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Growth, dendrobine content and photosynthetic characteristics of *Dendrobium nobile* under different solar irradiances

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Abstract

Dendrobium nobile is an ornamental and endangered traditional Chinese medicinal plant, which grows naturally on shaded rocks on high mountains or on shaded tree trunks. The aim of this study was to investigate how the seedlings of *D. nobile* acclimate to different light levels and to obtain an optimal light level for artificial planting and cultivation. The seedlings of *D. nobile* were grown at four light levels (15, 30, and 58% of solar irradiance, and as a control with 100% of solar irradiance): the plant growth, dendrobine content, photosynthesis, and chlorophyll fluorescence of these seedlings were investigated. The results indicated that plant height, stem diameter, leaf dry mass *per* unit area (LMA), and light-saturation point (LSP) were higher at 58% of solar irradiance than all other light levels. Meanwhile, net photosynthetic rate (P_N) and stomatal conductance (g_s) showed their highest ($P \leq 0.05$) values at midday (MM) and they were also higher at 58% of solar irradiance. Furthermore, stem dendrobine content was the highest ($P \leq 0.05$) at 58% of solar irradiance, compared to other light levels. In contrast, the highest value of light-saturated net photosynthetic rate (P_{Nmax}) was observed at 30% of solar irradiance. On the other hand, the minimal fluorescence yield of the dark-adapted state (F_o) declined and maximal quantum yield of PSII photochemistry (F_v/F_m) and Chl ($a + b$) concentration increased with decreasedlight levels. These results suggested that a moderate light level (58% of solar irradiance) would be fit for *D. nobile* and, as both growth and dendrobine content were comprehensively considered, it can be recommended for artificial planting or cultivation.

Keywords: gas exchange; medicinal plant; *Orchidaceae*; photo-inhibition; shade-loving.

Abbreviations: AM_morning; *A*n_net carbon assimilation rate; AQY_apparent quantum yield; Chl_Chlorophyll; *C*i_intercellular CO_2 concentration; CK_fully irradiated control plants; Fm_maximal fluorescence yield of the dark-adapted atate; Fo_minimal fluorescence yield of the dark-adapted atate; Fv/Fm_maximal quantum yield of PSII photochemistry; *g*s_stomatal conductance; LCP_light-compensation point; LMA_leaf dry mass per unit area; LSP_light-saturation point; MM_midday; PFD_photon flux density; PM_afternoon; P_{Nmax}_light-saturated net photosynthetic rate; P_N_net photosynthetic rate; PPFD_photosynthetic photon flux density; PSII_photosystem II; RH_relative humidity; SD_standard deviation.

Introduction

Dendrobium nobile is a traditional Chinese medicinal herb belonging to the *Orchidaceae* family (Zhang et al., 2007) and is one of the most important orchids known for its beautiful violet coloured flowers (Liang et al., 2012). These plants grow mainly on shade rocks on high mountains in Chishui County of China and the alkaloid content in its stem in this area is higher than in other areas in China (Hu, 1970). So, its stems were widely sold in China, South Korea, and Japan (Vasudevan and van Staden, 2010). Moreover, the horticultural production of *D. nobile* has been rapidly established in China. Since it has both ornamental and medicinal properties, wild *D. nobile* has become rarer and is at risk of extinction as a result of digging and the low natural reproduction rate of its seeds (Vasudevan and van Staden, 2010). In Dendrobium, many reports about these species or cultivars detailed the need for a shaded environment to obtain the highest alkaloid content and best development (Jiang et al., 2014). Leaves and flower of Dendrobium are sensitive to high irradiance and photo-inhibition occurred when exposed to full sunlight (He and Teo, 2007; He et al., 1998). Although Dendrobium was considered as shade-loving, different species or cultivars have optimal light levels for their growth and development (Jiang et al., 2014). A previous study has reported that *D. nobile* is a shade-loving plant (Wang et al., 2004) and even some growers in Chishui County have known that a black shade net is established during the growth of *D. nobile*; however, optimal light levels need to be researched for artificial planting and cultivation. For a shade-loving plant, the acclimation to lower light intensities is usually associated with some typical morphological and physiological changes, such as lower leaf mass *per* unit area, lamina thickness, leaf margin toothedness, larger leaf size, and higher growth rates (Valladares and Niinemets, 2008). At extremely low light levels, a shade-loving plant may be unhealthy in its growth and development. Similarly, its long-term exposure to full sunlight generally also results in slower growth, more foliage chlorosis, even some stressful reaction, and eventually fatal damage leading to a less productive harvest (Zhang et al., 2003). Thus, it is necessary for such shade-loving plants to get the optimal light intensity for their good growth and development. Some photosynthetic parameters can be used to estimate whether a plant had enjoyed healthy growth or was stressed. Generally, good growth is associated with higher net photosynthetic rate

(P_N), which mainly depends on photosynthetic photon flux density (PPFD) and intracellular CO_2 concentration (Ci). PPFD and Ci were limited by the light intensities and the stomatal conductance (g_s), respectively (Franck and Vaast, 2009). Moreover, a shade-loving plant has both lower light-saturation point (LSP), light-compensation point (LCP), and a higher apparent quantum yield (AQY), which can reflect the light radiation conditions required by the plant (Qi et al., 2012; Dai et al., 2009; Lorena et al., 2006). Thus it can capture and utilise fewer photons in a more efficient way to meet the demands of photosynthesis (Ghasemzadeh et al., 2010). In contrast, a stressed plant usually indicates damage to its photosynthetic apparatus (such as chloroplasts) (Pires et al., 2011; Gunadasa and Dissanayake, 2012) and photo-inhibition, which was characterised by a reduction in the maximal quantum yield of PSII photochemistry (F_v/F_m) (Zheng et al., 2011). A medicinal plant absorbs sunlight to produce secondary metabolites which accumulate in leaves or stems (Mosaleeyanon et al., 2005; Havaux and Kloppstech, 2001; Ghasemzadeh et al., 2010). Irradiance is known to regulate not only plant growth and development, but also affect the accumulation of secondary metabolites (Briskin and Gawienowski, 2001). Although a shade-loving plant was planted under a shade environment, the medicinal ingredients thereof were altered by changes in shade level (Wang et al., 2012). Consequently, to obtain the highest accumulation of a specific compound and good growth, optimal light levels need to be studied for a specific plant. For *D. nobile*, even though the planted area in Chishui County now covers approximately 100 ha, there is little information available about the relationship between growth, dendrobine content, and photosynthetic characteristics of *D. nobile* and light levels. Furthermore, the optimal light level for their growth and metabolism has still not yet been reported. Therefore, the objective of this study was to evaluate physiological parameters and dendrobine content of *D. nobile* at four irradiance levels for artificial planting and cultivation.

Results

Effects of shade on growth and dendrobine content in D. nobile

Shading limited PPFD throughout the day with the highest PPFD occurring at full solar irradiance and the lowest PPFD at 15% of solar irradiance, whereas, air temperature and relative humidity (RH) were not significant at the four light levels tested (Fig. 1). A significant effect of light level on plant height, stem diameter, and leaf dry mass *per* unit area (LMA) was found for seedlings of *D. nobile* (Fig. 2). Plant heights, stem diameters, and LMA had the highest values at 58% of solar irradiance after cultivation for three months (Figs 2*A*, *B*, *D*). In contrast, the differences in leaf number were not significant at the four light levels tested (Fig. 2*C*). The highest dendrobine contents (4.14 mg g^{-1} (DW)) were obtained at 58% of solar irradiance ($P \leq 0.05$), whereas, the lowest dendrobine contents were found at 100% of solar irradiance (Fig. 3).

Effects of shade on leaf gas exchange and light-response curves in D. nobile

Net photosynthetic rate (P_N) and stomatal conductance (g_s) were significantly different ($P \leq 0.05$) between morning (AM), midday (MM), and afternoon (PM) at the same light levels (Fig. 4). P_N at 58% of solar irradiance was significantly ($P \leq 0.05$) higher than at the other three light levels during AM and MM (Fig. 4*A*). Further, P_N at 30% of solar irradiance was significantly higher than that at 100% and 15% of solar irradiance at MM (Fig. 4*A*). Unlike P_N, g_s at 30% of solar irradiance was significantly higher than at the other three light levels (Fig. 4*B*). The light-saturation point (LSP) was not significantly different among the shading treatments analysed, although it had at its highest value at 58% of solar irradiance (Table 1). The light-compensation point (LCP) at full light was significantly ($P \leq 0.05$) higher than at the other three light levels (Table 1). In contrast, light-saturated net photosynthetic rate (P_{Nmax}) was the highest at 30% of solar irradiance (Table 1). In addition, the apparent quantum yield (AQE) behaved in a similar manner to P_{Nmax} (Table 1).

Effects of shade on Chl fluorescence and Chl concentration in D. nobile

Minimal fluorescence in the dark-adapted state (F_o) declined with decreasing light level, although it was not significantly different between shade and full solar irradiance (Fig. 5*A*). Whereas, maximal quantum yield of PSII photochemistry (F_v/F_m) at 100% of solar irradiance was significantly ($P \leq 0.05$) lower than that at other light levels (58%, 30%, and 15% of solar irradiance) (Fig. 5*B*). In addition, The Chl (*a* + *b*) concentration significantly ($P \leq 0.05$) increased with decreasing irradiance (Fig. 5*C*). In contrast, the Chl *a*/*b* ratio showed a decreasing change with decreasing light levels and no significant differences were observed among the four light levels tested (Fig. 5*D*).

Discussion

The vigorous growth was explained by the consequence of acclimation to environmental factors. The presently observed vigorous growth under shade conditions in *Orchidaceae* has been reported by several authors (Stancato et al., 2002). The vigorous growth at 58% of solar irradiance was shown by height, stem diameter, and leaf dry mass *per* unit area (Figs 2*A*, *B*, *D*), compared to that at full sunlight. These shade-loving traits, such as leaf area, have enabled *D. nobile* to increase light absorption and maintain photosynthesis under low light (Gunadasa and Dissanayake, 2012). Also, higher photosynthesis of *D. nobile* is associated with more accumulation of dendrobine in its stem at 58% of solar irradiance (Fig. 4*A*; Fig. 3). These results suggested that the dendrobine in *D. nobile* were up-regulated under reduced irradiance when photosynthetic performance was enhanced. A similar result was also observed in other species (Ali and Ashraf, 2011; Hura et al., 2011). Although a shade-loving plant requires less light for its growth and development, extremely low light may be not fit for its growth. Therefore, an optimal light intensity for shade-loving plants such as *Polyscias balfouriana* and *D. candidum* has been reported (Gunadasa and Dissanayake, 2012). Under different shade conditions, temperature (Fig. 1*B*), RH (Fig. 1*C*) and atmospheric CO_2 concentration were relatively constant, whereas PPFD (Fig. 1*A*) changed significantly during the day. Therefore, a limitation of photosynthesis was the consequence of limited PPFD. Furthermore, the enhanced P_N for *D. nobile* was associated with an increased g_s at reduced light levels (58% of solar irradiance) at AM and MM (Fig. 4*A*, *B*), which indicated that P_N was attributed to an increase of g_s at low light levels. Actually, in the case of extremely low light levels, such as 15% of solar irradiance, a lower P_N resulted from the lower PPFD (Fig. 1*A*; Fig. 4*A*). Whereas, the reduction in P_N at high light levels might have been caused by the limitation of g_s at MM (Fig. 4*A*, *B*). These daily patterns were observed in other shade-loving plants (Qi et al., 2012). On the other hand, at

Table 1. Light saturation point (LSP), light compensation point (LCP), light-saturated net photosynthetic rate (P_{Nmax}) and apparent quantum yield (AQY) of *D. nobile* at four light levels. Values represent mean ± SD ($n = 15$). Different lowercase letters in the same row indicate significant differences among shade conditions at $P \leq 0.05$.

Parameters	Solar irradiance [%]			
	100	58	30	15
LSP [μmol m^{-2} s^{-1}]	214.79±9.49^d	279.72±4.03^a	263.73±3.91^b	233.10±4.32^c
LCP [μmol m^{-2} s^{-1}]	21.98±4.13^a	12.65±3.05^b	12.98±1.41^b	5.99 ±3.46^c
*P*max [μmol m^{-2} s^{-1}]	3.18±0.32^c	4.11±0.64^b	6.11±2.18^a	3.47±1.55^c
AQE [CO_2 $photon^{-1}$]	0.038±0.001^b	0.047±0.002^a	0.048±0.003^a	0.035±0.002^b

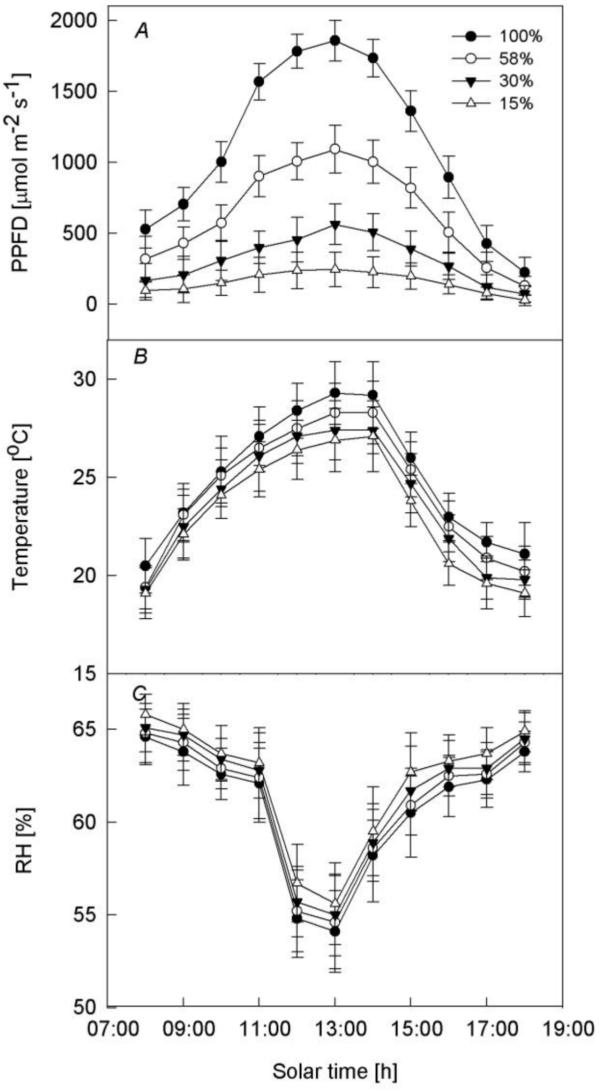

Fig 1. Daily changes in the photosynthetic photon flux density (PPFD; *A*), temperature (*B*), relative humidity (RH; *C*) at four light levels. Values represent mean ± SD ($n = 5$).

super-optimal light levels such as full light at MM (Fig. 4*A*), photo-inhibition might be caused because the absorption of excessive light energy (Dai et al., 2009), which resulted in a decreased light energy conversion efficiency to reduce P_N. The results suggested that *D. nobile* grown under high light levels may have closed stomata which decreased g_s and reduced its CO_2 assimilation (Franck and Vaast, 2009; Pires et al., 2011). At the same time, an extremely low light level can cause reductions of PPFD to limit P_N. In general, a shade-loving plant requiring a given light radiation condition can be reflected by LCP, LSP, AQE, and P_{Nmax}. The values of P_{Nmax}, LSP, and AQE were higher and LCP was lower in reduced light (at 58% and 30% of solar irradiance) (Table 1). A high P_{Nmax} indicated that plants can make full use of available light to synthesise organic materials and provide energy for plant growth. Actually, similar results have been reported elsewhere (Aleric and Kirkman, 2005). Whereas, a low LCP suggests that *D. nobile* is a shade-loving plant, which tends to have low photosynthetic capacity and have positive carbon balance in low light (Craine and Reich, 2005; Pires et al., 2011). Furthermore, a high AQE indicates shade-loving plant leaves have a higher light-use efficiency in low light (Huang et al., 2011). Leaf chlorophyll content is an important determinant of the light capturing capacity. Generally, plants grown in low light can increase their chlorophyll content (Chl *a*, Chl *b* and Chl *a* + *b*) in leaf (Dai et al., 2009), allowing them to compensate for the effects of low light availability. In contrast, the increase in light levels also usually leads to a reduction in leaf Chl content, especially for Chl *b*. In the present study, the Chl (*a* + *b*) content reduced with increasing light (Fig. 5*C*) and the Chl *a*/*b* ratio tended to be increased with increasing the sunlight available due to a higher relative proportion of Chl *b* in shaded habitats (Fig. 5*D*). The increase in leaf Chl content under low light levels demonstrates that the plants can maximise their ability to capture available light (Shao et al., 2014). F_o and F_v/F_m are good indicators of the effect of environmental stress on photosynthesis (Pires et al., 2011). Others (Maxwell and Johnson, 2000) reported that F_o is responsive to both photosynthetic pigments and the characteristics of the PSII reaction centre, as an increase in F_o might either result from the increase in Chl content or from the impaired PSII system centre (Zheng et al., 2011). In the present study, F_o was increased (Fig. 5*A*) and the Chl content reduced (Fig. 5*C*) in full sunlight, compared to shade conditions. The results suggested that *D. nobile* had an impaired PSII system centre in full sunlight. On the other hand, Baker (Baker, 2008) reported that, for the values of F_v/F_m, an efficient conversion of light energy varied from 0.75 to 0.85. Thus, an efficient conversion of light energy could have resulted from shading conditions for *D. nobile* ($0.824 \leq F_v/F_m \leq 0.840$) (Fig. 5*B*). Instead, an efficient conversion of light energy was influenced by full sunlight ($F_v/F_m = 0.732$) (Fig. 5*B*). These results suggested that photo-inhibition might have been caused by growth under full sunlight for *D. nobile*.

Materials and Methods

Plant material and experimental conditions

The experiment was conducted at the experimental station of the Key Laboratory of Plant Physiology and Development Regulation, Guizhou Normal University campus, in central Guizhou Province, China (26° 35′ N, 106° 43′ E). Single-noded stems of *D. nobile* were collected from Chishui County in the north of Guizhou Province and were micro-propagated in the laboratory. Seedlings from this micro-propagation were planted into a mixture with sawdust and vermiculite (4:1, v/v, purchased in Guiyang flower market) and left in a shaded greenhouse for one month. The young seedlings were then transplanted in 10 cm (top diameter) round black plastic pots

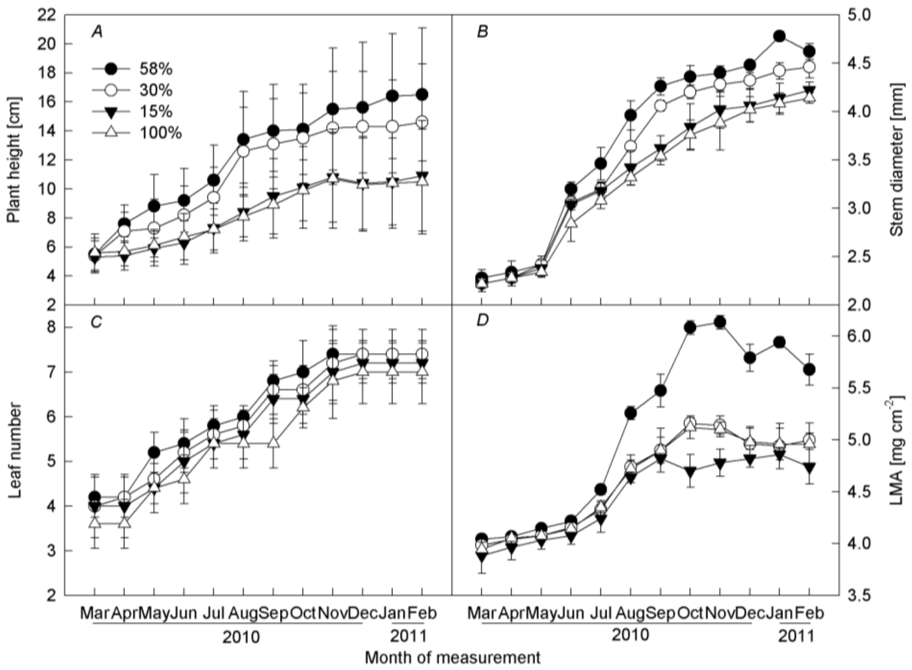

Fig 2. Plant heights (*A*), stem diameters (*B*), leaf numbers (*C*), and leaf dry mass *per* unit area (LMA; *D*) of *D. nobile* for each month from Mar. 2010 to Feb. 2011 at four light levels. Values represent mean ± SD ($n = 10$).

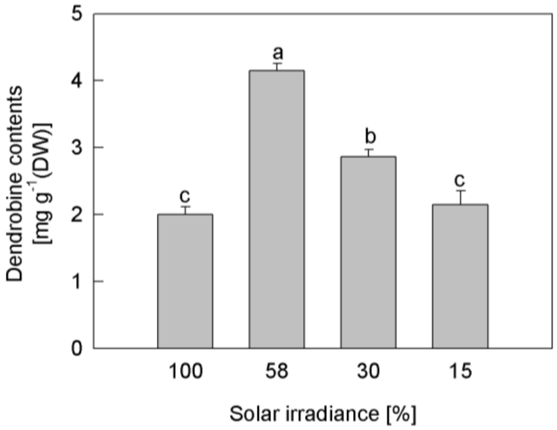

Fig 3. Dendrobine content of *D. nobile* at four light levels. Values represent mean ± SD ($n = 5$). Different lowercase letters above each bar indicate significant differences between shade conditions at $P \leq 0.05$.

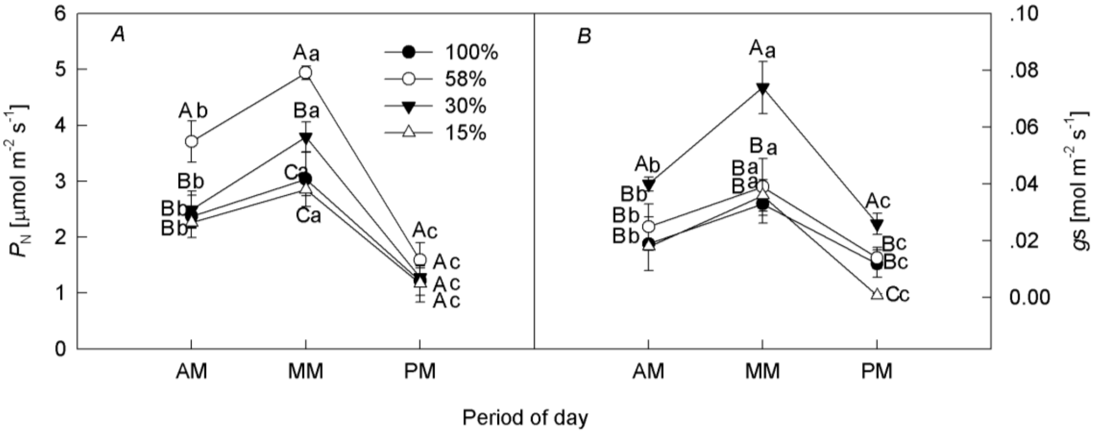

Fig 4. Net photosynthetic rate (P_N; *A*) and stomatal conductance (g_s; *B*) of *D. nobile* in AM, MM, and PM at four light levels. Values represent mean ± SD ($n = 10$). Different lowercase letters above each bar indicate significant differences between AM, MM, and PM under identical shade conditions. Different uppercase letters above each bar indicate significant simultaneous differences at four light levels ($P \leq 0.05$).

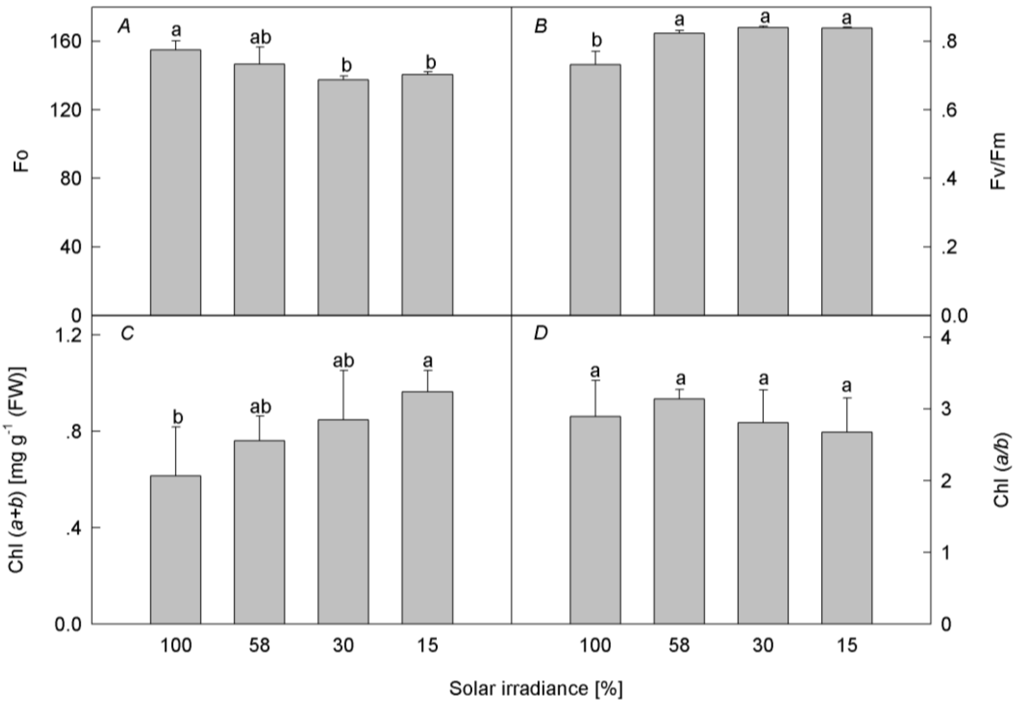

Fig 5. Minimal fluorescence yield of the dark-adapted state (F_o; *A*), maximal quantum yield of PSII photochemistry (F_v/F_m; *B*), chlorophyll (*a* + *b*) (Chl (*a* + *b*); *C*), and chlorophyll a/b (Chl (a/b); *D*) of *D. nobile* at four light levels. Values represent mean ± SD ($n = 10$). Different lowercase letters above each bar indicate significant differences between shade conditions at $P \leq 0.05$.

with a root substrate consisting of sawdust:vermiculite (4:1, v/v) for this shade experiment in Mar. 2010. Irrigation was supplied daily by an automatic micro-sprinkler system every morning. Artificial shade was established by use of different nylon nets, fixed on iron frames measuring 3 × 3 × 2 m, under field conditions, which accounted for 58% (1,092 μmol m^{-2} s^{-1}, maximal means), 30% (564 μmol m^{-2} s^{-1}, maximal means), and 15% (245 μmol m^{-2} s^{-1}, maximal means) of full sunlight, along with a control treatment (CK) under full sunlight (1,856 μmol m^{-2} s^{-1}, maximal means). Fifty pots of seedlings of *D. nobile* were cultivated in each shading frame. The photosynthetic photon flux density (PPFD), temperature, and relative humidity (RH) values (Fig. 1) at four solar irradiances were recorded hourly at the upper extremity of plants over 10 cm between 08:00 and 18:00 h using a portable photosynthesis system *LI-6400* (*LI-6400, LI-COR Inc.*, Lincoln, NE, USA) with a standard chamber (also an *LI-6400*). These data were obtained on five separate sunny days during July to Aug. 2010.

Measurement of growth and dendrobine contents

From Mar. 2010 to Feb. 2011, ten seedlings were measured using a ruler for plant height, a vernier caliper for stem diameter, and manual counting of leaf numbers each month. The leaf area of ten leaves was measured using an automatic leaf area meter LI-3100 (*LI-COR Bioscience Inc.*, Lincoln, NE, USA), then dried at 80 °C to a constant mass which was used to determine the leaf dry mass *per* unit area (LMA). The five pots of stems for each light level were collected in Feb. 2011 and dried at 105 °C for 30 min, and then at 60 °C until reaching a constant mass. Samples of mass 0.25 g were powdered to 300 μm in diameter and used for dendrobine content determination. Samples were suspended in 1 mL of ammonia solution for 30 min and extracted with 25 mL of chloroform in a 100 mL conical flask. The flasks were weighed and placed in a water bath at 65 °C for 2 h, then allowed to cool at room temperature; chloroform was added and the material filtered. Then, 10 mL of the aforementioned filtered solution were vacuum-concentrated to dry. The methanol was added to a 2 mL of constant volume and filtered with 0.45 μm filter membrane. The dendrobine content was determined using a gas chromatograph mass spectrometer (*GCMS-QP2010*, *Shimadzu Co.*, Kyoto, Japan) with a standard dendrobine curve.

Gas exchange and PFD-response curves

Net photosynthetic rate (P_N) and stomatal conductance (g_s) were performed on leaves from ten plants in the morning (AM: 8:30, 9:30, and 10:30), midday (MM: 12:30, 13:30, and 14:30), and afternoon (PM: 16:30, 17:30, and 18:30), using a portable photosynthesis system *LI-6400* (*LI-6400, LI-COR Inc*., Lincoln, NE, USA) with a standard chamber (2 × 3 cm). Light-response curves were recorded on leaves from ten plants from 09:00 to 11:00 h using a portable photosynthesis system *LI-6400* (*LI-6400*, *LI-COR Inc*., Lincoln, NE, USA) with a red-blue light chamber *LI-6400-02B* operated at 400 μmol s^{-1} air flow rate starting from the highest light intensity of 1,000 μmol m^{-2} s^{-1} to complete darkness, at regular intervals of 2 min, to give the stomata time to equilibrate at each light level. The leaf was induced at 1,000 μmol m^{-2} s^{-1} for 60 min before measurement. P_N was recorded at 1,000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 80, 50, 30, 20, and 0 μmol m^{-2} s^{-1}. Leaf temperature, relative humidity (RH), and CO_2 concentration in the leaf chamber were maintained at 25 °C, 65%, and 400 μmol m^{-2} s^{-1}, respectively. The light-saturated photosynthetic rate (P_{Nmax}), LSP, LCP, and apparent quantum efficiency (AQY) were calculated using Photosyn Assistant Software (*Photosyn Assistant*, *V1.1*, Dundee Scientific, UK).

Chl fluorescence measurements

Chlorophyll (Chl) fluorescence emission was measured simultaneously on leaves from ten plants using a portable photosynthesis system (*LI-6400*, *LI-COR Inc*., Lincoln, NE, USA) with a leaf chamber (also an *LI-6400-40*), an LED-based fluorescence accessory. To assess the emission of Chl fluorescence in dark-adapted leaves, the leaf tissue was placed in standard *Hansatech* leaf clips for 30 min. Following dark-adaptation, the leaf tissue was illuminated with a weak-modulated measuring beam (0.25 kHz, < 0.1 μmol m^{-2} s^{-1}, 630 nm, 1 s) to obtain the minimal fluorescence yield in the dark-adapted state (F_o). A saturating white-light pulse (20 kHz, 6,000 μmol m^{-2} s^{-1}, 630 nm, 1 s) was applied to ensure maximal fluorescence yield of the dark-adapted state (F_m). The maximum photochemical efficiency of PSII (F_v/F_m) was calculated by the equipment as:

[$F_v/F_m = (F_m - F_o)/F_m$] (Baker 2008; Pires et al. 2011).

Chl content determination

Fresh leaves (collected from the same leaves used for the gas-exchange measurements) were ground in ice-cold 80% (v/v) acetone using a previously chilled mortar and pestle and were centrifuged at 4 °C at 1,000 rpm for 2 min. The supernatant was then collected and analysed in a spectrophotometer (*UV-2550, Shimadzu,* Kyoto, Japan) at 645 and 663 nm and the chlorophyll contents were calculated using the equations proposed by Arnon (Arnon 1949).

Statistical analysis

Statistical analysis was performed using *SPSS 18.0* (*Statsoft Inc.*, College Station, TX, USA). The means and calculated standard deviations (SD) were reported and analysed using one-way *ANOVA* and Tukey’s tests.

Conclusion

These results demonstrated that *D. nobile*, at 58% and 30% of solar irradiance, appeared to fare best at capturing and using light resources, and achieving plant growth as evinced by plant height and stem diameter measurements. Although seedlings at 30% of solar irradiance attained the highest P_{max} and LCP, the net photosynthetic rate, and stomatal conductance throughout the entire day was higher and the samples stored more dendrobine in stem at 58% of solar irradiance light levels than at 30% thereof. The lower contents of Chl ($a + b$), higher F_o and lower F_v/F_m in full sunlight induced photo-inhibition in *D. nobile*. The data on photosynthetic characteristics, growth, and dendrobine content at 58% of solar irradiance might be important for artificial cultivation.

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