Effects of Simulated Dark Shipping on Photosynthetic Status and Post-shipping Performance in *Phalaenopsis* Sogo Yukidian ‘V3’

Jiunn-Yan Hou

Department of Horticulture, National Taiwan University, Taipei 10617, Taiwan

Tim L. Setter

Department of Crop and Soil Science, Cornell University, Ithaca, NY 14853

Yao-Chien Alex Chang¹

Department of Horticulture, National Taiwan University, Taipei 10617, Taiwan

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ABSTRACT. *Phalaenopsis* plants are routinely shipped long distances in total darkness. To determine how these long dark periods affect photosynthetic status in *Phalaenopsis* Sogo Yukidian ‘V3’, changes of net CO₂ uptake, photosystem II (PS II) efficiency, and abscisic acid (ABA) concentration after a long-term simulated dark shipping were investigated. Net CO₂ uptake rate, malate concentration, and titratable acidity in potted *Phalaenopsis* Sogo Yukidian ‘V3’ decreased after a 21-day simulated dark shipping at 20 °C, but recovered gradually with time after shipping. It took 6 to 9 days to recover to a normal photosynthetic status after shipping. The value of Fv/Fm was little affected by shipping. Therefore, net CO₂ uptake rate would be a better indicator for estimating the recovery time after shipping. After shipping, fresh weight loss, leaf ABA concentration, and number of yellowed leaves of bare-root plants were higher than those of potted plants, and increased with longer durations (7, 14, and 21 days) of the simulated dark period. The spiking (the emergence of flowering stems) date was delayed when plants were stored in a bare-root condition. The concentration of ABA in leaves rose in the first 3 days after simulated shipping and then decreased within the next 3 to 8 days. Plants that received photosynthetic photon flux (PPF) at 399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ after shipping had lower PS II efficiency and reduced net CO₂ uptake rate than those given less PPF levels. We recommend a post-shipping acclimation for 6 to 9 days with gradual light increase (34–72–140–200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) or maintaining a light level of 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF for *Phalaenopsis* to achieve a better photosynthetic status after prolonged dark storage.

Phalaenopsis orchid is one of the most valued potted ornamental plants in the world. It is usually micropropagated, produced, and sold in more than one country, thus the production of *Phalaenopsis* has become an international specialized industry. Plants are often transported intercontinentally in a bare-root condition due to quarantine requirements. Therefore, the transportation duration must be kept short by way of air freight. Taiwan has been certified by the U.S. Department of Agriculture since 2005 to export *Phalaenopsis* with potting medium to the United States under a specified process. Shipping *Phalaenopsis* with potting medium reduces stress during transport, thereby permitting shipment by sea freight, which much lowers costs. However, shipping plants from Taiwan to the United States by marine transport takes about 2 to 3 weeks, and the effects of long-term dark storage on *Phalaenopsis* physiology were not known.

Mature leaves of *Phalaenopsis* exhibit typical crassulacean acid metabolism (CAM) photosynthetic pathway (Endo and

Ikusima, 1989; Guo and Lee, 2006; Ota et al., 1991). Some CAM plants such as *Opuntia basilaris* (Szarek et al., 1973) and *Xerosicyos danguyi* (Bastide et al., 1993; Rayder and Ting, 1983) shift their photosynthetic pattern from CAM to CAM-idling during a long period of drought. CAM-idling is defined as a damped form of CAM in which plants maintain diurnal fluctuation of organic acid by recycling respiratory CO₂ without stomata opening. Under such a circumstance, the total organic acid concentration in plants gradually dropped during the drought period but rapidly recovered after rewatering (Bastide et al., 1993). The plasticity of photosynthetic status was also observed in *Doritaenopsis* Tinny Tender, in which net CO₂ uptake rate declined with increasing period of drought and had a sudden revival after rewatering (Cui et al., 2004). However, up to the present, there has been no research related to the effects of long-term dark storage on photosynthetic status of *Phalaenopsis*.

Chlorophyll fluorescence is a subtle reflection of the primary processes of photosynthesis that take place in the chloroplasts (DeEll et al., 1999). Up to now, effects of dark storage (Su et al., 2001), light intensity (Lin and Hsu, 2004), growth stage (Hsu, 2007), and diurnal cycle (Pollet et al., 2009) on chlorophyll fluorescence changes in *Phalaenopsis* had been studied. The quantum efficiency of *Phalaenopsis equestris* leaf was unaffected after exposure to 25 °C with 70% or 10% relative humidity in a dark growth chamber for up to 30 d, but was reduced after exposure to 35 °C (Su et al., 2001). The study

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¹Corresponding author. E-mail: alexchang@ntu.edu.tw.

showed that *Phalaenopsis* is tolerant of long-term dark storage under a favorable environment, though it did not provide information on subsequent vegetative and flowering performance.

Plants, transferred from shipping container to greenhouse, often experience a sharp light intensity change. This can easily cause leaf yellowing or sunburn when higher than tolerable light intensities are provided after dark storage. As a heavily self-shading plant, lower mature leaves of *Phalaenopsis* are adaptive to low light, and receive less than one-sixth the light intensity of upper leaves; however, they possess the ability to reacclimate to high light (Lin and Hsu, 2004). Net photosynthetic rate of *Phalaenopsis* saturates at 130 to 180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux (PPF) (Lootens and Heursel, 1998; Ota et al., 1991). Commercial growers generally provide 280 to 380 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF to their *Phalaenopsis* plants (Chen and Wang, 1996). *Phalaenopsis* exposed immediately to the regular culturing light intensity after dark shipping may be injured.

The objectives of this study were to investigate the photosynthetic status after simulated dark shipping (SDS), to determine the effects of bare-root treatment and dark-storage duration on leaf hormonal content and post-shipping quality, and to determine the optimal light intensity for maximizing photosynthetic efficiency right after long-term dark storage of *Phalaenopsis* Sogo Yukidian 'V3'.

Materials and Methods

PLANT MATERIALS. The white-flowered clone *Phalaenopsis* Sogo Yukidian 'V3', purchased from Clone International Biotech (Pingtung, Taiwan), was used in this study. Plants with a leaf span of 35 to 40 cm, mature and able to flower, were grown in 10.5-cm-diameter clear, soft plastic pots (0.75 L) that were tightly filled with sphagnum moss. Plants were fertigated as needed with a 20N-8.6P-16.6K water-soluble fertilizer (Peters Professional 20-20-20; Scotts, Marysville, OH) at 1 $\text{g}\cdot\text{L}^{-1}$. Fertigation was stopped ≈ 7 d before SDS to reduce the risk of disease occurrence. Heating and cooling (double-shaded cloth) systems in the greenhouse were programmed to maintain an average day/night temperature of 28/25 °C and a maximum PPF of 370 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

MEASUREMENT OF NET CO₂ UPTAKE RATE AND CHLOROPHYLL FLUORESCENCE. Measurements of net CO₂ uptake rate and chlorophyll fluorescence were conducted on the newly matured leaf (the second leaf from the apex). Measurement area was located at the middle of each leaf near the midrib.

Net CO₂ uptake rate was measured with a portable photosynthesis system (LI-6400; LI-COR, Lincoln, NE). Because *Phalaenopsis* are CAM plants, the measurements were done in dark at 2100 HR in the greenhouse. Each measurement was conducted on 6 cm² of leaf area. External air was scrubbed of CO₂ and then mixed with a supply of pure CO₂ to create a standard concentration of 350 $\mu\text{mol}\cdot\text{mol}^{-1}$. Air flow rate was controlled at 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. During the measurement, the leaf was kept in the leaf chamber for 20 to 30 s to equilibrate with the ambient microconditions. Net CO₂ uptake rate and stomatal conductance (g_s) were recorded.

Leaf chlorophyll fluorescence was measured at 1300 HR. Minimal fluorescence (F_o) was determined after a 40-min dark adaptation, and maximal fluorescence (F_m), quantum yield, photochemical quenching (Q_p), and non-photochemical quenching (Q_n) were measured after a saturation pulse with a photosynthesis yield analyzer (MINIPAM; Heinz Walz,

Effeltrich, Germany). The fluorescence ratio F_v/F_m, where F_v = F_m - F_o, was then calculated.

DETERMINATION OF MALATE CONCENTRATION AND TITRABLE ACIDITY. Leaf discs (≈ 0.2 g each sample) were collected at 0700 HR and were frozen immediately in liquid N₂ after weighing. The frozen tissues were ground with 5 mL of distilled water. The crude extract was then transferred to a test tube and boiled for 10 min. After cooling to room temperature, the crude extract solution was clarified by centrifugation at 10,000 g_n for 5 min. The subsequent analysis was done as described by Guo and Lee (2006) with slight modification. The reactions were conducted by mixture of aliquot of extract solution, malate-dehydrogenase, and nicotinamide adenine dinucleotide (β-NAD), and then were incubated at 30 °C for 1 h. The absorbance at 340 nm was determined with a spectrophotometer (U2800; Hitachi, Pleasanton, CA). Standard curves were made to calculate malate concentration in leaves.

The sampling and extraction for titratable acidity were similar to the malate analysis. The following procedure followed Chu et al. (1990) with slight modifications. A 4-mL aliquot of the supernatant was titrated with 0.01 N NaOH (prepared freshly in distilled water) to an endpoint of pH 8.3.

DETERMINATION OF LEAF RELATIVE WATER CONTENT. The procedure was according to Andrade (2003). Leaf discs (≈ 0.2 g) were sampled from a newly matured leaf and were weighed immediately as fresh weight (FW). Turgid weight (TW) was measured after hydrating the leaf discs for 24 h at 4 °C in plastic vials with distilled water. Stable dry weight (DW) was determined after samples had been dehydrated at 65 °C for 48 h. Relative water content (RWC) is defined as (FW - DW)/(TW - DW).

DETERMINATION OF ABA CONCENTRATION. The sampling procedure was similar to that for RWC described above. Leaf discs (≈ 0.2 g) were frozen in liquid N₂ after recording fresh weight. The samples were extracted with 200 μL of 80% methanol at 25 °C for 6 h. A 100- μL aliquot of each extract was put in a 96-well plate and dried at 45 °C. The samples were redissolved and separated with reverse-phase chromatography on columns packed with C₁₈-silica material (J.T. Baker Chemicals, Philipsburg, NJ). The analysis of ABA was by the method of indirect enzyme-linked immunosorbant assay (ELISA) as described by Setter et al. (2001).

EXPT. 1. EFFECT OF LONG-TERM DARK STORAGE ON PHOTOSYNTHETIC STATUS. The experiment was designed to investigate changes of photosynthetic status affected by SDS. On 27 July, plants designated to receive SDS treatment (referred to as "shipped plants") were put into cartons with shredded newspaper and then placed in a dark growth chamber at a constant 20 °C for 21 d. Relative humidity was 40% to 50% inside the carton during storage, as collected by a datalogger (H08-004-02; Onset, Buzzards Bay, MA). Control plants did not receive SDS and were continually grown in the greenhouse. After the completion of storage for 21 d, shipped plants were returned to the greenhouse and were arranged in a completely randomized design. Changes of net CO₂ uptake rate, malate concentration, titratable acidity, and parameters of chlorophyll fluorescence were determined before the 21-d SDS and 0, 1, 2, 3, 6, 9, 12, and 15 d after SDS. Samples for measuring malate concentration and titratable acidity were taken from the middle and near the midrib of the third leaf (from the apex) at 0700 HR. Each treatment consisted of eight single-plant replications. The experiment was repeated three

times with similar results. Results from the third experiment are reported.

EXPT. 2. EFFECTS OF BARE-ROOTING AND DARK STORAGE DURATION ON POST-SHIPING PERFORMANCE. The experiment was to compare post-shipping performance between *Phalaenopsis* being stored with or without potting medium for various durations. On 6 Dec., 13 Dec., and 20 Dec., 14 potted plants and 14 bare-root plants were moved to a growth chamber for SDS at 20 °C. Bare-root plants were taken out of the sphagnum moss media and placed in a ventilated room for 1 d before storage. On 27 Dec., all plants were moved out from the dark growth chamber so that the plants received a 7-, 14-, or 21-d SDS. Plants were weighed with (potted plants) or without potting medium (bare-root plants) before and after storage. After SDS, the bare-root plants were replanted into 10.5-cm-diameter pots with sphagnum moss and all plants were moved to a phytotron with 30 °C day/25 °C night and a light intensity of 200 to 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF. Control plants did not receive SDS and were continually grown in the phytotron. The number of yellowed leaves was counted on Day 0 after storage. A leaf in which more than 20% of its surface was yellow, or the basal part had become yellow, was defined as a “yellowed leaf.” Net CO_2 uptake rate and g_s were determined before and 0, 1, 2, 3, 4, 6, 8, 10, 12, and 14 d after storage. Leaves for determining relative water content and ABA concentration were sampled from the same plant. Sampling for relative water content was carried out on 0, 3, and 6 d after SDS, and for the ABA concentration 0, 3, 6, and 11 d after SDS. Spiking is defined as the emergence of flower stem from the base of a leaf. On 14 Jan., plants were all transferred to a greenhouse for subsequent floral development, and were arranged in a completely randomized design with 14 single-plant replications. Measurements of net CO_2 uptake rate and g_s were conducted on five replications.

EXPT. 3. DETERMINATION OF THE OPTIMAL LIGHT INTENSITY RIGHT AFTER A LONG-TERM DARK STORAGE. To determine the effect of the light level after shipping on *Phalaenopsis*, 35 plants were subjected to a 21-d SDS. The shipping treatment procedures were similar to those described in Expt. 1. Plants were then moved to a growth chamber with 30 °C day/25 °C night, and given 34, 72, 140, 200, or 399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF at a 12-h photoperiod. Light was provided by high-pressure sodium lamps, and various PPFs were achieved by using shade clothes and the adjustment of bench height. Control plants did not receive SDS and were grown in a growth chamber with day/night temperature of 30/25 °C with a PPF of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Net CO_2 uptake rate, g_s , and parameters of chlorophyll fluorescence were taken before storage and 0, 1, 2, 3, 4, 5, 9, 12, and 15 d after storage. The relative chlorophyll concentration and the leaf color were, respectively, measured with a chlorophyll meter (SPAD502; Minolta, Tokyo) and a spectrophotometer (CM-2600d; Konica Minolta Sensing, Osaka, Japan) on the 15th d from the end of SDS. Plants were transferred to a 25 °C day/20 °C night phytotron for flowering and were arranged in a completely randomized design. There were seven single-plant replications in each treatment.

STATISTICAL ANALYSIS. Data were subjected to analysis of variance by using a completely randomized design. Means separation between treatments was obtained using the least significance difference (LSD) test at $P \leq 0.05$. Statistic analyses were performed using Costat (version 6.1; CoHort Software, Monterey, CA).

Results

EXPT. 1. EFFECT OF LONG-TERM DARK STORAGE ON PHOTOSYNTHETIC STATUS. After dark storage, plants were in good appearance except that the youngest growing leaves were light green and pale from the base to the apex (data not shown). Net CO_2 uptake rates in leaves of shipped plants was below zero on Day 0 from the end of SDS, significantly lower than those of controls (Fig. 1A). Net CO_2 uptake rate increased gradually with time after SDS and recovered to a similar level as the control plants on Day 6. Malate concentration and titratable acidity also decreased during SDS and gradually increased with time after SDS (Fig. 1, B and C). The results indicated that photosynthesis capacity of *Phalaenopsis* markedly decreased after a 21-d SDS, but had recovered to a regular status in 6 to 9 d after being placed in a normal culture environment.

No differences were found in F_v/F_m , F_o , and F_m in leaves on Day 0 between shipped and control plants (Fig. 2). The values of F_v/F_m in controls and shipped plants fluctuated at 0.82 to 0.83, and 0.81 to 0.82, respectively, during the evaluation period (Fig. 2A). These represent typical healthy, non-photoinhibited leaves (Bolhar-Nordenkamp et al., 1989). The higher value of F_v/F_m in control plants was due to a higher value of F_m in the first 2 d after SDS and a lower value of F_o on the other days. There was no significant difference between control and shipped plants in Q_p on Day 0 from the end of SDS;

Fig. 1. Changes of net CO_2 uptake rate (A), malate concentration (B), and titratable acidity (C) in *Phalaenopsis* Sogo Yukidian ‘V3’ before and 0, 3, 6, 9, 12, and 15 d after a 21-d simulated dark shipping. Controls did not receive simulated shipping and were grown in a greenhouse with day/night temperature of 28/25 °C. All shipped plants were moved to the same greenhouse after the simulated dark shipping. Bars indicate SE ($n = 8$). * indicates $P \leq 0.05$, ** indicates $P \leq 0.01$, and *** indicates $P \leq 0.001$.

Fig. 2. Changes of Fv/Fm (A), Fo (B), and Fm (C) in *Phalaenopsis* Sogo Yukidian 'V3' 0, 3, 6, 9, 12, and 15 d after a 21-d simulated dark shipping. Controls did not receive simulated shipping and were grown in a greenhouse with day/night temperature of 28/25 °C. Shipped plants were placed in the same greenhouse after the simulated dark shipping. Bars indicate SE (n = 8). * indicates $P \leq 0.05$, ** indicates $P \leq 0.01$, and *** indicates $P \leq 0.001$.

however, slightly lower values of Q_p on Days 3 to 15 in shipped plants than in controls were observed (data not shown). The results suggest that the PS II of *Phalaenopsis* functioned normally after a 21-d SDS. Reductions of Fv/Fm and Q_p in shipped plants during the recovery period may have been caused by sharp changes of light intensity and temperature between shipping and cultivation. The reduction of photosynthetic capacity in *Phalaenopsis* after SDS was not due to the damage of PS II system. Therefore, Fv/Fm was not a suitable indicator for investigating the effect of dark storage on photosynthetic status in *Phalaenopsis*. The gas exchange rate was thus used as an indicator to investigate the photosynthetic status in *Phalaenopsis* after SDS in the next experiment.

EXPT. 2. EFFECTS OF BARE-ROOTING AND DARK STORAGE DURATION ON POST-SHIPING PERFORMANCE. The absence of potting medium during SDS greatly affected post-shipment quality of *Phalaenopsis* Sogo Yukidian 'V3'. A higher number of yellowed leaves was observed in bare-root plants than potted plants (Table 1). Fresh weights decreased with shipping time, which were 96%, 92%, and 91% of its initial weight after a 7-, 14-, and 21-d SDS, respectively, in potted plant and 83%, 77%, and 72% in bare-root plants (data not shown). The relative leaf water content had a similar tendency with fresh weight, and more severe water loss was found in bare-root plants. Bare-root plants, after being stored for 21 d, had the lowest relative water content (79.0%); drooping leaves and shriveled leaf surfaces

were also observed. The reduction of the relative water content was not permanent. Leaf relative water content gradually increased after potting and growing plants in a greenhouse and showed no differences with control plants 6 d after the end of SDS (Table 1). The concentrations of ABA in leaves ascended with storage time and were higher in bare-root plants than in potted plants on Day 0 from the end of SDS. High values of ABA concentration in bare-root plants were observed on Day 3 from the end of SDS. Values sharply increased in the first 3 d after the end of SDS and then declined to normal levels with time. *Phalaenopsis* stored without potting medium resulted in a higher number of yellowed leaves, greater water loss, and higher ABA accumulation than in the undisturbed plants (Table 1).

Net CO_2 uptake rate and g_s were close to 0 in all shipped plants on Day 0 upon completion of the SDS, and were much lower than those in the controls (Fig. 3). Net CO_2 uptake rate of controls on Day 2 showed a steep decrease because it was a rainy day during which light intensity was only 37 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF at noon of that day. Net CO_2 uptake rate in shipped plants increased with time and recovered to a regular level within 6 to 8 d after the end of SDS (Fig. 3, A and B). This shows that *Phalaenopsis* has good recovery ability of photosynthesis after shipping for up to 21 d, regardless of whether they were potted or bare-rooted.

The subsequent flowering quality after SDS of plants was strongly affected by how *Phalaenopsis* plants were stored. The shipped plants were placed in a phytotron with 30 °C day/25 °C night for 18 d after the end of SDS, and were then moved to a greenhouse for flower forcing by a natural cool climate. The spiking time was delayed by bare-root treatment and duration of storage (Table 2). A delay in spiking time of 40 d was recorded between the bare-root plants stored for 21 d and the controls. More bare-root plants had two stalks but fewer branches and lower flower counts on the primary stalk. As a result, total flower count was similar in all plants. A reduction of primary stalk flower count and growing fewer stalk branches reduced the quality for commercial sale.

EXPT. 3. DETERMINATION OF THE OPTIMAL LIGHT INTENSITY RIGHT AFTER A LONG-TERM DARK STORAGE. In previous experiments, we observed that the net CO_2 uptake rate was greatly reduced after a long-term SDS, and a period of time was required to recover to a regular level. The effect of light intensity on *Phalaenopsis* during the recovery period after a 21-d SDS was determined. All shipped plants had low net CO_2 uptake rate at the end of SDS (Fig. 4A). One day after the end of SDS, net CO_2 uptake rate was similar between all shipped plants under various levels of PPF. Net CO_2 uptake rate of plants under 140 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF rapidly increased to the level of control plants within 4 d after the end of SDS (Fig. 4A). Net CO_2 uptake rate of plants in 34 and 72 $\mu mol \cdot m^{-2} \cdot s^{-1}$ treatments increased with time during the early days of recovery period; however, they reached a plateau after 4 to 5 d and were not able to achieve the level of control plants within 15 d after the end of SDS (Fig. 4A). Recovery of net CO_2 uptake rate and g_s was greatly inhibited by a high light intensity (399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF). Net CO_2 uptake rose slowly with time during recovery period (Fig. 4). The relationship between PPF and net CO_2 uptake rate changed with time after SDS (Fig. 5). No correlation between PPF and net CO_2 uptake rates on Day 1 ($R^2 = 0.23$, $P = 0.02$) was seen; all shipped plants had comparably low net CO_2 uptake rates, regardless of PPF. Net

Table 1. Number of yellowed leaves, changes of relative water content, and abscisic acid (ABA) concentration in *Phalaenopsis* Sogo Yukidian 'V3' after being stored potted or bare-rooted at 20 °C for 7, 14, or 21 d (n = 5).

Duration in simulated shipping (d)	Shipping condition	Yellowed leaves (no.) ^z	Relative water content (%)			ABA concn (pmol·g ⁻¹ FW)			
			Time after the end of simulated dark shipping (d) ^y			Time after the end of simulated dark shipping (d)			
			0	3	6	0	3	6	11
Control ^x	Potted	0.1	87.9	90.2	90.0	14.6	22.2	14.4	17.0
7	Bare-root ^w	0.7	85.9	87.6	90.4	12.6	35.6	12.6	21.6
	Potted	0.3	92.5	91.1	91.3	7.6	17.4	13.8	19.8
14	Bare-root	1.3	86.2	82.9	86.9	30.4	66.8	18.2	16.8
	Potted	0.2	88.7	91.5	88.7	8.2	15.6	11.6	25.8
21	Bare-root	1.4	79.0	80.5	86.7	23.4	74.4	13.2	18.0
	Potted	0.4	90.5	91.5	90.4	14.2	12.8	15.6	19.6
Significance									
Duration		NS	*	NS	NS	*	NS	NS	NS
Shipping condition		***	***	**	NS	***	***	NS	NS
Duration × shipping condition		NS	*	NS	NS	*	NS	**	NS

^zThere were 14 replications in each treatment for yellow-leaf investigation.

^yBare-root plants were repotted at the end of simulated dark shipping.

^xControls did not receive simulated shipping and were grown in a phytotron with day/night temperature of 30/25 °C. All shipped plants were placed in the same phytotron after the simulated dark shipping.

^wSphagnum moss was removed to bare-root the plants.

NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Fig. 3. Changes of net CO₂ uptake rate (A and B) and stomatal conductance (g_s) (C and D) in *Phalaenopsis* Sogo Yukidian 'V3' 0, 1, 2, 3, 4, 6, 8, 10, 12, and 14 d after being stored potted (A and C) or bare-rooted (B and D) at 20 °C for 7, 14, or 21 d. Controls did not receive simulated shipping and were grown in a phytotron with day/night temperature of 30/25 °C. All shipped plants were placed in the same phytotron after the simulated dark shipping. Bars indicate SE (n = 7).

CO₂ uptake rates in shipped plants had a marked increase on Day 2 except for treatment at 399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF (Fig. 5). A quadratic relationship between PPF and net CO₂ uptake rate was found on Days 2 through 9, and the peaks of the curves shifted to the right side with time after storage, which means that the requirement of light after dark storage increased with time in *Phalaenopsis* (Fig. 5). However, the curves had a marked decline at 399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF (Fig. 5). The results showed that reduction of net CO₂ uptake rate in plants after a long-term SDS was able to recover to a normal status at a medium light intensity (140–200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF), but was

restricted by low (34–72 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) and high (399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) light intensities.

Data of chlorophyll fluorescence were similar in all plants before and after SDS, indicating that PS II of plants was not damaged during storage (Figs. 6 and 7). The values of Fv/Fm were slightly and markedly reduced in plants under 200 and 399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF, respectively, during post-shipping acclimation (Fig. 6A). This was mainly due to a reduction of Fm (Fig. 6C). Quantum yield showed a similar trend with Fv/Fm (Fig. 7A). Photochemical quenching (Qp) in plants under 399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF decreased in the early period of post-shipping acclimation, then increased with time (Fig. 7B). Non-photochemical quenching in plants had a contrary trend with Qp under 399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF (Fig. 7C).

With increasing light intensity being provided, SPAD values of shipped plants declined and L, a, and b values increased after 15 d of light acclimation (Table 3). It could be inferred that an excessive light intensity resulted in degradation of chlorophyll and color fading in leaves.

The plants were placed in phytotron with 30 °C day/25 °C night for light acclimation for 15 d after storage, and then were moved to phytotron with 25 °C day/20 °C night in same light environment for flowering forcing. Plants provided with low light intensities (34 and 72 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) in post-shipping light acclimation spiked early; however, only 71% plants spiked under such low light intensities 60 d after the beginning of flowering forcing. Shipped plants provided with 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF all spiked in 60 d after the start of flowering forcing (Table 4).

Discussion

The value of Fv/Fm refers to the maximal quantum efficiency of PS II, which is typically in the range of 0.75 to 0.85 for non-stressed plants (Bolhar-Nordenkamp et al., 1989). In our study, the values ranged 0.81 to 0.83 in shipped plants at the end

Table 2. Subsequent flowering quality of *Phalaenopsis* Sogo Yukidian 'V3' after being stored potted or bare-rooted at 20 °C for 7, 14, or 21 d.

Duration in simulated shipping (d)	Shipping condition	Flower spikes (no.)	Total flower count (no.)	Primary flower stalk ^z			
				Time from the end of simulated dark shipping to spiking (d)	Stalk length (cm)	Stalk diam (mm)	Flowers (no.)
Control ^y	Potted	1.1	19.6	9	88.4	4.5	18.8
7	Bare-root ^x	1.3	17.9	29	87.3	4.9	15.2
	Potted	1.1	18.3	8	87.5	4.8	17.6
14	Bare-root	1.6	18.9	40	82.3	5.2	14.3
	Potted	1.0	18.4	18	91.5	5.1	17.6
21	Bare-root	1.5	16.4	49	84.7	5.0	12.3
	Potted	1.2	18.0	27	84.3	5.1	16.5
Significance							
Duration		NS	NS	*	NS	NS	NS
Shipping condition		***	NS	***	NS	NS	***
Duration × shipping condition		NS	NS	NS	NS	NS	NS

^zThe first stalk emerged from stem.

^yControls did not receive simulated shipping and were grown in a phytotron with day/night temperature of 30/25 °C. Shipped plants were placed in the same phytotron for 18 d after the simulated dark shipping. All plants were then moved to a greenhouse for flower forcing by a natural cool climate.

^xSphagnum moss was removed to bare-root the plants.

NS, *, *** Not significant or significant at $P \leq 0.05$ or 0.001, respectively (n = 14).

Fig. 4. Effect of light acclimatization at various levels of photosynthetic photon flux (PPF) on net CO₂ uptake rate (A) and stomatal conductance (gs) (B) in *Phalaenopsis* Sogo Yukidian 'V3'. Measurements were taken before and 0, 1, 2, 3, 4, 5, 9, 12, and 15 d after a 21-d simulated dark shipping at 20 °C. Controls did not receive simulated shipping and were grown in a growth chamber with day/night temperature of 30/25 °C and with a PPF of 200 μmol·m⁻²·s⁻¹. All shipped plants were moved to the same growth chamber as controls but received various PPF as indicated after the simulated dark shipping. Bars indicate SE (n = 7).

of 21-d dark storage and were similar to those of unshipped control plants (Figs. 2A and 6A). The results revealed that *Phalaenopsis* leaves could maintain the function of PS II during dark storage and rapidly recover photosynthesis afterward.

The bare-rooted *Phalaenopsis* was previously considered to not be hampered by a single environmental constraint, but was severely suppressed when given a combination of stresses (dark treatment and dehydration) (Su et al., 2001). Wang (2007) suggested that when losing less than one-fifth of the fresh

Fig. 5. The relationship between photosynthetic photon flux (PPF) and net CO₂ uptake rate of *Phalaenopsis* Sogo Yukidian 'V3' on Day 1 (—○—, $y = 0.0002 + 0.0028x - 0.0000066x^2$, $R^2 = 0.23$, $P = 0.02$), Day 2 (—●—, $y = 1.07 + 0.0096x - 0.0000292x^2$, $R^2 = 0.81$, $P < 0.0001$), Day 3 (—△—, $y = 1.31 + 0.011x - 0.0000305x^2$, $R^2 = 0.55$, $P < 0.0001$), Day 4 (—▲—, $y = 1.75 + 0.013x - 0.000083x^2$, $R^2 = 0.85$, $P < 0.0001$), Day 5 (—□—, $y = 1.56 + 0.019x - 0.000051x^2$, $R^2 = 0.74$, $P < 0.0001$), and Day 9 (—■—, $y = 1.52 + 0.028x - 0.000071x^2$, $R^2 = 0.69$, $P < 0.0001$) from the completion of simulated dark shipping. Bars indicate SE (n = 7).

weight, bare-root *Phalaenopsis* Atien Kaala could be stored at 20 to 25 °C for 14 d without affecting subsequent performance. However, our study suggests that the spiking date was greatly delayed when plants were stored in bare-root condition (Table 2). Accumulation of ABA was found in *Xerosicyos* under a period of drought, and the water stress-induced ABA increase in CAM plants may be related to stomatal closure, which reduces transpiration and elevates the level of respiratory CO₂ recycling (Bastide et al., 1993). It may also stimulate leaf senescence (Pourtau et al., 2004). The combination of water loss and ABA accumulation in bare-root *Phalaenopsis* may account for the higher number of yellowed leaves in bare-root plants than in potted plants after dark storage (Table 1).

Diurnal change of titratable acidity is considered an indicator of the capacity of CAM photosynthesis. Some CAM plants such as *Opuntia basilaris* (Szarek et al., 1973) and

Fig. 6. Effects of light acclimatization at various levels of photosynthetic photon flux (PPF) on F_v/F_m (A), F_o (B), and F_m (C) in *Phalaenopsis Sogo Yukidian* 'V3'. Measurements were taken before and 0, 1, 2, 3, 4, 5, 9, 12, and 15 d after a 21-d simulated dark shipping at 20 °C. Controls did not receive simulated shipping and were grown in a growth chamber with day/night temperature of 30/25 °C and with a PPF of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Shipped plants were placed in the same growth chamber as controls but received various PPF as indicated after the simulated dark shipping. Bars indicate SE (n = 7).

Xerosicyos danguyi (Bastide et al., 1993; Rayder and Ting, 1983) under severe drought for a long period of time may convert to CAM-idling in which organic acids fluctuate without exogenous CO_2 uptake and the magnitude of diurnal change of titratable acidity was reduced with prolonged stress time. Without CO_2 uptake, plants under CAM-idling maintain the capability of diurnal titratable acidity by recycling respiratory CO_2 (Rayder and Ting, 1983). The current study indicated that g_s and net CO_2 uptake rate of *Phalaenopsis* were low when plants were stored in dark for 7, 14, and 21 d, regardless of whether they had potting medium or not (Figs. 1, 3, and 4). Malate and titratable acid concentrations in shipped plants decreased markedly after a 21-d dark storage, probably due to a lack of exogenous CO_2 uptake (Fig. 1, B and C). From these results, it can be speculated that *Phalaenopsis* closes stomata during long-term dark storage to decrease water loss and increase the recycling ratio of respiratory CO_2 .

Nighttime CO_2 uptake was reduced by malate accumulation, which inhibited the activity of nocturnal phosphoenolpyruvate carboxylase (PEPC) (Baker et al., 1997; Ting, 1985). Daytime malate consumption of CAM plants was inhibited by a low light intensity (Ting, 1985). Our results suggested that the require-

Fig. 7. Effect of light acclimatization at various levels of photosynthetic photon flux (PPF) on quantum yield (A), photochemical quenching (B), and non-photochemical quenching (C) in *Phalaenopsis Sogo Yukidian* 'V3'. Measurements were taken before and 0, 1, 2, 3, 4, 5, 9, 12, and 15 d after a 21-d simulated dark shipping at 20 °C. Controls did not receive simulated shipping and were grown in a growth chamber with day/night temperature of 30/25 °C and with a PPF of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Shipped plants were placed in the same growth chamber as controls but received various PPF as indicated after the simulated dark shipping. Bars indicate SE (n = 7).

ment of light intensity in *Phalaenopsis* after a long-term dark storage increased with time (Fig. 5). Low light intensities (34 and 72 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) did not influence net CO_2 uptake in shipped plants in the first 1 or 2 d after dark storage, but resulted in low net CO_2 uptake rate subsequently comparing with unshipped plants (Figs. 4A and 5). Based on these considerations, the appropriate light intensity for acclimation after dark shipping would be 140 to 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF, under which the net CO_2 uptake rate in shipped plants would successfully recover to a normal status (Fig. 4A). However, under a light intensity of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF, maximal quantum efficiency in shipped plants remained lower than that of unshipped plants during the recovery period (Fig. 6A). This might have been caused by the sudden light change from 0 to 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF. A gradual increase of light intensity (34–200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) for 6 to 9 d is suggested during the post-shipping light acclimation. Providing a high light intensity (399 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) resulted in a steep increase of Qn in the first few days after dark storage and Qn declined gradually afterward (Fig. 7C); perhaps excessive irradiance dissipated into

Table 3. Leaf chlorophyll concentration estimated by SPAD value and leaf color determined by Lab values of *Phalaenopsis* Sogo Yukidian 'V3', measured at the end of a 15-d light acclimatization at various levels of photosynthetic photon flux (PPF) after a 21-d simulated dark shipping.

PPF ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	SPAD value	L value ^x	a value ^w	b value ^y
Control ^z	59.4 b ^y	38.0 b	-8.2 b	17.4 b
34	71.8 a	32.7 d	-8.0 b	11.8 d
72	61.1 b	34.7 c	-8.6 b	14.1 cd
140	61.1 b	37.6 b	-8.5 b	17.4 b
200	52.5 c	37.3 b	-8.1 b	16.4 bc
399	39.3 d	43.1 a	-5.9 a	24.3 a

^zControls did not receive simulated shipping and were grown in a growth chamber with day/night temperature of 30/25 °C and with a PPF of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Shipped plants were placed in the same growth chamber as controls but received various PPF as indicated after the simulated dark shipping.

^yMeans followed by a different letter in columns are significantly different at $P \leq 0.05$ by LSD test ($n = 7$).

^xThe L value refers to lightness, which ranges from 0 for black to 100 for white.

^wThe a value refers to redness, which is positive for red and negative for green.

^yThe b value refers to yellowness, which is positive for yellow and negative for blue.

Table 4. Subsequent spiking percentage of *Phalaenopsis* Sogo Yukidian 'V3', after a 21-d simulated dark shipping and a 15-d light acclimatization at various levels of photosynthetic photon flux (PPF).

PPF ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Spiking (%)		
	Time after the beginning of flower forcing (d) ^z		
	20	40	60
Control ^y	0	83	100
34	29	71	71
72	43	57	71
140	0	86	100
200	0	57	86
399	0	43	71

^zPlants were transferred to a phytotron with day/night temperature of 25/20 °C for flowering after the 15-d of light acclimatization.

^yPlants did not receive simulated shipping and were grown in a growth chamber with day/night temperature of 30/25 °C and with a PPF of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ($n = 7$).

heat in the antenna of PS II (DeEll et al., 1999). An abrupt decline of maximal quantum efficiency and quantum yield showed that PS II of chlorophyll was damaged by excessive light during the period investigated (Figs. 6A and 7A). Degradation of chlorophyll led to low net CO₂ uptake rate (Figs. 4A and 5) and yellowed leaves (Table 3).

In conclusion, the net photosynthesis in *Phalaenopsis* after a long-term dark storage required 6 to 9 d to recover to a normal status regardless of whether they were with potting medium or not. Also, providing a gradual increase of light intensity (34–72–140–200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) or maintaining it at 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF during the recovery period for 6 to 9 d is suggested for *Phalaenopsis* to achieve a better PS II efficiency and net CO₂ uptake rate. The post-shipping quality was greatly reduced when plants were stored bare-rooted and was worsened by prolonged storage. As a result, the bare-root plants dehydrated, leaf abscission increased, and spiking was delayed.

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