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Photosynthetic Light Utilization Efficiency, Water Relations and Leaf Growth of C_3 and CAM Tropical Orchids under Natural Conditions

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Abstract

Native orchid species of Singapore in their natural conditions experience stress from high irradiance, high temperatures and periods of extended low rainfall, which impact orchid plant physiology and lead to reduced growth and productivity. In this study, it was found that there was a reduction in photochemical efficiency of photosystem II (PSII) in 6 native orchid species under high light (HL) and *Bulbophyllum membranaceum* under low light (LL). There was chronic photoinhibition in these 6 orchid species over a period of 3 months after transplanting onto the tree trunks without watering and fertilization, especially in *Coelogynes mayeriana* and *Bulbophyllum membranaceum* under both HL and LL. This chronic photoinhibition caused by sustained period of water deficit in their natural conditions was later reversed by natural re-watering conditions from higher rainfall. These results indicate that water deficit has a greater impact on photosynthetic light utilization efficiency than excess light. The present study also showed that after natural re-watering, relative water content (RWC) of leaves and pseudobulbs generally increased. During the natural re-watering, total leaf area also gradually increased and reached maximum expansion after 7 weeks under both HL and LL, with some exceptions due to leaf abscission or decline in total leaf area, possibly a strategy for water conservation.

Keywords

Orchid, CAM, C_3, Photosynthetic Light Utilization Efficiency, Water Relations and Leaf Growth

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1. Introduction

Singapore has a tropical rainforest climate with no distinctive seasons, temperatures ranging from 22˚C to 35˚C, high relative humidity averages ranging from 60% to 100%, and annual rainfall about 2300 mm, with November to January being the wettest months. Considerable efforts have been made in the past decade to re-introduce native orchid species back into Singapore as part of conservation [1]-[3]. Many of these re-introduced species are epiphytes adapted to relatively dry habitats, native to low-elevation forests, and are dependent upon their microclimate. Crucial to these epiphytic orchids is the requirement for water economy where high evapotranspiration combined with scarce water supply challenges plant water balance [4]. Epiphytic orchids grow well under optimal environmental conditions and propagate naturally. However, when they experience stress from high irradiance, high temperatures and periods of extended low rainfall, these factors significantly impact their physiology and lead to reduced growth and productivity.

High irradiance and high temperature cause a reduction in photosynthetic quantum yield, loss in leaf chlorophyll, and a greater susceptibility to photoinhibition in orchids [5]-[7]. The reduction in photosynthesis can be attributed to dynamic photoinhibition, photooxidation, photobleaching of chlorophyll and chloroplast damage leading to deletrious effects of the leaf photosynthetic apparatus [7]-[9]. Furthermore, orchid leaves exposed to drastic increase in photon flux densities experienced a drop in CO_2 gas exchange and leaf chlorosis [10].

Epiphytic orchids experiencing stress from water deficit show significant decrease in relative water content (RWC) in leaves and pseudobulbs, reduction in photosynthetic light utilization, and greater susceptibility to photoinhibition caused by high irradiance [11]. In other plants, periodic drought stress also leads to a closure of stomata [12], through the reduction of shoot water potential directly or reduced turgor [13]. As a result, CO_2 depletes in the intercellular spaces and at the chloroplast level, limiting photosynthesis. Plants grown under low irradiance may also experience a reduction in the photosynthetic rate, resulting in a decline in growth rate [5] [14]. Therefore it is necessary to strike a good balance in maximising photon energy utilization of leaves to increase photosynthetic activity whilst minimising photoinhibition [6].

Orchids are also able to tolerate and acclimatise to stressful conditions, dynamically achieved by the physiological and morphological adaptations. In some orchid species, carbon fixation through Crassulacean acid metabolism [15] provides a means of water conservation during times of water deficit, while preventing loss of carbon [16]-[19]. Thick-leaved orchids, such as *Bulbophyllum vaginatum*, *Dendrobium leonis* and *Phalaenopsis cornu-cervi*, have features that are characteristic of plants that fix carbon primarily through Crassulacean acid metoblism [15], while thin-leaved orchids such as *Coelogynes rochussenii*, *Coelogynes mayeriana* and *Bulbophyllum membranaceum* are plants that fix carbon primarily through C_3 pathway [20]-[22].

There is little information about the photosynthetic light utilization efficiency and water relations of tropical orchid species in their natural environments, especially with regards to their responses to varying environmental conditions and the stress that comes with it. The information would be useful for improving the approach towards conservation of orchid species in their natural environments. Therefore, to complement the conservation efforts in Singapore, this study aims to better understand the photosynthetic light utilization efficiency, water relations and leaf growth of six selected C3 and CAM native tropical orchids under natural conditions. The results benefit sustainable conservation of these orchid species under natural conditions.

2. Materials and Methods

2.1. Plant Cultivation under Natural Conditions

Mature plants of *Coelogynes rochussenii*, *Coelogynes mayeriana*, *Bulbophyllum vaginatum*, *Bulbophyllum membranaceum*, *Dendrobium leonis* and *Phalaenopsis cornu-cervi* planted by the National Parks Board (NParks) Singapore in National Institute of Eduction (NIE), Singapore, were used in this study. These epiphytic orchid plants were planted on the trunks of rain trees, *Albizia saman* (syn. *Samanea saman*), under natural high light (HL) and low light (LL) conditions on 12 January 2015. LL was achieved by natural shading provided by the trees and positioning the plants out of direct sunlight. These orchids were neither watered nor fertilized after planting, and depended upon rainfall as the only source of water. The maximal PPFD ranged from 90 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for LL and from 400 to 1300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for HL, with an ambient day temperature of about 30˚C to 35˚C during the photoperiod. 6 plants of each species for each light condition were planted on the trees.

2.2. Measurement of Photosynthetic Photon Flux Density (PPFD)

PPFD was measured using a photosynthetically available radiation quantum sensor and reading unit (Skye Instruments Ltd, Llandrindod, UK). The stabilized PPFD, within a range of 0 to 1999 μmol·m^{-2}·s^{-1}, was measured from 6 different positions above the leaves for HL and LL respectively, and an average was calculated from the 6 values measured.

2.3. Measurement of Chl Fluorescence F_v/F_m Ratio

Diurnal changes of F_v/F_m ratios were taken every two hours from 0800 to 1600 h with the Plant Efficiency Analyser, PEA (Hansatech Instruments Ltd, England) on sunny and cloudy days, while weekly F_v/F_m ratios were taken at the time corresponding to the maximal midday PPFD. 6 samples were taken from each species under each light condition. The 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 chlorophyll (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 μmol·m^{-2}·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.

2.4. Measurement of Chl Reading by Hand Held Chlorophyll Meter (SPAD-502)

Leaf Chl content was read by using a hand-held chlorophyll meter (SPAD-502, Minolta, Japan), which measures the absorbances of the leaf at wavelengths 645 nm and 663 nm. Using these two absorbances, the meter calculates a numerical SPAD value which is proportional to the amount of Chl present in the leaf, and expresses in terms of SPAD units. 6 samples were taken from each species under each light condition.

2.5. Measurement of Midday RWC

Square cuts (1 cm by 1 cm) were made out of the leaves and 5 mm thick slices were made out of the pseudobulbs. These sample were first weighed with an analytical balance to determine fresh weight (FW), followed by immersion in water in the dark for 3 hours (for pseudobulb of *C.* mayeriana and leaf of *B. membranaceum*) or 24 hours (for the rest of the samples) prior to measurement of their saturated weight (SW). The samples were then dried in the oven at 80˚C for at least 72 hours to obtain their dry weight. RWC was expressed as RWC = (FW − DW)/(SW − DW) × 100%. 10 samples each for leaves and for pseudobulbs were obtained from each species.

2.6. Measurement of Total Leaf Area

Young, budding leaves from 6 plants of each orchid species, cultivated under the respective light conditions (HL or LL), were selected and tracked over 16 weeks. The individual leaf area was measured every week by capturing the image of selected leaves against a measured scale and then using Image J software to calculate the leaf area from the image according the method outlined by Schneider *et al.* [23]. The total leaf area was calculated as a sum of the individual leaf areas. Total leaf area is one of the parameters for leaf growth.

2.7. Statistical Analysis

One-way ANOVA was used to test for significant differences among different growth irradiances, using Tukey's multiple comparison tests to discriminate the means (IBM SPSS Statistics for Macintosh, Version 22.0, 2013).

3. Results

3.1. Photosynthetic Light Utilization Efficiency

Diurnal PPFD was much higher for the plants grown under HL than LL on a sunny day at 1000 h, 1200 h and 1400 h (**Figure 1(a)** and **Figure 1(b)**) and the diurnal F_v/F_m ratio at these timings were also correspondingly lower in HL than LL (**Figure 1(c)** and **Figure 1(d)**), with the biggest difference in *B. vaginatum* and *B. membranaceum*. This suggests a reduction in the efficiency of open PSII reaction centres of the orchids under exposure

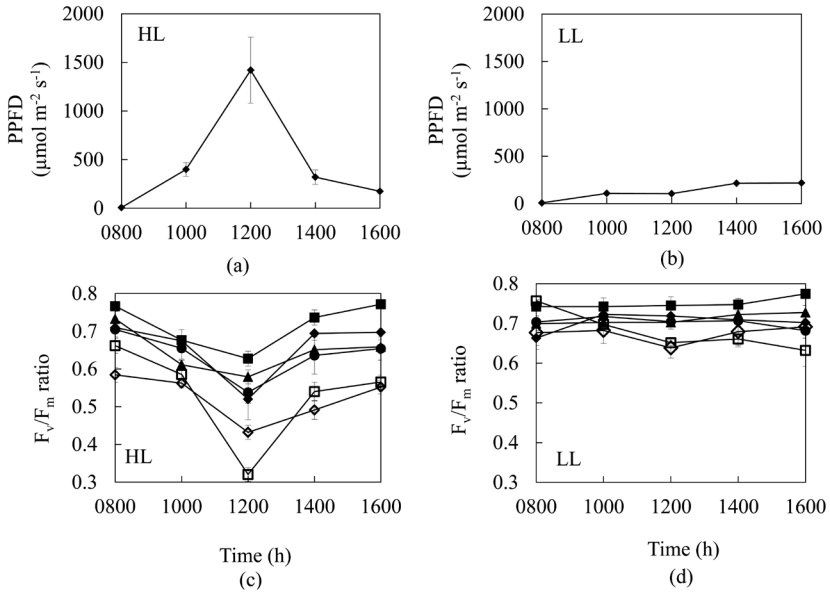

Figure 1. Diurnal changes of PPFD and F_v/F_m ratio on sunny days for *C. rochussenii* (▲), *C. mayeriana* (■), *B. vaginatum* (◇), *B. membranaceum* (□), *D. leonis* (●) and *P. cornu-cervi* (◆) grown under HL and LL.

to HL (PPFDs of 400 to 1300 μmol·m^{-2}·s^{-1}) towards midday, particularly more severe in *B. vaginatum* and *B. membranaceum*, among the 6 orchid species examined. A reduction in the efficiency of open PSII reaction centres was also shown in *B. membranaceum* under exposure to LL (with a maximal PPFD of 200 μmol·m^{-2}·s^{-1}) during midday. F_v/F_m ratio at 1600 h of all plants grown under HL except *C. mayeriana*, and *B. membranaceum* grown under LL did not recover to the same levels as at 0800 h, suggesting that these plants were suffering from chronic photoinhibition.

However, there was evidence of recovery from this chronic photoinhibition as shown in the difference in F_v/F_m ratios at midday, between 1 and 3 months of growing under HL and LL. Significant increase in the midday F_v/F_m ratios from 1 to 3 months of cultivation were observed in all species under both HL (**Figure 2(a)**) and LL (**Figure 2(b)**) except for *C. mayeriana* under LL (One-way ANOVA, $p < 0.05$, $n = 6$). Between species, *B. vaginatum* and *B. membranaceum* after 1 month under HL show significantly lower F_v/F_m ratios as compared with the other species, but recovered to levels comparable to *D. leonis* and *P. cornu-cervi* after 3 months under HL (Tukey's multiple comparison test, $p < 0.05$, $n = 6$). No significant difference in F_v/F_m ratio was found between species after 1 month and 3 months under LL. Further evidence of recovery from this chronic photoinhibition was provided in the data collected over 16 weeks for the changes to midday maximal PPFD and F_v/F_m ratio. Over the 16 weeks of cultivation under HL, midday maximal PPFD exceeded 800 μmol·m^{-2}·s^{-1} only in weeks 2 to 7 and week 13 (**Figure 3(a)**) while little fluctuation in midday PPFD (<200 μmol·m^{-2}·s^{-1}) was seen in LL (**Figure 3(b)**). Based on rainfall data of the micro-climate of the area where the orchids were cultivated, weeks 2 to 7 coincided with a period of low rainfall (**Figure 3(a)**), while the drop in midday maximal PPFD (< 800 μmol·m^{-2}·s^{-1}) from week 8 onwards, with the exception of week 13, coincided with an increase in weekly total rainfall. Corresponding with the fluctuations in midday maximal PPFD under HL and the weekly total rainfall, the F_v/F_m ratio decreased from weeks 2 to 8 for all 6 orchid species, under both HL (**Figure 3(c)** and **Figure 3(e)**) and LL (**Figure 3(d)** and **Figure 3(f)**), and started increasing back to original levels or higher thereafter. The decrease in F_v/F_m ratio was greater in HL than LL, for *B. vaginatum*, *B. membranaceum* and *P. cornu-cervi*, *C. rochussenii*, *C. mayeriana* and *D. leonis*. Therefore, the period of higher weekly total rainfall from week 8 onwards provided re-watering conditions, and this corresponded with plants showing recovery from chronic photoinhibition, where the F_v/F_m ratio that was decreasing below 0.700 in the first 8 weeks started to increase back to levels above 0.700 after week 8.

In contrast to the changes in F_v/F_m ratio over the 16 weeks of cultivation, difference between Chl content under HL and LL were lesser, with the exception of *B. membranaceum* which showed a higher Chl content under

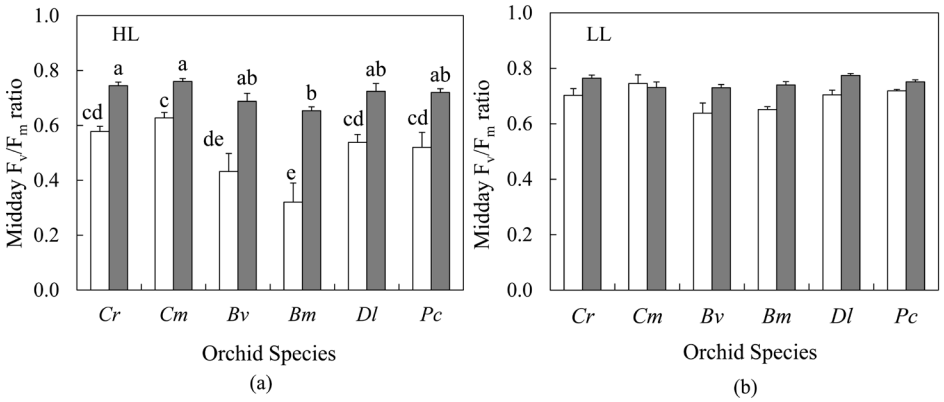

Figure 2. F_v/F_m ratio at midday maximal PPFD after 1 month (open bars) and 3 months (solid bars) of cultivation under HL and LL (*Cr*: *C. rochussenii*, *Cm*: *C. mayeriana*, *Bv*: *B. vaginatum*, *Bm*: *B. membranaceum*, *Dl*: *D. leonis*, *Pc*: *P. cornu-cervi*). Significant difference observed between the F_v/F_m ratio after 1 and 3 months, under HL and LL, in all species except for *Cm* under LL (One-way ANOVA, $p < 0.05$, $n = 6$). Different letters above bars indicate significant difference in F_v/F_m ratio between species after 1 month ((a), (b)) and 3 months ((c), (d), (e)) under HL (Tukey's multiple comparison, $p < 0.05$, $n = 6$). No significant difference found between species after 1 month and 3 months under LL.

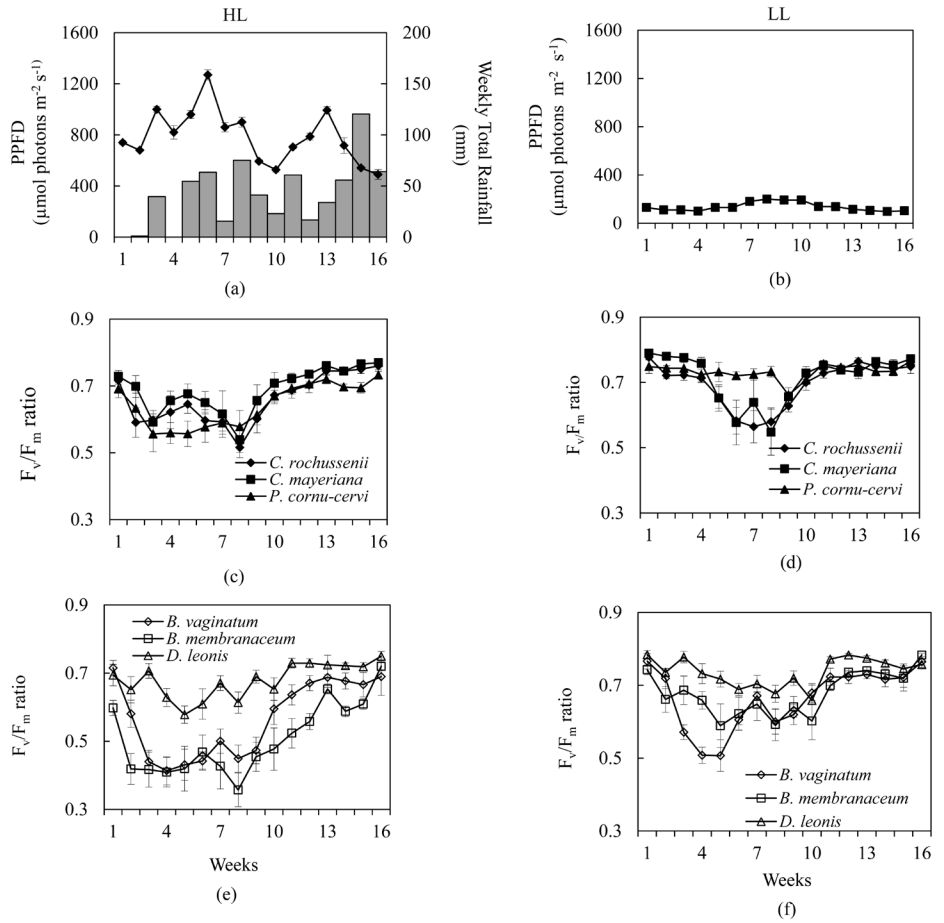

Figure 3. Changes of midday PPFD and F_v/F_m ratios of different orchid species over 16 weeks under HL and LL. Rainfall data adapted with permission from Meteorological Services Singapore (Historical Daily Records, 2015).

LL (**Figure 4(d)**) than HL (**Figure 4(c)**). Chl content fluctuateed little between 20 to 40 SPAD units for *C. rochussenii*, *C. mayeriana* and *P. cornu-cervi* (**Figure 4(a)** and **Figure 4(b)**), whereas Chl content showed greater fluctuations between 30 to 70 SPAD units for *B. vaginatum* and *D. leonis* (**Figure 4(c)** and **Figure 4(d)**).

3.2. Water Relations

To further understand the significance of the natural re-watering conditions provided by the higher weekly total rainfall from week 8 onwards, the water status of the plants was examined. RWC of leaves (**Figure 5(a)** and **Figure 5(b)**) and pseudobulbs (**Figure 5(c)** and **Figure 5(d)**) increased significantly in all 6 orchid species, between 1 to 4 months of cultivation under HL and LL, with the exception of the pseudobulbs of *B. vaginatum* and *B. membranaceum* under HL (One-way ANOVA, $p < 0.05$, $n = 10$). Between species, leaves of *B. vaginatum*, *B. membranaceum*, *D. leonis and P. cornu-cervi* after 1 month under HL and LL had significantly lower RWC compared to C. *rochussenii* and *C. mayeriana* (Tukey's multiple comparison test, $p < 0.05$, $n = 10$). However, after 4 months, RWC in the leaves of *P. cornu-cervi* under HL and LL and *B. membranaceum* under LL increased back to levels comparable with *C. rochussenii* and *C. mayeriana*. After 1 and 4 months under HL and LL, pseudobulbs of *C. rochussenii*, *B. vaginatum* and *B. membranaceum* remained significantly lower than that of *C. mayeriana*.

3.3. Leaf Growth

The difference in leaf growth under HL and LL was also examined through the changes in total leaf area for all 6 species. Total leaf area generally increased for the first 7 weeks for all 6 orchid species, with the exception of *D. leonis* cultivated under HL (**Figure 6(e)**), where there was a sharp drop in total leaf area during weeks 5 to 7. The sharp drop was due to a loss of the selected leaves, and this could be further attributed to the sustained low rainfall period coupled with HL from weeks 2 to 9. Total leaf area continued to be sustained at around the same level from week 7 onwards for the rest of the plants, with the exception of *C. rochussenii* (**Figure 6(a)**) and *B. membranaceum* (**Figure 6(d)**) cultivated under LL, and *C. mayeriana* (**Figure 6(b)**) and *P. cornu-cervi* (**Figure 6(f)**) cultivated under HL. For the plants with total leaf area remaining unchanged from week 7 onwards, it could be attributed to the leaves reaching their maximum expanded area.

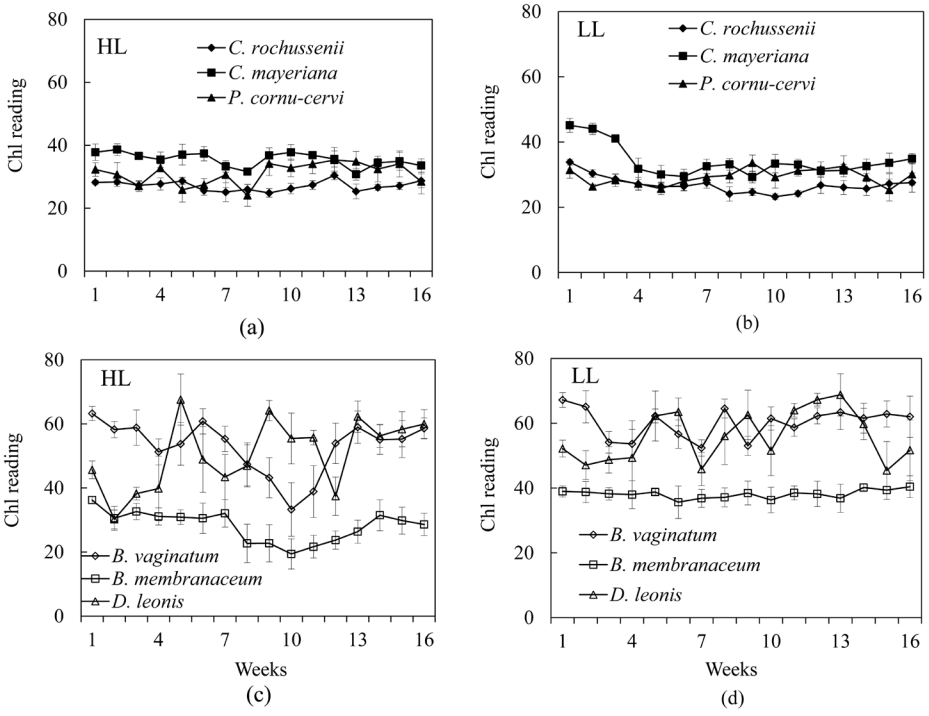

Figure 4. Changes of Chl reading of different orchid species over 16 weeks under HL and LL.

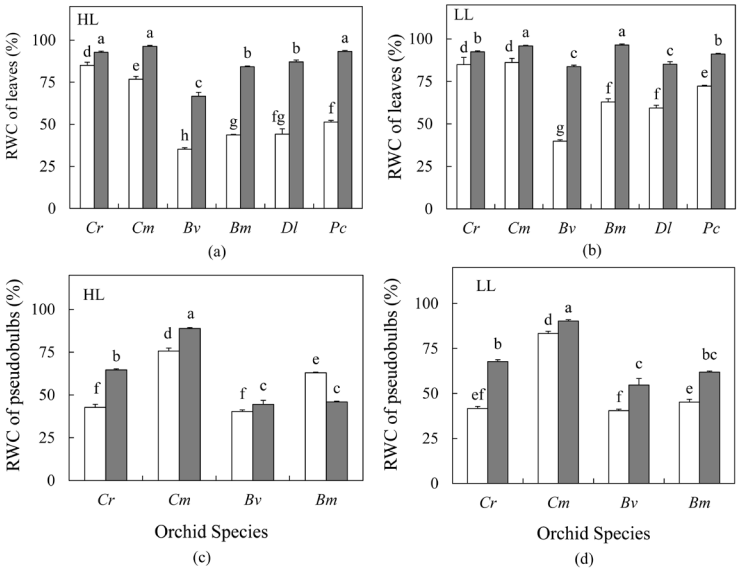

Figure 5. RWC of leaves and pseudobulbs after 1 month (open bars) and 4 months (solid bars) of cultivation under HL and LL (*Cr*: *C. rochussenii*, *Cm*: *C. mayeriana*, *Bv*: *B. vaginatum*, *Bm*: *B. membranaceum*, *Dl*: *D. leonis*, *Pc*: *P. cornu-cervi*). Significant difference was observed between 1 and 4 months under HL and LL, in all species except for *Bv* pseudobulb under HL (One-way ANOVA, $p < 0.05$, $n = 10$). Different letters above bars indicate significant difference in RWC between species after 1 and 4 months under HL ((a), (b), (c)) and LL ((d), (e), (f)) (Tukey's multiple comparison, $p < 0.05$, $n = 10$).

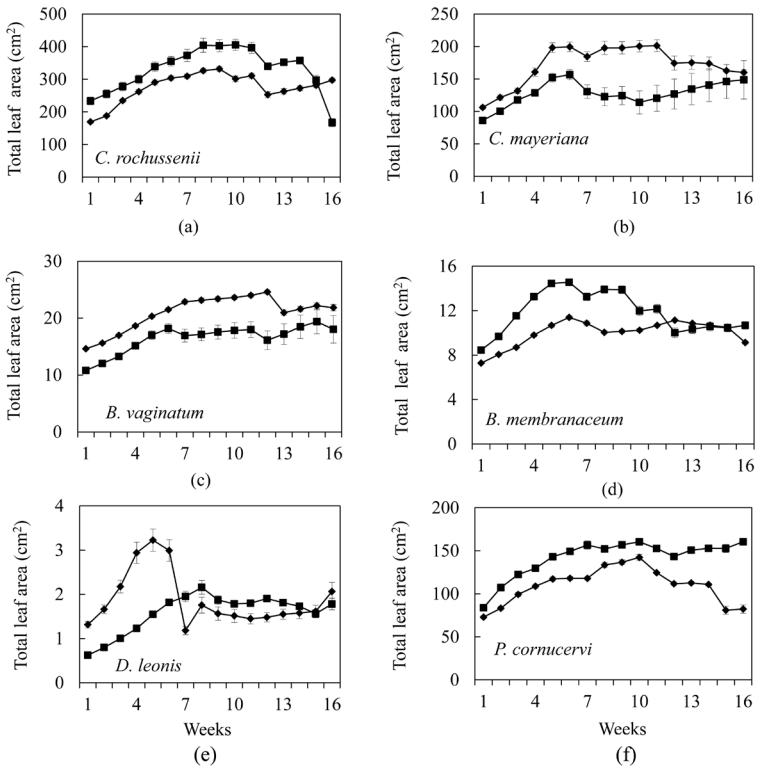

Figure 6. Total leaf area of different orchid species over 16 weeks under HL (◆) and LL (■).

4. Discussion

Some 70% of the native orchid species of Singapore are mostly vascular epiphytes [1]. Vascular epiphytes are dependent upon their micro-climate in photosynthetic light utilization and water economy [4]. In a review by Ort [24], it was reported that light intensities often exceed photosynthetic capacity in plants, while stomatal conductance limits CO_2 uptake into leaves, rendering even moderate irradiances in excess of photosynthetic capacity. Plants grown under high irradiance may lead to photoinhibition of the leaves, and this will result in a reduction in growth and productivity. Both C_3 and CAM orchids have been reported to show stress from exposure to high irradiances under natural conditions, such as decrease in photosynthetic light utilization, photoinhibition, photooxidative damage, and leaf chlorosis [5] [10] [11] [25]-[29]. Our present study reports a reduction in the efficiency of open PSII reaction centres of all the 6 orchid species under exposure to HL and C_3 orchid *B. membranaceum* under exposure to LL, towards midday (**Figure 1**), indicating photoinhibition. This photoinhibition is particularly more severe in *B. vaginatum* and *B. membranaceum* grown under HL. All plants under HL were also not able to fully recover from the photoinhibition over the first month (**Figure 2(a)**, open bars), indicating chronic photoinhibition.

However, photoinhibition of PSII *in vivo* provides photoprotection and is an acclimation strategy for plants under stress from excessive irradiances [30]. Orchids have protective stategies to overcome the stress from high irradiances and to help balance maximising photon energy utilization while minimising photoinhibition [6]. Previous studies reported higher tolerance to high PPFD and faster recovery from photoinbition in C_3 *Oncidium* orchid as compared to CAM *Phalaenopsis* orchid [29], greater photoprotection in leaves of C_3 orchid *C. rochussenii* under HL compared to LL [28], and recovery from photoinhibition in C_3 orchid *Gramatophyllum speciosum* in HL [27]. The results of our study seem to contradict these cases because the diurnal F_v/F_m ratios decline sharply towards 1200 h in all the orchids exposed to HL (**Figure 1(c)**) as compared to LL (**Figure 1(d)**). All 6 species also showed chronic photoinhibition after 1 month under HL, but recovered after 3 months (**Figure 2**). However, in all previous studies, water deficit was not severe and this could be the reason why the C_3 orchids could better utilize photosynthetic light energy as compared to the CAM orchids.

Ort [24] argued that at high irradiances, water status take physiological precedence over maximizing photosynthesis. It has been reported that in thin-leaved, C_3 epiphytic orchid *Catasetum viridiflavum*, and other epiphytes, re-watered plants show recovery from photoinhibition despite high PPFD, indicating that water deficit has a greater impact on photoinhibition than high light [31] [32]. Furthermore, strong photoinhibition and reduced photosynthetic capacity was observed in epiphytic CAM orchid hybrid, *Cattleya forbesii* Lindl. × *Laelia tenebrosa* Rolfe., following sustained water deficit for 42 days [11]. It has also been reported that under water deficit stress, *C. rochussenii* under HL had a reduced photosynthetic light utilization than plants under LL, and water deficit stress took precedence over light stress in reducing the Chl reading of *C. rochussenii*. [28]. Drought stress coupled with high light occurs when water supply is insufficient to sustain plant growth [27] [33]. Our present study showed that a sustained period of water deficit caused a decline in photosynthetic light utilization in all 6 orchid species, followed by a period of natural re-watering conditions that enabled recovery from this chronic photoinhibition (**Figure 3**). The changes in photosynthetic light utilization were less likely to be attributed to changes in Chl content since there was little difference in Chl content between HL and LL (**Figure 4**), with the exception of *B. membranaceum* which showed a higher Chl content under LL than HL. Therefore, the results suggest that water deficit has a greater impact on photosynthetic light utilization efficiency than HL, and that *B. vaginatum* and *B. membranaceum* thrive better in LL, well-watered conditions.

Water deficit that drives closure of stomata result in reduction in CO_2 uptake and subsequent down-regulation of photosynthetic efficiency [34], which is alleviated in epiphytic orchids by carbon fixation through CAM, while achieving water economy [4] [35] [36]. Epiphytic orchids have also been reported to lose water from the leaf slowly, yet maintain high values of RWC [37]. However, another study reported a reduction in RWC and osmotic potential during a period of water shortage in some tropical epiphytes [38] [39]. Moreover, studies had shown that pseudobulbs helped to reduce the loss of leaf water content during periods of water deficit stress by storing water in the pseudobulbs and then supplying this water to the leaves, thereby providing orchids with the ability to survive [11] [40]-[43]. A previous study had also reported that *C. rochussenii* grown under LL had a significantly higher leaf RWC than those under HL [28]. Our present study showed that after a period of natural re-watering, RWC of leaves and pseudobulbs increased significantly in all 6 orchid species, with the exception of the pseudobulbs of *B. vaginatum* and *B. membranaceum* under HL and *C. mayeriana* under LL (**Figure 5**).

Large pseudobulbs in C_3 orchids *C. rochussenii* and *C. mayeriana* could have stored water and helped maintain leaf water content so as to alleviate photoinhibition. The small size and thin leaves of *B. membranaceum* could have disadvantaged it in its ability to store water in the pseudobulbs to overcome periods of water deficit and for the leaves to overcome the stress from high irradiance, which explains the severity of photoinhibition. On the other hand, in both *D. leonis* and *P. cornu-cervi*, having fleshy leaves and the ability to fix carbon through CAM could be significant strategies in response to photoinhibition. Therefore, both of these species are more adaptable to both HL and LL conditions, and could have a wider range of tolerable irradiance.

Water deficits lead to reduction in leaf area, leaf abscission, and dieback of individual plant parts. However, reduction in leaf area and leaf abscission strongly reduced water loss, and epiphytic orchids that encounter shortages of water may stop growing when water is in short supply, only to resume growth when water supply is restored [31] [44]. Our study showed a general increase in total leaf area for the 6 species for the first 7 weeks under HL and LL and maintained onwards as they had reached maximum leaf expansion. However, the drop in total leaf area in some species, such as *D. leonis*, was due to leaf abscission and decline in total leaf area. This could be a strategy for water conservation through reducing the total number of leaves, and therefore number of stomata, as well as reducing the surface area, leading to lesser water loss by evapotranspiration.

5. Conclusion

The results suggest that water deficit has a greater impact on photosynthetic light utilization efficiency than excess light. Over a period of water deficit, 6 orchid species experienced a decrease in F_v/F_m ratio and RWC of leaves and pseudobulbs, which could both be reversed by natural re-watering conditions from higher rainfall. Over 16 weeks, total leaf area increased and leaves reached maximum expansion after 7 weeks under both HL and LL, with some exceptions due to leaf abscission or decline in total leaf area, possibly a strategy for water conservation. Future work would be required to fully understand the adaptation strategies in response to stress from HL and water deficit that may occur in the natural environment. This will aid in improving the approach of re-introduction of native orchid species in Singapore.

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