mean spike length of 4.4 cm (Table 1). Evidently, lowering of the irradiance delayed the flower stalk development.

Chl *a* fluorescence: During the CK treatment, F_v/F_m slightly decreased during the day time and reached a minimum at 18:00; then gradually increased during the night time (Fig. 1*A*). Likewise, this trend also occurred in the SH plants; the mean F_v/F_m remained at 0.80–0.82 during the dark period (22:00–03:00 h), slightly decreased to 0.76–0.79 during the light period (09:00–18:00 h), and both treatments showed limited changes throughout the day (Fig. 1*A*).

As compared to F_v/F_m, Φ_{PSII} showed a distinct day-night fluctuation (Fig. 1*B*). Under the CK treatment, it showed approximately 37% decrease from 12:00 to 18:00 h, then remained at low levels during the dark period, and peaked at 09:00 and 12:00 h. In contrast, under the SH treatment, the high values were similar to CK and remained such during the day time, while the lower values appeared during the dark period. The major disparity between the CK and SH treatments occurred at 18:00 h, CK was significantly lower than that under SH.

Table 1. Effect of lower irradiance on the spiking characteristics of *Phalaenopsis aphrodite*. Data are means ± SE of 36 individual plants from 3 batch experiments, with 12 individual plants each. *Different letters* show significant difference between control and shade treatments ($p \leq 0.05$).

Condition	Spiking ratio [%]	Spike number per spiking plant	Spike length [cm]
Control	94 ± 6^a	1.7 ± 0.2^a	4.4 ± 0.8^a
Shade	8 ± 14^b	1.0 ± 0.0^b	0.6 ± 0.1^b

Similar to Φ_{PSII}, NPQ values also showed a marked day-night fluctuation with a peak during the night time and the lowest values during the day time for both treatments. A marked difference also appeared at 18:00 h, where CK values were significantly higher than those under SH (Fig. 1*C*).

Gas exchange: During the CK treatment, g_s exhibited a significant day-night fluctuation. Namely, stomata started opening after 15:00 h and remained opened throughout the night time, then closed from 09:00 to 15:00 h (Fig. 2*A*). Similar day-night fluctuation of g_s was reported in our previous work (Chen *et al*. 2008). However, under the SH treatment, the stomata opened only at night and remained closed throughout the day time with significantly decreased g_s compared with CK.

Similarly, the pattern of net CO_2 uptake rate (P_N) (Fig. 2*B*) corresponded well with g_s (Fig. 2*A*). During the CK treatment, CO_2 fixation slightly increased during the late afternoon, with a peak at 21:00, then gradually decreased and became zero in the early morning. In contrast, during the SH treatment, CO_2 fixation proceeded only during the night time (Fig. 2*B*). The total net carbon gain estimated by integrating the CO_2 uptake rate curve for the CK treatment was *ca*. 234.7 mmol m^{-2}, significantly higher than that of the SH treatment, which was *ca*. 32.6 mmol m^{-2}.

O_2 evolution: In both treatments, oxygen consumption occurred at 06:00 h and a peak of O_2 evolution was reached at 12:00 h. The value was four times higher in the CK than that of SH plants (Table 2). During transition from day to night (18:00 h), O_2 evolution significantly decreased in plants of both treatments and some SH plants even started consuming O_2.

Organic acids: During the CK treatment, the highest malate concentration, *ca*. 7.1 mg g^{-1}(FM), was reached at 06:00 h and the lowest, *ca*. 0.3 mg g^{-1}(FM), was found at 18:00, which showed steady decarboxylation from 06:00 to 18:00 h and a distinct nocturnal accumulation (Fig. 3*A*). Under the SH treatment, the malate concentration was similar to the CK concentration at 00:00 and 12:00 h. However, at 06:00 and 18:00 h, the concentrations of *ca*. 3.5 and 2.4 mg g^{-1}(FM) were significantly lower and higher, respectively, than those of CK. Malate decarboxylation primarily occurred from 12:00 to 18:00 h and the magnitude was only *ca*. 1.2 mg g^{-1}(FM) (Fig. 3*A*). Thus, lowering of irradiance significantly reduced the nocturnal malate accumulation and delayed the day-time decarboxylation.

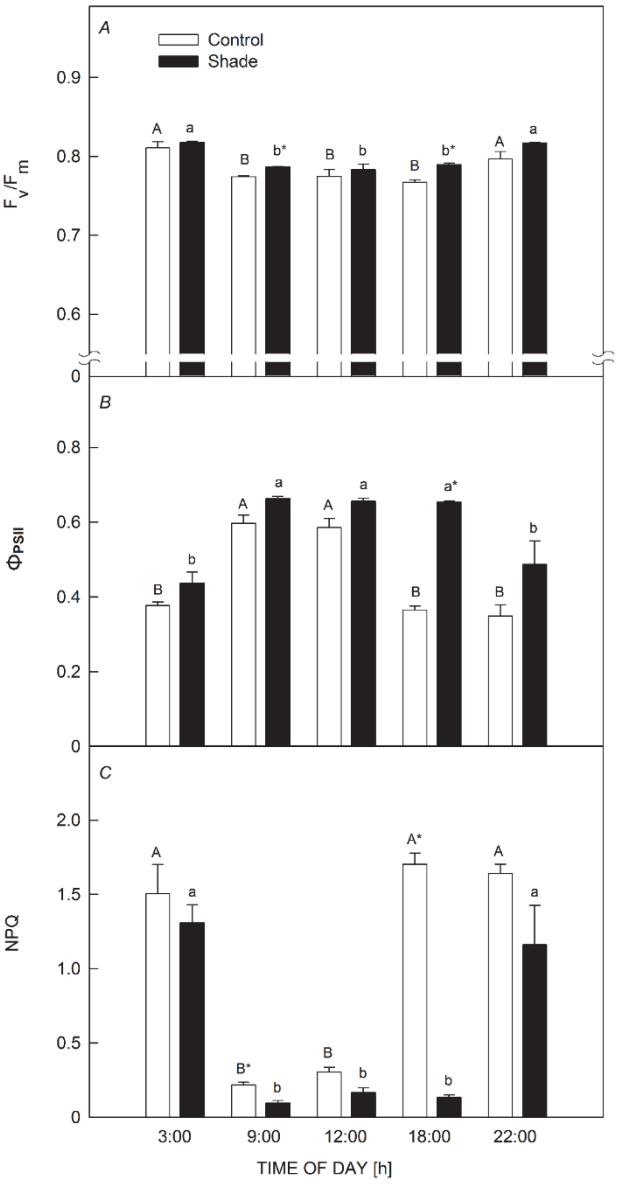

Fig. 1. Day-night fluctuation of the maximum quantum yield (F_v/F_m) (*A*), actual quantum yield of Φ_{PSII} (*B*), and actual nonphotochemical quenching (NPQ) (*C*) of *Phalaenopsis aphrodite* under control (open bars) and shade (full bars) conditions. Data are mean ± SE of three individual plants. Different *capital* and *lowercase letters* show significant difference ($p \leq 0.05$) in day-night changes under control and shade conditions, respectively. The asterisks indicate significant differences between control and shade treatments at the same sampling time ($p \leq 0.05$).

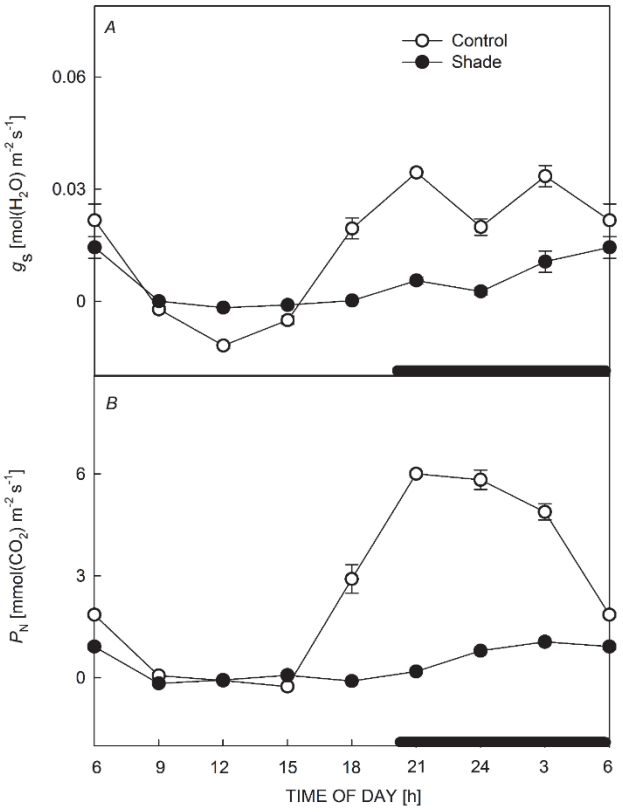

Fig. 2. Day-night fluctuation patterns of (*A*) stomatal conductance (g_s) and (*B*) net CO_2 uptake rate (P_N) of *Phalaenopsis aphrodite* under control (*opened circles*) and shade (*closed circles*) conditions. The dark bars indicate night time. Data are mean ± SE of three individual plants.

When compared to malate, citrate concentrations remained constant and did not show any day-night fluctuation in both treatments (Fig. 3*B*). However, lowering of irradiance decreased citrate concentrations approximately to 30–50% of CK throughout the day (Fig. 3*B*). Clearly, the metabolic fates of citrate and malate differ in *Phalaenopsis* exposed to various light intensities.

Carbohydrates: The concentrations of glucose and fructose did not show day-night fluctuation in the leaf and

Table 2. Day-time fluctuation of O_2 evolution of *Phalaenopsis aphrodite* under control and shade conditions. Data are mean ± SE of four individual plants. Different *capital* and *lowercase letters* show significant difference ($p \leq 0.05$) in day-time changes under control and shade conditions, respectively. The asterisks indicate significant differences between control and shade treatments at the same sampling time ($p \leq 0.05$).

Condition	O_2 evolution [μmol m^{-2} s^{-1}]		
	06:00 h	12:00 h	18:00 h
Control	-17.5 ± 8.8^{C}	$23.3 \pm 1.3^{A*}$	$9.8 \pm 1.6^{B*}$
Shade	-33.8 ± 3.1^{c}	5.6 ± 1.0^{a}	-2.2 ± 2.9^{b}

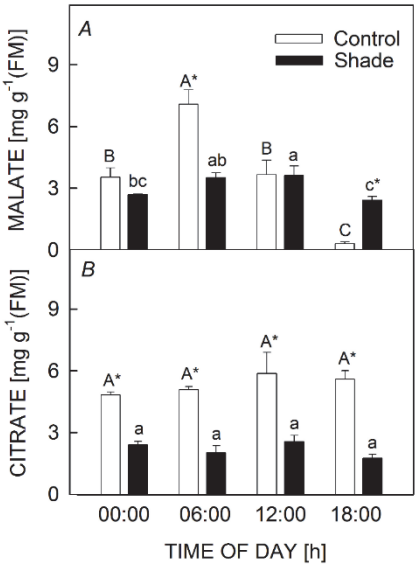

Fig. 3. Day-night fluctuation of malate (*A*) and citrate (*B*) contents in *Phalaenopsis aphrodite* under control- (*open bars*) and shade (*full bars*) conditions. Data are mean ± SE of three individual plants. Different *capital* and *lowercase letters* show significant difference ($p \leq 0.05$) in day-night changes under control and shade conditions, respectively. The asterisks indicate significant differences between control and shade treatments at the same sampling time ($p \leq 0.05$).

glucose concentrations were always 32–43% lesser than that of fructose throughout the day during the CK treatment (Fig. 4*A,B*). The concentration of sucrose increased from 06:00 to 12:00 h, then it was gradually declining until 18:00 h, and slightly increased from 18:00 to 00:00 h (Fig. 4*C*). This moderate day-night change pattern of sucrose was completely different from that of starch (Fig. 4*D*). The lowest concentration of starch was detected at 06:00 h, *ca*. 2.1 mg g^{-1}(FM), then slightly increased to 4.1 mg g^{-1}(FM) at 12:00 h, and greatly increased to peak at 18:00 h [*ca*. 13.0 mg g^{-1}(FM)]. Clearly, most of the starch deposition appeared from 12:00 to 18:00 h.

When compared with CK, lowering of irradiance greatly reduced the concentrations of carbohydrates in the leaf and a magnitude of the decrease strongly depended on the sampling time. Glucose increased from sevenfold at 18:00 h to 20-fold at 00:00 h (Fig. 4*A*). Fructose was enhaced from threefold at 06:00 to 11-fold at 00:00 h (Fig. 4*B*), sucrose increased from eightfold at 06:00 h to 12-fold at 00:00 h (Fig. 4*C*), while starch accumulated from threefold at 12:00 h to 8-fold at 18:00 h (Fig. 4*D*). The slight day-night fluctuation for sucrose was abolished and the daytime starch deposition was substantially reduced to only 1.1 mg g^{-1}(FM). However, fructose showed a slight day-night change in its content and its mean content was higher than that of glucose at all four sampling points, even though the contents of glucose and

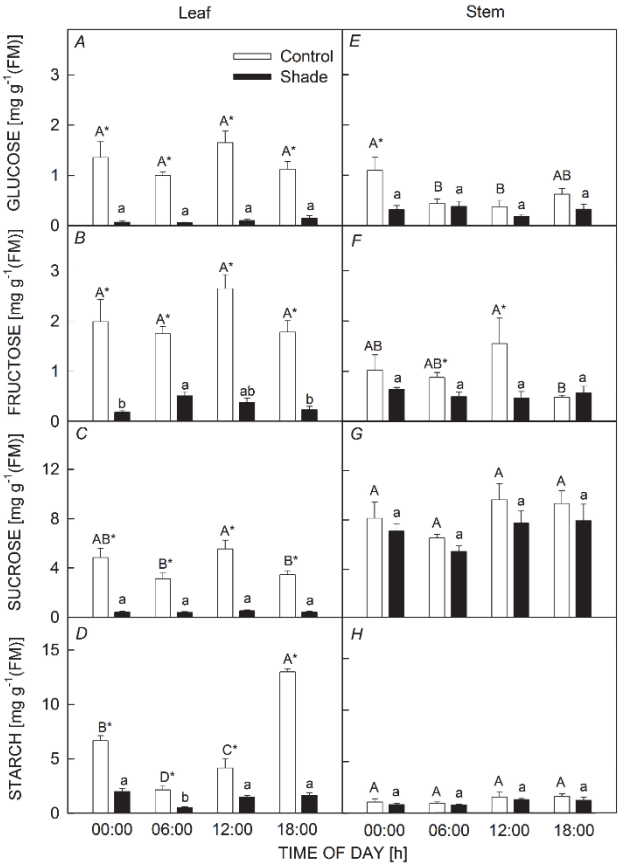

Fig. 4. Day-night fluctuation of glucose (*A,E*), fructose (*B,F*), sucrose (*C, G*), and starch (*D, H*) contents in leaf (*A–D*) and stem (*E–H*) of *Phalaenopsis aphrodite* under control (*open bars*) and shade (*full bars*) conditions. Data are mean ± SE of three individual plants. Different *capital* and *lowercase letters* show significant difference ($p \leq 0.05$) in day-night changes under control and shade conditions, respectively. The asterisks indicate significant differences between control and shade treatments at the same sampling time ($p \leq 0.05$).

sucrose remained rather constant. Thus, lowering of irradiance profoundly changed the carbohydrate metabolism in *Phalaenopsis* leaf.

Despite the marked differences in carbohydrate concentration in leaves under both treatments, during the SH treatment, stems showed the lower content of glucose only at 00:00 h (Fig. 4*E*) and that of fructose at 06:00 and 12:00h when compared with CK (Fig. 4*F*). However, the contents of sucrose (Fig. 4*G*) and starch (Fig. 4*H*) were not affected by the SH treatment and remained rather constant throughout the day as well as the starch content did not show day-night changes in both treatments. Compared to the source leaf, the stem showed lowered glucose, fructose, and starch contents and two- to threefold increased sucrose content in the CK plants. However, under SH, elevations in the glucose and fructose content were slight and varied with the sampling time, but the sucrose content rose from 14-fold at 06:00 h to 20-fold at 18:00 h.

Discussion

Lower irradiance decreases photosynthetic performance: Numerous studies showed that the photosynthetic rate significantly declined with shading, which resulted in a reduced content of total soluble carbohydrates and lesser fluctuation in the starch concentration (Souza *et al*. 2004, Chang *et al*. 2008, Ceusters *et al*. 2011, Wu *et al*. 2013). For example, *Aechmea* 'Maya', an obligate CAM plant, significantly decreased P_N, the concentrations of sucrose and starch, and the day-night fluctuations of starch and malic acid under severe irradiance limitation for 6 d (Ceusters *et al*. 2011). In our experiment, lowering of irradiance also significantly reduced g_s and delayed stomata opening (Fig. 2*A*). Likewise, a declined P_N during the night time (Fig. 2*B*) and supplemental CO_2 *via* malate decarboxylation during the day time (Fig. 3*A*), together with reduced O_2 evolution (Table 2), resulted in greatly reduced free glucose, fructose, sucrose, and starch concentrations, and lowered the amplitude of day-time starch deposition in the leaf (Fig. 4*A–D*).

Compared to carbohydrates, the content of citrate decreased (Fig. 3*B*) and the amplitude of night-day change of malate (Fig. 3*A*) was lessened by lowering of irradiance. This mitigation of malate and citrate concentration changes also occurred in *A.* 'Maya' under severe irradiance limitation (approximately 15% of mean control daily integrated irradiance) for 6 d (Ceusters *et al*. 2011) and in two *Phalaenopsis*-type orchids under low irradiance (approximately 15% of mean control irradiance) for 15 weeks (Wu *et al*. 2013). Citrate and malate are important intermediates of the Krebs cycle that serves as the biochemical hub of the cell and closely relates with respiration capacity. In order to survive, citrate and malate must be maintained at a suitable concentration, even though the carbohydrate contents are severely depleted by limiting irradiance.

Lowering irradiance does not affect the photosynthetic apparatus: Chl *a* fluorescence analysis has been widely used to assess PSII functioning under abiotic and biotic stresses (Roháček and Barták 1999, Maxwell and Johnson 2000, Baker 2008). Quiles (2005) suggested that typical values for F_v/F_m were from 0.75–0.85 for nonstressed plants. When the plants were subjected to 20 μmol(photon) m^{-2} s^{-1}, the day-night trend of F_v/F_m was similar to CK and values were from 0.76–0.82 throughout the day (Fig. 1*A*). Evidently, the PSII apparatus of *P. aphrodite* under low irradiance for six weeks has not been affected, which agrees with *Phalaenopsis* having the inherent ability to acclimate to low-light arboreal environments because they inhabit tropical rainforests.

Moreover, Φ_{PSII} and NPQ values were always high during the day time (Fig. 1*B*) and the night time (Fig. 1*C*), respectively, for both treatments. High Φ_{PSII} during the day time implied that the light absorbed by the PSII antennae could be efficiently utilized in the PSII photochemistry (Genty *et al*. 1989, Demmig-Adams *et al*. 1996) and high NPQ during the night time indicated that the excess radiant energy could be efficiently dissipated as heat in the PSII antenna complex (Bilger and Björkman 1990, Demmig-Adams *et al*. 1996). Consistent with F_v/F_m (Fig. 1*A*), Φ_{PSII} and NPQ did not show significant differences between the treatments. Thus, it further confirmed that the PSII apparatus of *P*. *aphrodite* was not affected by low irradiance treatment for six weeks.

The most prominent differences in Φ_{PSII} and NPQ between the treatments appeared at 18:00; namely, the stomata were already opened in CK (Fig. 2*A*), which resulted in lower Φ_{PSII} and higher NPQ (Fig. 1*B*,*C*) when compared with low irradiance treatment where the stomata were still closed (Fig. 2*A*). In fact, this distinct day-night and reciprocal fluctuation between Φ_{PSII} and NPQ was also manifested in C_3 plants, such as *Helianthus annuus*, *Vinca major* (Demmig-Adams *et al*. 1996), *Phaseolus radiates* (Yang *et al*. 2004), C_4 plant, such as *Setaria italic* (Yang *et al*. 2004), and CAM plants, such as *Clusia hilariana* (Franco *et al*. 1999) and *Phalaenopsis* (Pollet *et al*. 2009). Pollet *et al*. (2009) explained that low Φ_{PSII} and high NPQ at the transition from day to night was closely associated with the inactivation of the Calvin cycle. Indeed, in our CK plants, the sucrose concentration was lower at 18:00 than that at 12:00 h (Fig. 4*C*). However, the hexose content was not changed (Fig. 4*A* and *B*) and the starch amount greatly increased from 12:00 to 18:00 h (Fig. 4*D*). The starch content could increase due to the increase in the amount of accessible precursors for starch biosynthesis during this period, including CO_2 source derived from ambient (Fig. 2*B*) and malate decarboxylation (Fig. 3*A*), the carbon-skeleton source derived from malate decarboxylation (Fig. 3*A*), sucrose degradation (Fig. 4*C*), and the high rate of light reaction as evidenced by the high O_2 evolution (Table 2).

Lowering irradiance does not affect the sucrose concentration in the stem: One of the most intriguing findings is that lowering irradiance did not affect the sucrose concentration in the stem and its concentrations were unusually higher than that in the leaf throughout the day (Fig. 4*C*,*G*). Due to the dormant spike bud of *Phalaenopsis* directly connected with the stem, the correlation between the spike emergence and sucrose concentration is seemingly not so tight, even though many studies have mentioned a positive relationship between sucrose concentration and spiking of *Phalaenopsis* (Konow and Wang 2001, Kataoka *et al*. 2004, Guo and Lee 2006, Tsai *et al*. 2008, Wu *et al*. 2013).

Sucrose is an important compatible solute (Wang *et al*. 2000, Roussos *et al*. 2010). It is explicable that the different leaves of *Phalaenopsis* under a limiting irradiance maintain only a threshold content of sucrose for survival and transport the surplus into the stem to sustain the viability of the dormant spike bud(s). In fact, such a high sucrose content in the stem also occurs after warm-night treatment, which is also a strategy for delaying spike development (Liu *et al*. 2013). Hence, the shortened stem of *Phalaenopsis* plays a storage function having a strong sink activity to store enough sucrose from all source leaves (Liu *et al*. 2013), to supply a carbon source for spike development when *Phalaenopsis* is under suitable flowering conditions, and to maintain the viability of dormant spike bud(s) when *Phalaenopsis* is exposed to a spiking inhibition condition.

Conclusion: We reported that lowering of irradiance greatly decreased photosynthetic performance and delayed the spiking, but did not affect the potential function of photosynthetic apparatus and retained enough supply of sucrose in the stem, where the spiking takes place. Our results confirmed that *Phalaenopsis* possesses the inherent ability to acclimate to low-light arboreal environments. We suggested that the correlation between sucrose and spiking was not apparently so tight and the crucial factors for spiking of *Phalaenopsis* remain to be ascertained.

References

Baker N.R.: Chlorophyll fluorescence: A probe of photosynthesis *in vivo*. – Annu. Rev. Plant Biol. **59**: 89-113, 2008.

Bilger W., Björkman O.: Role of the xanthophylls cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in *Hedera canariensis*. – Photosynth. Res. **25**: 173-185, 1990.

Callaway T.R., Martin S.A., Wampler J.L. *et al*.: Malate content of forage varieties commonly fed to cattle. – J. Dairy Sci. **80**: 1651-1655, 1997.

Ceusters J., Borland A.M., Godts C. et al.: Crassulacean acid metabolism under severe light limitation: a matter of plasticity in the shadows? – J. Exp. Bot. **62**: 283-291, 2011.

Chang X., Alderson P.G., Wright C.J.: Solar irradiance level alters the growth of basil (*Ocimum basilicum* L.) and its content of volatile oils. – Environ. Exp. Bot. **63**: 216-223, 2008.

Chen W.H., Tseng Y.C., Liu Y.C. *et al*.: Influence of cool-night on the photosynthetic efficiency and the nonstructural carbohydrate and organic acid pools in *Phalaenopsis aphrodite*. – Plant Cell Rep. **27**: 1667-1675, 2008.

Chugh S., Guha S., Rao I.U.: Micropropagation of orchids:

A review on the potential of different explants. – Sci. Hortic.-Amsterdam **122**: 507-520, 2009.

Demmig-Adams B., Adams Ⅲ W.W., Baker D.H. *et al*.: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plantarum **98**: 253-264, 1996.

Endo M., Ikusima I.: Diurnal rhythm and characteristics of photosynthesis and respiration in the leaf and root of a *Phalaenopsis* plant. – Plant Cell Physiol. **30**: 43-47, 1989.

Endo M., Ikusima I.: Changes in concentrations of sugars and organic acids in the long-lasting flower clusters of *Phalaenopsis*. – Plant Cell Physiol. **33**: 7-12, 1992.

Franco A.C., Herzog B., Hübner C. *et al*.: Diurnal changes in chlorophyll a fluorescence, CO_2-exchange and organic acid decarboxylation in the tropical CAM tree *Clusia hilariana*. – Tree Physiol. **19**: 635-644, 1999.

Genty B., Briantais J.M., Baker N.R.: The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Guo W.J., Lee N.: Effect of leaf and plant age and day/night temperature on net CO_2 uptake in *Phalaenopsis amabilis var. Formosa.* – J. Am. Soc. Hortic. Sci. **131**: 320-326, 2006.

Hisamatsu T., Sugiyama Y., Kubota S., Koshioka M.: [Delaying anthesis by dark treatment in *Phalaenopsis*.] – J. Jap. Soc. Hortic. Sci. **70**: 264-266, 2001. [In Japanese]

Kataoka K., Sumitomo K., Fudano T., Kawase K.: Changes in sugar content of *Phalaenopsis* leaves before floral transition. – Sci. Hortic.-Amsterdam **102**: 121-132, 2004.

Konow E.A., Wang Y.T.: Irradiance levels affect *in vitro* and greenhouse growth, flowering, and photosynthetic behavior of a hybrid *Phalaenopsis* orchid. – J. Am. Soc. Hortic. Sci. **126**: 531-536, 2001.

Kubota S., Yoneda K.: [Effects of light intensity preceding day/night temperatures on the sensitivity of *Phalaenopsis* to flower.] – J. Jap. Soc. Hortic. Sci. **62**: 595-600, 1993. [In Japanese]

Liu C.H., Liu Y.C., Chen W.H., Wang H.L.: [Effects of underneath-bench shading treatment on spiking and flowering of *Doritaenopsis*.] – J. Agr. Assoc. Taiwan **11**: 501-513, 2010. [In Chinese]

Liu Y.C., Tseng K.M., Chen C.C. *et al*.: Warm-night temperature delays spike emergence and alters carbon pool metabolism in the stem and leaves of *Phalaenopsis aphrodite*. – Sci. Hortic.-Amsterdam **161**: 198-203, 2013.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence. - A practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Osmond C.B.: Crassulacean acid metabolism: a curiosity in context. – Annu. Rev. Plant Physio. **29**: 379-414, 1978.

Pollet B., Steppe K., Van Labeke M.C., Lemeur R.: Diurnal cycle of chlorophyll fluorescence in *Phalaenopsis*. – Photosynthetica **47**: 309-312, 2009.

Pollet B., Vanhaecke L., Dambre P. *et al*.: Low night temperature acclimation of *Phalaenopsis*. – Plant Cell Rep. **30**: 1125-1134, 2011.

Popp M., Janett H.P., Lüttge U., Medina E.: Metabolite gradients and carbohydrate translocation in rosette leaves of CAM and C3 bromeliads. – New Phytol. **157**: 649-656, 2003.

Quiles M.J.: Photoinhibition of photosystem I and II using chlorophyll fluorescence measurements. – J. Biol. Edu. **39**: 136-138, 2005

Roháček K., Barták M.: Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. – Photosynthetica **37**: 339-363, 1999.

Roussos P.A., Denaxa N.K., Damvakaris T. *et al*.: Effect of alleviating products with different mode of action on physiology and yield of olive under drought. – Sci. Hortic.-Amsterodam **125**: 700-711, 2010.

Sakanishi Y., Imanishi H., Ishida G.: [Effect of temperature on growth and flowering of *Phalaenopsis amabilis*.] – Bull. Univ. Osaka Prefecture, **B 32**: 1-9, 1980. [In Japanese]

Souza A., de Paula A., Figueiredo-Ribeiro R.C.L.: Effects of irradiance on non-structural carbohydrates, growth, and hypoglycemic activity of *Rhynchelytrum repens* (Willd.) C.E. Hubb. (Poaceae). – Braz. J. Biol. **64**: 697-706, 2004.

Tsai W.T., Wang Y.T., Lin H.L.: Alternating temperature affects spiking of a hybrid *Phalaenopsis*. – Acta Hortic. **766**: 307-314, 2008.

Wang H.L., Lee P.D., Chen W.L. *et al*.: Osmotic stress-induced changes of sucrose metabolism in cultured sweet potato cells. – J. Exp. Bot. **51**: 1991-1999, 2000.

Wang Y.T.: *Phalaenopsis* light requirement during the induction of spiking. – HortScience **30**: 59-61, 1995.

Wang Y.T.: *Phalaenopsis* light requirements and scheduling of flowering. – Orchids **66**: 934–939, 1997.

Wu P.H., Liu C.H., Tseng K.M. *et al.*: Low irradiance alters carbon metabolism and delays flower stalk development in two orchids. – Biol. Plantarum **57**: 764-768, 2013.

Yang J.D., Zhao H.L., Zhang T.H.: Diurnal patterns of net photosynthetic rate, stomatal conductance, and chlorophyll fluorescence in leaves of field-grown mungbean (*Phaseolus radiatus*) and millet (*Setaria italica*). – J. Crop Hortic. Sci. **32**: 273-279, 2004.