

## **Orchid Conservation in Singapore under Natural Conditions: Responses of *Grammatophyllum Speciosum* to Growth Irradiances**

**Jie He<sup>1\*</sup>, Teh Zhi Yong<sup>1</sup> & Tim Wing Yam<sup>2</sup>**

<sup>1</sup>Natural Sciences and Science Education Academic Group, National Institute of Education, Nanyang Technological University, Singapore

<sup>2</sup>Singapore Botanic Gardens, National Parks Board, 1 Cluny Road, Singapore

\*Correspondence: Dr. Jie He, Natural Sciences and Science Education Academic Group, National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637 616.  
Email: jie.he@nie.edu.sg

DOI: 10.12735/ips.v1n1p11

URL: <http://dx.doi.org/10.12735/ips.v1n1p11>

### **Abstract**

*Grammatophyllum speciosum* orchid plants were grown both on the ground and on trees under high light (HL) and low light (LL) conditions in Pasir Ris Park, Singapore. This paper studied photosynthetic energy utilization investigated by chlorophyll (Chl) fluorescence and photosynthetic pigments; plant water status measured by leaf relative water content (RWC) and stomatal conductance ( $g_s$ ). All plants had midday leaf RWC greater than 80%, indicating no severe water deficit occurred in any plants. Except for plants grown on trees under LL, midday depression of  $F_v/F_m$  ratios occurred in all other plants, indicating dynamic photoinhibition. There was no chronic photoinhibition in any plants as  $F_v/F_m$  ratios recovered to healthy level at early morning. Grown on the ground under HL, *G. speciosum* had higher photochemical quantum yield ( $\Delta F/F_m'$ ), greater electron transport rate (ETR) and non-photochemical quenching (NPQ), indicating their higher efficiency in utilization and dissipation excess energy. Plants grown on the ground under HL had lower Chl and Chl/Car ratio, but higher content of carotenoids and Chl a/b ratio compared to the other plants, implying their acclimation to HL conditions. The highest  $g_s$  was recorded at 1000 h, suggesting the avoidance of drought stress by widely opening stomata only for a short period of time in the early morning. Higher  $g_s$  was related to higher leaf temperature, indicating that the role of  $g_s$  in moderating leaf temperature. Understanding the effects of growth irradiance on photosynthesis of *G. speciosum* would be an important step towards a sustainable conservation of native orchids in Singapore.

**Keywords:** growth irradiance, *Grammatophyllum speciosum* orchids, photosynthesis, relative water content

**Abbreviations:** Car, carotenoids; Chl, chlorophyll; DW, dry weight; ETR, electron transport rate;  $F_o$ , minimal fluorescence yield of a “dark-adapted” sample;  $F_m$  and  $F_v$ , maximal and variable fluorescence yields obtained from a dark-adapted sample upon application of a saturation pulse of radiation, respectively;  $F_m'$ , the maximum fluorescence at the steady state;  $F_t$ , the current fluorescence yield;  $\Delta F/F_m'$ , the effective photochemical quantum yield;  $g_s$ , stomatal conductance; NPQ, non-photochemical quenching; PPFD, photosynthetic photon flux density; RWC, relative water content

## 1. Introduction

Orchids can be found thriving in many terrestrial ecosystems, especially the tropics. Their modes of growth are epiphytic, lithophytic and terrestrial (Roberts & Dixon, 2008). They can vary in size and weight. The world's biggest orchid, *G. speciosum*, also known as the tiger orchid, can weigh up to several tons and span several metres. This orchid is widely distributed in Southeast Asian countries such as Indonesia, Malaysia, Thailand and the Philippines. Though it is once a native species in Singapore, it has gradually become extinct due to habitat loss. As part of Singapore's orchid conservation program, *G. speciosum* has been reintroduced into the country's natural and urban settings (Yam, Chua, Tay, & Ang, 2010). Currently, it is cultivated both on the ground and on trees of some parks. In general, these tiger orchids appear healthy; more than 80% are growing well and quite a number of them flowered in 2013 (Yam, 2013). However, certain *G. speciosum* plants show visible signs of leaf yellowing, which could be caused by a combination of excess light irradiances and insufficient water.

Excessive light is one of the environmental stresses experienced by orchid plants (He, Khoo, & Hew, 1998; Khoo, He, & Hew, 1998). When plants capture more light than what they could use in photosynthesis, they are likely to undergo dynamic photoinhibition that is defined as reversible reduction in light utilisation efficiency to avoid photoinhibitory damage on the photosynthetic systems (Chow, 1994; Osmond, 1994). On the other hand, long term exposure to high irradiance, photooxidation will occur, resulting in photobleaching of Chl and chloroplast damage (Osmond, 1994). These effects, in contrast are irreversible and pose long term damage to the photosynthetic apparatus (Osmond, 1994). Drought stress coupled with high light occurs when water supply is insufficient to sustain plant growth. The effects of drought stress include reduction in growth rate (Ashraf, 2010) decrease in water content and cell turgor potential (Gomes *et al.*, 2008), closure of stomata (Pinheiro & Chaves, 2011) and decline in photosynthesis (Herbinger *et al.*, 2002).

Using *G. speciosum* orchids grown both on the ground and on trees under HL and LL in Pasir Ris Park, Singapore, the main objectives of this project were to study photosynthetic utilization of radiant energy by the measurements of Chl fluorescence and photosynthetic pigments. Studies of water status measured as midday leaf RWC,  $g_s$  and leaf temperature were also included in this paper. Understanding the effects of growth irradiance on photosynthesis of *G. speciosum* under natural conditions would be an important step towards a sustainable conservation of native orchid in Singapore.

## 2. Materials and Methods

### 2.1. Plant Materials

Mature plants of *G. speciosum* planted by National Parks Board (NParks) Singapore in Pasir Ris Park were used in this study. The orchid plants were grown both on the ground and on trees under HL and LL conditions in October 2010. Trees on which the *G. speciosum* were grown were the rain trees, *Albizia saman* (syn. *Samanea saman*). Each tiger orchid has a stem length of 2 metres and had an initial size of 10 pseudobulbs on the ground and 30 pseudobulbs on trees. These orchids were neither watered nor fertilized after planting in the park. The maximal PPFD and ambient temperature in the park ranged between 200 to 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and 30 to 35°C respectively during the photoperiod.

### 2.2. Measurement of Photosynthetic Photon Flux Density (PPFD)

PPFD was measured using a photosynthetically available radiation quantum sensor (SKP 215, Skye Instruments Ltd, Llandrindod, UK) by placing it beside the leaves of each species. The readings of PPFD were recorded by a reading unit (SKP 200, Skye Instruments Ltd, Llandrindod, UK) after it stabilized, with a range between 0 and 1999  $\mu\text{mol m}^{-2}\text{s}^{-1}$ .

### 2.3. Measurement of Midday RWC

Leaves under different growth conditions were each punched into 5 discs (1 cm diameter) and immediately weighed with an analytical balance to determine FW. The leaf discs were then floated on water in the dark for 24 hours prior to measurement of their saturated weight (SW). The samples were thereafter dried in the oven at 80° C for 72 hours to obtain their DW. RWC was expressed as  $RWC = (FW-DW)/(SW-DW) \times 100\%$ .

### 2.4. Measurement of Chl Fluorescence $F_v/F_m$ Ratio

Diurnal changes of  $F_v/F_m$  ratios were taken every two hour from 0800 to 1800 h with the Plant Efficiency Analyser, PEA (Hansatech Instruments Ltd, England) on sunny and cloudy days. Leaves were pre-darkened with clips for 15 min prior to measurements. Dark adapted leaves were placed under the light pipe to measure  $F_o$ , initial Chl fluorescence during the very early onset of illumination with excitation light.  $F_m$ , maximum Chl fluorescence was determined by 0.8 seconds of saturated pulse ( $> 6000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The variable fluorescence yield  $F_v$ , was determined by  $F_m - F_o$ . The efficiency of excitation energy captured by open PSII reaction centres in dark adapted leaves was estimated by the fluorescence  $F_v/F_m$  ratio.

### 2.5. Measurements of Different Chl Fluorescence Parameters

Leaves were harvested at 0900h for Chl fluorescence analysis. The effective photochemical quantum yield ( $\Delta F/F_m'$ ), electron transport rate (ETR) and non-photochemical quenching (NPQ) were determined using the Imaging-PAM Chl Fluorometer (Walz, Effeltrich, Germany) at 25°C in the laboratory. Leaves were pre-darkened under a piece of black cloth for 15 min prior to measurements. By using the PAM Chl Fluorometer, images of fluorescence emission were digitized within the camera and via a Firewire interface (400 megabits/s) (Firewire-1394, Austin, TX, USA) to a personal computer for storage and analysis. Measuring light pulses were applied at low frequency (about 1 Hz) for measurement of initial Chl fluorescence,  $F_o$  images in a quasi-dark state. During actinic illumination and saturation pulses, the frequency of measuring light pulses and image capture was automatically increased to about 10 Hz. The Imaging-PAM continuously measured the current fluorescence yield (F). In the absence of actinic illumination and upon application of a 0.8-s of maximal saturation pulse ( $2,400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), the dark-level or initial fluorescence yield ( $F_o$ ) and the maximum fluorescence yield ( $F_m$ ) were determined respectively. After that rapid light curve measurements in the presence of actinic illuminations (Schreiber, Gademann, Ralph, & Larkum, 1997) were obtained through the application of a series of 10-s light exposures with increasing irradiance from 1 to  $1,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . A 0.8-s of maximal saturation pulse ( $2,400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was applied after each actinic illumination to obtain maximal fluorescence yield ( $F_m'$ ). Hence, in the presence of actinic illumination, the current fluorescence yield, and the maximum fluorescence ( $F_m'$ ) at the steady state, were determined, from which the effective PSII quantum yield,  $\Delta F/F_m' [(F_m' - F) / F_m']$  and ETR ( $\text{PPFD} \times \Delta F/F_m' \times 0.5 \times 0.84$ ) (Rascher, Liebig, & Lüttge, 2000) could be calculated. The number of 0.5 represents a supposition that the excitations are equally distributed between PSII and PSI. Correction factor 0.84 takes into account that only a fraction of incident light is really absorbed by photosynthesis (Rascher *et al.*, 2000). NPQ was defined as:  $\text{NPQ} = (F_m - F_m')/F_m'$ .

### 2.6. Determination of Chl and Carotenoids (Car) Content

0.05g of fresh leaves was cut into smaller pieces. Photosynthetic pigments of Chl and Car were extracted from these samples using dimethylformamide and quantified spectrophotometrically at wavelengths of 480, 647 and 664nm (Wellburn, 1994).

### 2.7. Measurement of $g_s$ and Leaf Temperature by Porometer

The  $g_s$  and leaf temperature were measured on fully expanded leaves by clamping a portion of the leaf in the leaf porometer's chamber (SC-1, Decagon, U.S.) with a fixed diffusion path to the leaf

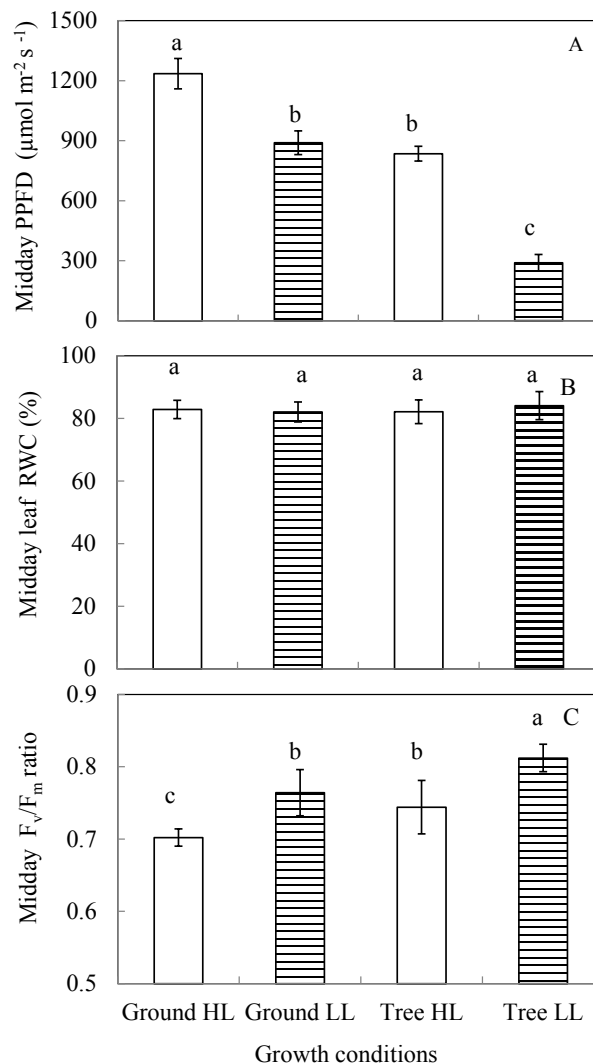
surface. The  $g_s$  was determined via a steady state technique where the vapour pressure at two locations in the diffusion path was measured.

## 2.8. Statistical Analysis

For Figures 1, 3, and 4, one-way ANOVA was used to test for significant differences among different growth irradiances, using Tukey's multiple comparison tests to discriminate the means (MINITAB, Inc., Release 15, 2007). For Figures 2 and 5, a  $t$ -test was used to test for differences between HL and LL grown leaves (MINITAB, Inc., Release 15, 2007).

## 3. Results

### 3.1. PPFD, Leaf RWC and Chl Fluorescence $F_v/F_m$ Ratio during Midday

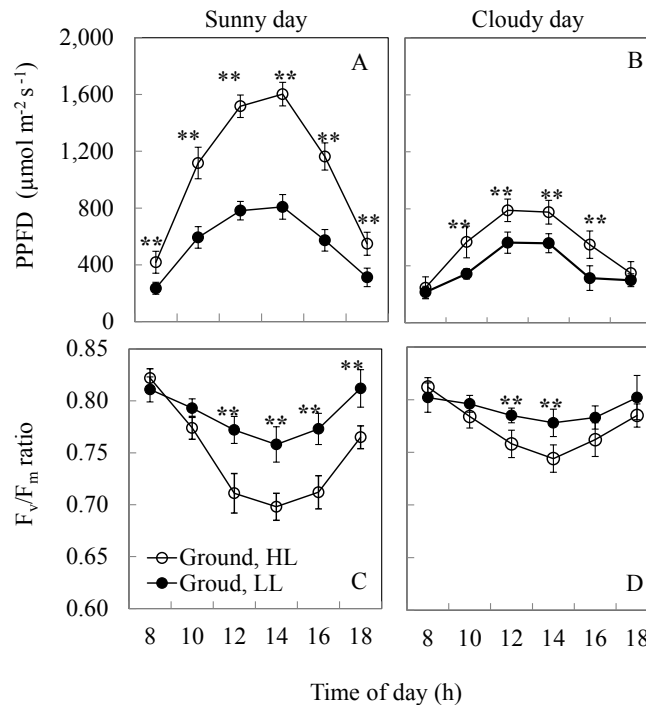


**Figure 1.** Midday PPFD (A), RWC (B) and  $F_v/F_m$  ratio (C) of *G. speciosum* grown on the ground and on trees under HL and LL conditions. Each value is the mean of 6 leaves. Vertical bars represent standard errors. Means with different letters above the bars are statistically different ( $p < 0.05$ ) by Tukey's multiple comparison tests.

PPFD of Ground HL was much higher than that of Tree HL (Figure 1A,  $p < 0.05$ ). Ground HL was indeed under an open field condition. However, for *G. speciosum* plants grown on tree under HL, there was some shading from the host plants, rain trees. PPFDs of both Ground LL and Tree LL were respective, lower than those of Ground HL and Tree HL (Figure 1A,  $p < 0.05$ ). All plants had midday leaf RWC greater than 80%, indicating there was no severe water deficit occurred in any plants regardless of growth irradiance (Figure 1B,  $p > 0.05$ ). Plants grown under Ground LL and Tree LL had a higher midday  $F_v/F_m$  ratio than Ground HL and Tree HL respectively (Figure 1C,  $p < 0.05$ ). These results imply that plants grown under HL experienced midday depression of photosynthesis as  $F_v/F_m$  ratio was less than 0.8 compared to those grown under LL both on the ground and on trees.

### 3.2. Diurnal Changes of PPFD and $F_v/F_m$ Ratios on Sunny and Cloudy Days

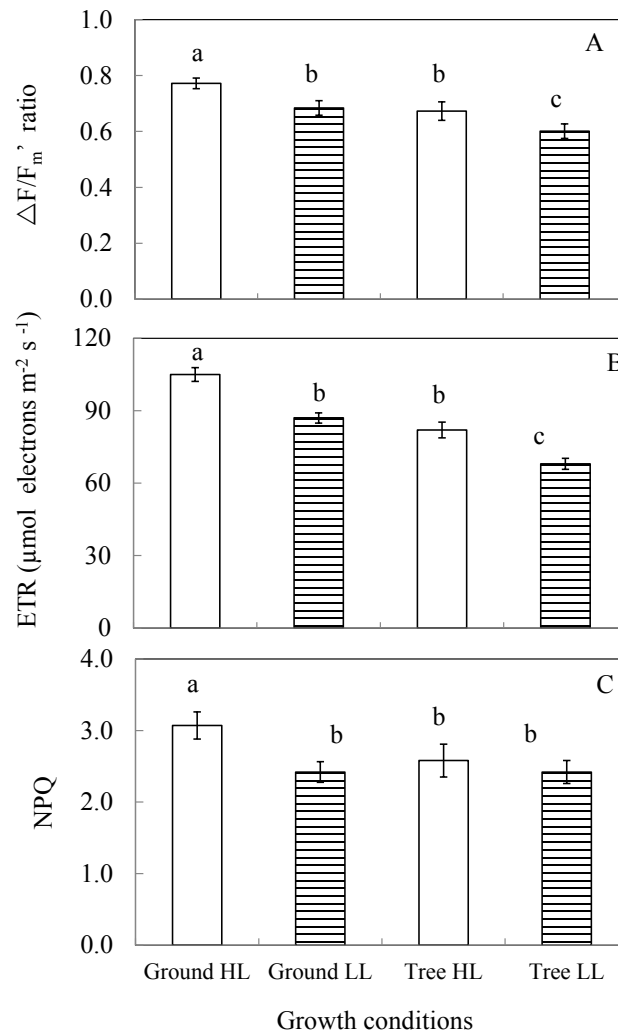
These measurements were only made plants grown on the ground. PPFD was higher for plants grown under HL than LL on a sunny day at any given time (Figure 2A,  $p < 0.05$ ). There was no significant difference in PPFD between Ground HL and Ground LL at 0800h and 1800h on a cloudy day (Figure 2B,  $p > 0.05$ ). However, PPFD of Ground HL was higher than that of Ground LL for the rest of the day (Figure 2B,  $p < 0.05$ ). There was no significant difference in  $F_v/F_m$  ratio between Ground HL and Ground LL at 0800 h and 1000h on a sunny day (Figure 2C,  $p > 0.05$ ).  $F_v/F_m$  ratios of plants grown under Ground HL were lower than those grown under Ground LL for the rest of the day (Figure 2C,  $p < 0.05$ ). On a cloudy day, significant differences in  $F_v/F_m$  ratios were only observed between Ground HL and Ground LL during midday, that was, at both 1200h and 1400h (Figure 2D,  $p < 0.05$ ). The above measurements were repeated two more days and similar results were obtained.



**Figure 2.** Diurnal changes of  $F_v/F_m$  ratio of *G. speciosum* grown on the ground under HL and LL conditions on sunny (A) and cloudy (B) days. Each value is the mean of 6 measurements from 6 different plants. Vertical bars represent standard errors. \*\* Above the lines are statistically different ( $p < 0.05$ ) as determined by *t*-test.

### 3.3. $\Delta F/F_m'$ , ETR and NPQ

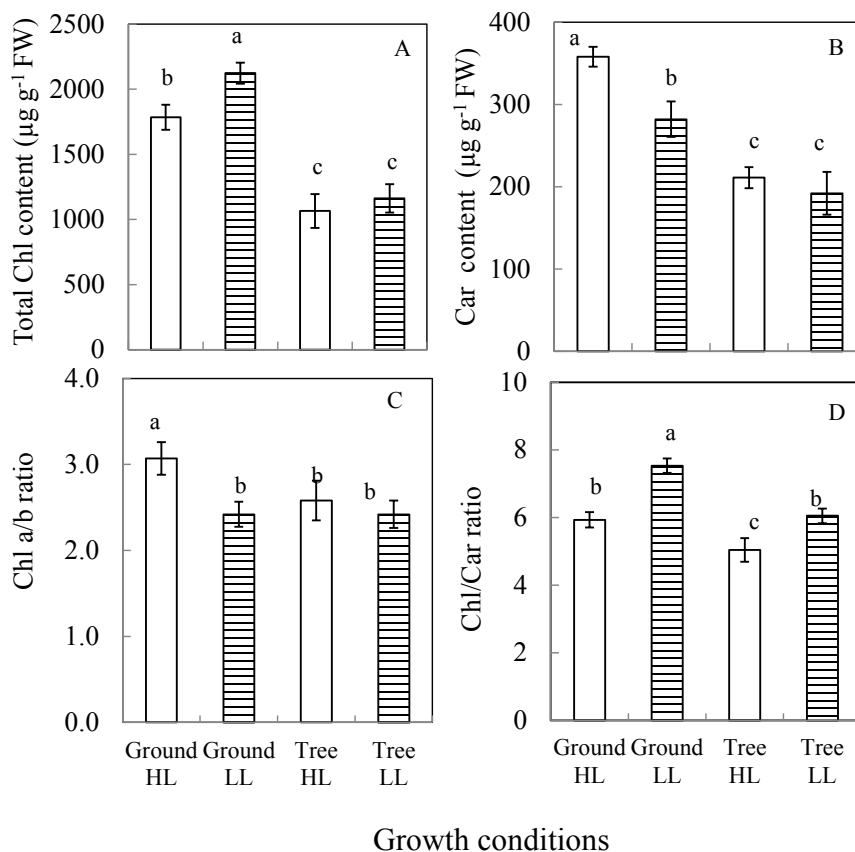
$\Delta F/F_m'$ , ETR and NPQ were used to explore the utilization of light energy. Light response curves of these three parameters were measured from 1 to 1,200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ .  $\Delta F/F_m'$  values decreased while NPQ reading increased with increasing PPFD in all plants (data not shown). Initially, there was a steep increase of ETR values with increasing PPFD until 505  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , after which it decreased sharply (data not shown). To compare the utilization and dissipation of light energy among *G. speciosum* plants grown under different condition, Figure 3 summarizes the values of  $\Delta F/F_m'$ , ETR and NPQ measured at a PPFD of 505  $\mu\text{mol m}^{-2} \text{s}^{-1}$  when ETR of all plants reached the highest readings. Plants grown under Ground HL condition had the highest  $\Delta F/F_m'$  (Figure 3A), ETR (Figure 3B) and NPQ (Figure 3D) followed by plants grown under Ground LL and Tree HL ( $p < 0.05$ ). Plants grown on trees under LL had the lowest readings of these three variables. These results indicate that Ground HL plants had the highest capacities in utilization and dissipation excess energy.



**Figure 3.**  $\Delta F/F_m'$  (A), ETR (B) and NPQ (C) of *G. speciosum* grown on the ground and trees under HL and LL conditions. Each value is the mean of 4 measurements from 4 different leaves. Vertical bars represent standard errors. Means with different letters above the bars are statistically different ( $p < 0.05$ ) by Tukey's multiple comparison tests.

### 3.4. Photosynthetic Pigments

Plants grown on the ground had much higher Chl and Car contents than those plants grown on trees under both HL and LL (Figures 4A, 4B  $p < 0.05$ ). Ground HL plants had lower Chl but higher Car content compared to those of ground LL plants, implying their acclimation to high growth irradiance. Lower Chl/Car ratios (Figure 4D), but higher Car content (Figure 4B,  $p < 0.05$ ) and higher Chl a/b ratio (Figure 4C,  $p < .05$ ) of plants grown on the ground under HL compared to ground LL further support high light acclimation of these plants. However, there were no significant differences in Chl and Car contents, Chl a/b (Figure 4C  $p > 0.05$ ) between Tree HL and Tree LL plants although Chl/Car ratio was higher in Tree LL plants (Figure 4D,  $p > 0.05$ ).



**Figure 4.** Total Chl content (A), total Car content (B), Chl a/b ratio (C) and Chl/Car ratio (D) of *G. speciosum* grown on the ground and on trees under HL and LL conditions. Each value is the mean of 4 measurements from 4 different leaves. Vertical bars represent standard errors. Means with different letters above the bars are statistically different ( $p < 0.05$ ) by Tukey's multiple comparison tests.

### 3.5. $g_s$ and Leaf Temperature

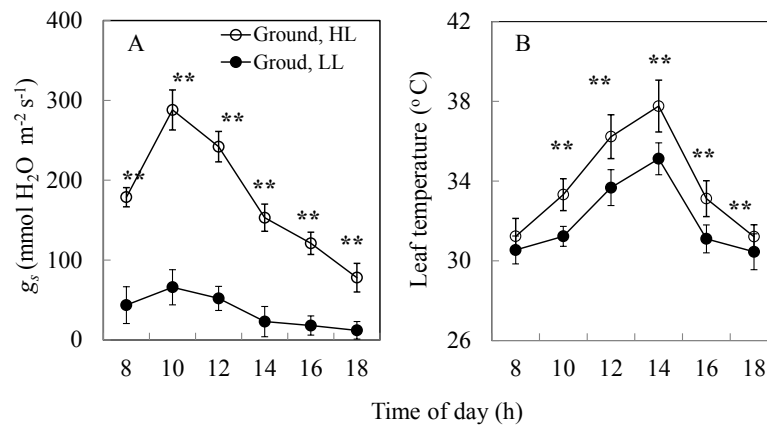
Higher readings of  $g_s$  were recorded in plants grown on the ground and on the tree under HL than under LL during midday (Table 1,  $p < 0.05$ ). Higher values of  $g_s$  were related to their higher leaf temperatures, indicating that higher  $g_s$  could moderate leaf temperature (Table 1,  $p < 0.05$ ). Diurnal changes of  $g_s$  and leaf temperature were only made on plants grown on the ground on sunny days.  $g_s$  was higher for plants grown under HL than LL at any given time throughout the day (Figure 5A,  $p < 0.05$ ). Highest values of  $g_s$  was obtained at 1000h, suggests that *G. speciosum* plants avoid drought stress by widely opening their stomata only during a short time in the early morning and closing or

partial closing them from midday. Higher  $g_s$  in plants grown under HL at any given time than under LL was related to their high leaf temperature (Figure 5B). This further confirmed that increased  $g_s$  could moderate leaf temperature.

**Table 1.** Midday  $g_s$  and leaf temperature of *G. speciosum* on the ground and on trees under HL and LL conditions

Light condition	$g_s$ (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	Leaf temperature (°C)
Ground, HL	230.7 ± 28.33 <sup>a</sup>	37.18 ± 0.09 <sup>a</sup>
Ground, LL	55.67 ± 12.07 <sup>b</sup>	34.85 ± 0.12 <sup>b</sup>
Tree, HL	43.21 ± 9.07 <sup>c</sup>	33.7 ± 0.09 <sup>c</sup>
Tree, LL	9.03 ± 0.34 <sup>d</sup>	32.1 ± 0.06 <sup>d</sup>

**Notes:** Each value is the mean of 6 leaves from 6 different leaves. Means with different letters are statistically different ( $p < 0.05$ ) by Tukey's multiple comparison tests.



**Figure 5.** Diurnal changes of  $g_s$  (A) and leaf temperature (B) of *G. speciosum* grown on the ground under HL and LL conditions. Each value is the mean of 6 measurements from 6 different plants. Vertical bars represent standard errors. \*\*Above the lines are statistically different ( $p < 0.05$ ) as determined by  $t$ -test.

#### 4. Discussion

*G. speciosum* orchid native to Singapore, has become extinct, mainly due to habitat loss. *G. speciosum* is one of the species reintroduced through our conservation programme. While light is mandatory for growth and survival in plant, the amount of light received may have a great impact on plant performance. HL intensity can cause detrimental damage to the photosynthetic machinery of plants compared to LL, especially combined with other stress factors such as drought (Lambers, Chapin III, & Pons, 2008). On the other hand, LL intensity compared to HL conditions could reduce photosynthetic rates and a subsequent reduction in overall plant growth (Khoo *et al.*, 1998; He & Teo, 2007; Lambers *et al.*, 2008). In this study, the water status of orchid plants grown on the ground and on trees under HL and LL was first determined. Midday leaf RWC was more than 80%, indicating that no severe drought stress occurred in any plant (Figure 1B) (Lizana *et al.*, 2006; Lugojan & Ciulca, 2011) even though they were not irrigated. Leaves from plants grown on the ground under HL had much higher  $g_s$  than that of Ground LL (Table 1, Figure 5A) and the highest



values of  $g_s$  were obtained at about 1000h (Figure 5A), implying that these plants had developed a mechanism to cope with water limitation. For plants grown on the ground under HL, they were able to dissipate heat quickly via transpiration through their stomata (Crawford, McLachlan, Hetherington, & Franklin, 2012) and this was supported by the fact that *G. speciosum* orchid plants grown on the ground under HL in an open field had much higher  $g_s$  that moderated the increase in leaf temperature (Table 1, Figure 5A). Ground LL plants did not experience as much heat as Ground HL and thus lower leaf temperature and lower  $g_s$  (Table 1, Figure 5B). These findings supported the role of  $g_s$  in moderating leaf temperature (Hamerlynck & Knapp, 1996).

Depending on the plant species, developmental stage, severity and duration of water deficit stress, plants may either develop tolerance by osmotic adjustment and changing cellular elasticity, or develop avoidance such as stomatal closure and reducing leaf surface area (Anjum *et al.*, 2011). It is predicted that drought-resistant plants maximize their fitness by decreasing both leaf size and  $g_s$  to water vapor in response to limited water availability (Zangerl & Bazzaz, 1984; Donovan & Ehleringer, 1992; Dudley, 1996; Nativ, Ephrath, Berliner, & Saranga, 1999). *G. speciosum* orchid plants have long, narrow arching leaves. In the present study, the highest  $g_s$  was recorded at 1000 h (Figure 5A), suggesting that *G. speciosum* orchid plants had developed the avoidance of drought stress by widely opening stomata only for a short period of time in the early morning (Ort, 2001) to conserve water.  $g_s$  modulated transpiration rate and water loss (Farquhar & Sharkey, 1982). Zangerl and Bazzaz (1984) hypothesized that in order to conserve water, plants could adapt physiologically by decreasing  $g_s$  and thereby increasing their water use efficiency (WUE, the ratio of carbon gained per unit water lost). Leaf temperature increased (Figure 5B) as transpiration decreased from 1000h (Figures 5A). However, Ground HL plants still had significantly higher  $g_s$  compared to that of Ground LL plants, indicating these plants were able to modulate leaf temperature via transpiration. The closure of some stomata would limit  $CO_2$  availability thereby causing photosynthetic rate to decline. In our further study with the same *G. speciosum* orchid plants found that decreasing  $g_s$  to increase WUE concomitantly decreased photosynthesis by reducing  $CO_2$  intake through stomata (unpublished data).

One important direct consequence of reduction in  $CO_2$  supply is the decrease of photosynthetic electron consumption, causing a down-regulation of photosynthetic efficiency (Cornic & Fresneau 2002). When the plants capture more light than they use in photosynthesis, especially under shortage of  $CO_2$  supply, they are likely to undergo dynamic photoinhibition. Dynamic photoinhibition is defined as reversible reduction in light utilisation efficiency to avoid photoinhibitory damage on the photosynthetic systems (Osmond, 1994). In the present study, Ground HL, Ground LL and Tree HL plants had reversibly reduced their light utilisation efficiency during midday. This was supported by the depression of midday  $F_v/F_m$  ratio in these plants (Figure 1C) to avoid photoinhibitory damages to the photosystems (Chow, 1994; Osmond, 1994; Osmond & Chow, 1988). Long term exposure to high growth irradiance, a term defined as chronic photoinhibition, will result in photobleaching and chloroplast damage (Osmond, 1994).

To investigate if chronic photoinhibition occurred in *G. speciosum* orchid plants, subsequently, the light utilisation efficiency on sunny and cloudy days was investigated in plants grown on the ground under both HL and LL. All plants had  $F_v/F_m$  ratios greater than 0.8 at 0800h although dynamic photoinhibition, the different extents of midday depression of  $F_v/F_m$  ratios were observed on both sunny and cloudy days (Figures 2C, 2D) when PPFD was between 500 to 1500  $\mu mol m^{-2} s^{-1}$ . HL plants had a lower  $F_v/F_m$  ratio than LL plants during midday of sunny day (Figure 2C). This suggested that these plants had experienced greater dynamic photoinhibition but completely recovered through the night as indicated by similar  $F_v/F_m$  ratio at 0800 h as other plants (Figures 2C, 2D, Chow, 1994; Osmond, 1994).

Lower  $\Delta F/F_m'$ , ETR and NPQ (Figure 3) of *G. speciosum* plants that were grown on the ground and on trees under LL compared to those grown under HL, indicate that these plants had lower light

utilization. Although plants grown under HL had greater dynamic photoinhibition, these plants, however, had higher  $\Delta F/F_m'$  and ETR, exhibiting their higher capacities of utilizing and dissipating light energy. Dynamic photoinhibition is the protective down regulation of photosynthesis by diversion of excitation energy away from photosystem II by increasing non-photochemical quenching, NPQ (He & Lee, 2004). When plants suffer from photoinhibition there is an accompanied increase in Car to offer photoprotection (Armstrong & Hearst, 1996; Puthur, 2005).

Plants adapted to LL and HL are well known to have respectively low and high Chl a/b ratio (Anderson, 1986; Anderson & Osmond, 1987). In this study, higher Chl a/b ratio was observed in Ground HL plants than in Ground LL plants (Figure 4C). However, no significant differences were observed in Chl a/b ratio between Tree HL and LL plants. The low Chl content in the leaves of Ground HL plants exposed to maximal PPFD above  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  could be a result of increased Chl degradation or adaptation to HL conditions (Anderson, 1986). Lower Chl/Car ratio (Figure 4D) found in Ground HL plants was due to both the lower Chl content and the increased total Car content (Figure 4B), indicating that when these plants were under reversible dynamic photoinhibition (reduced midday  $F_v/F_m$  ratio, Figures 1C, 2C), they up-regulated their Car level to dissipate excess photons through the xanthophyll cycle (Demmig-Adams & Adams III, 1992; 2006; Chow, 1994). Shading improved Chl content of Ground LL plants (Figure 4A) and this was previously reported in *Rhododendron* (Anderson, Norcini, & Knox, 1991) and *Euonymus* (Newman & Follett, 1988). However, there was no significant difference in Chl content between Tree HL and Tree LL plants. It may be due to other limited factors such as nitrogen deficiency which merits our further study.

## 5. Conclusion

*G. speciosum* orchids grown on the ground and on trees under natural conditions did not suffer drought stress. They were able to adapt both high and low irradiances and thrive well both on the ground and on trees. *G. speciosum* orchids were experienced high irradiances, high air temperatures and low water availability when they are conserved under natural condition without additional watering and fertilizing. These conditions impose a selection pressure on plants for stomatal regulation of transpiration to maintain water balance and leaf temperature. Photosynthetic utilization of radiant energy and non-photochemical energy dissipation in photosystem PSII provide a dynamic mechanism to reduce photodamage during midday when growth irradiances and leaf temperatures are high and partial closure of the stomata. Although plants grown on the ground under HL had more severe photoinhibition, they were able to recover quickly overnight. Higher efficiency in utilization and dissipation excess energy was tightly correlated with both Chl and Car concentrations. The findings of the present study further supported that *G. speciosum* orchids are growing well under different growth irradiances in Singapore (Yam, 2013) due to their different physiological acclimations to different growth environments.

## Acknowledgement

This project was supported by the teaching vote, Ministry of Education, Singapore. We also thank Singapore botanic gardens and national parks board for providing the plant materials.

## References

- [1] Anjum, S. A., Xie, X., Wang, L., Saleem, M. F., Chen, M., & Wang, L. (2011). Review: Morphological, physiological and biochemical response of plants to drought stress. *African Journal of Agricultural Research*, 6(9), 2026-2032.

- [2] Anderson, J. M. (1986). Photoregulation of the composition, function, and structure of thylakoid membranes. *Annual Review of Plant Physiology*, 37, 93-136.
- [3] Anderson, J. M., & Osmond, C. B. (1987). Shade-sun responses: Compromises between acclimation and photoinhibition. In D. J. Kyle, C. B. Osmond, & C. J. Arntzen (Eds.), *Photoinhibition* (pp. 1 – 38). Amsterdam: Elsevier.
- [4] Anderson, P. C., Norcini, J. G., & Knox, G. W. (1991). Influence of irradiance on leaf physiology and plant growth characteristics of *Rhododendron x* 'Pink Ruffles'. *Journal American Society Horticultural Sciences*, 116(5), 881-887.
- [5] Armstrong, G. A., & Hearst, J. E. (1996). Carotenoids 2: Genetics and molecular biology of carotenoid pigment biosynthesis. *FASEB Journal*, 10(2), 228-237.
- [6] Ashraf, M. (2010). Inducing drought tolerance in plants: Recent advances. *Biotechnology Advances*, 28(1), 169-183.
- [7] Chow, W. S. (1994). Photoprotection and photoinhibitory damage. *Advanced molecular Cell Biology*, 10, 151-196.
- [8] Cornic, G., & Fresneau, C. (2002). Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during a mild drought. *Annual Botany*, 89, 887-894.
- [9] Crawford, A. J., McLachlan, D. H., Hetherington, A. M., & Franklin, K. A. (2012). High temperature exposure increases plant cooling capacity. *Current Biology*, 22(10), R396–R397.
- [10] Demmig-Adams, B., & Adams III, W.W. (1992). Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology*, 43, 599 – 626.
- [11] Demmig-Adams, B., & Adams III, W. W. (2006). Photoprotection in an ecological context: The remarkable complexity of thermal energy dissipation. *New Phytologist*, 172(1), 11-21.
- [12] Donovan, L. A., & Ehleringer, J. R. (1992). Contrasting water-use patterns among size and life-history classes of a semi-arid shrub. *Functional Ecology*, 6(4), 482–488.
- [13] Dudley, S. A. (1996). Differing selection on plant physiological traits in response to environmental water availability: A test of adaptive hypotheses. *Evolution*, 50(1), 92–102.
- [14] Farquhar, G. D., & Sharkey, T. D. (1982). Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, 33, 317 – 345.
- [15] Gomes, F. P., Oliva, M. A., Mielke, M. S., de Almeida, A.-A. F., Leite, H. G., & Aquino, L. A. (2008). Photosynthetic limitations in leaves of young Brazilian green dwarf coconut (*Cocos nucifera* L.'nana') palm under well-watered conditions or recovering from drought stress. *Environmental and Experimental Botany*, 62(3), 195 – 204.
- [16] Hamerlynck, E., & Knapp, A. K. (1996). Photosynthetic and stomatal responses to high temperature and light in two oaks at the western limit of their range. *Tree Physiology*, 16(6), 557 – 565.
- [17] He, J., & Lee, S. K. (2004). Photosynthetic utilization of radiant energy by temperate lettuce grown under natural tropical condition with manipulation of root-zone temperature. *Photosynthetica*, 42(3), 457 – 463.
- [18] He, J., Khoo, G. H., & Hew, C. S. (1998). Susceptibility of CAM *Dendrobium* leaves and flowers to high light and high temperature under natural tropical conditions. *Environmental and Experimental Botany*, 40(3), 255 – 264.

- [19] He, J., & Teo, L. C. D. (2007). Susceptibility of green leaves and green flower petals of CAM orchid *Dendrobium* cv. Burana Jade to high irradiance under natural tropical conditions. *Photosynthetica*, 45(2), 214–221.
- [20] Herbinger, K., Tausz, M., Wonisch, A., Soja, G., Sorger, A., & Grill, D. (2002). Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Physiology and Biochemistry*, 40(6-8), 691-696.
- [21] Khoo, G. H., He, J., & Hew, C. S. (1998). Photosynthetic utilization of radiant energy by CAM *Dendrobium* flowers. *Photosynthetica*, 34(3), 367–376.
- [22] Lambers, H., Chapin III, F. S., & Pons, T. L. (2008). *Plant physiological ecology* (pp. 75-81). The Netherlands: Springer.
- [23] Lizana, C., Wentworth, M., Martinez, J. P., Villegas, D., Meneses, R., Murchie, E. H., . . . Pinto, M. (2006). Differential adaptation of two varieties of common bean to abiotic stress: I. Effects of drought on yield and photosynthesis. *Journal of Experimental Botany*, 57(3), 685-697.
- [24] Lugojan, C., & Ciulca, S. (2011). Evaluation of relative water content in winter wheat. *Journal of Horticulture, Forestry and Biotechnology*, 15(2), 173-177.
- [25] Nativ, R., Ephrath, J. E., Berliner, P. R., & Saranga, Y. (1999). Drought resistance and water-use efficiency in *Acacia saligna*. *Australian Journal of Botany*, 47(4), 577–586.
- [26] Newman, S. E., & Follett, M. W. (1988). Irrigation frequency and shading influences on water relations and growth of container-growth *Euonymus japonica* ‘Aureo-marginat’. *Journal Environmental Horticulture*, 6(3), 96-100.
- [27] Ort, D. R. (2001). When there is too much light. *Plant Physiology*, 125(1), 29 – 32.
- [28] Osmond, C. B. (1994). What is photoinhibition? Some insights from comparisons of shade and sun plants. In N. R. Baker, & J. R. Bowyer (Eds.), *Photoinhibition of photosynthesis: From molecular mechanisms to the field* (pp. 1-24). Oxford, U.K.: Bios Scientific Publisher.
- [29] Osmond, C. B., & Chow W. S. (1988). Ecology of photosynthesis in the sun and shade: Summary and prognostications. *Australian Journal of Plant Physiology*, 15(2), 1– 9.
- [30] Pinheiro, C., & Chaves, M. M. (2011). Photosynthesis and drought: can we make metabolic connections from available data? *Journal of Experimental Botany*, 62(3), 869 –882.
- [31] Puthur, J. T. (2005). Influence of light intensity on growth and crop productivity of *Vanilla planifolia* Andr. *General and Applied Plant Physiology*, 31(3-4), 215– 224.
- [32] Rascher, U., Liebig, M., & Lüttge, U. (2000). Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. *Plant Cell & Environment*, 23(12), 1397-1405.
- [33] Roberts, D. L., & Dixon, K. W. (2008). Orchids. *Current Biology*, 18(8), R325-329.
- [34] Schreiber, U., Gademann, R., Ralph, P. J., & Larkum, A. W. D. (1997). Assessment of photosynthetic performance of *Prochloron* in *Lissoclinum patella* in hospite by chlorophyll fluorescence measurements. *Plant Cell Physiology*, 38(8), 945–951.
- [35] Wellburn, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, 144(3), 307-313.
- [36] Yam, T. W., Chua, J., Tay, F., & Ang, P. (2010). Conservation of the native orchids through seedling culture and reintroduction - A Singapore experience. *Botanical Review*, 76(2), 263–274.

- [37] Yam, T. W. (2013). *Native orchids of Signapore: Division, indentification and conservation*. Singapore Botanic Gardens, National Parks Board, Singapore: Stallion Press (Singapore) Pte Ltd.
- [38] Zangerl, A. R., & Bazzaz, F. A. (1984). Effects of short-term selection along environmental gradients on variation in populations of *Amaranthus retroflexus* and *Abutilon theophrasti*. *Ecology*, 65(1), 207–217. <http://dx.doi.org/10.2307/1939472>

### Copyrights



Copyright for this article is retained by the author(s), with first publication rights granted to the journal. This is an open-access article distributed under the terms and conditions of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

