Plant Omics Journal POJ

POJ 5(3):253-260 (2012) ISSN:1836-3644

Optimization of shade condition and harvest time for *Dendrobium candidum* plants based on leaf gas exchange, alkaloids and polysaccharides contents

Yueping Zheng[1], Wu Jiang[1], Evandro Nascimento Silva[2], Lingzhi Mao[1], David B. Hannaway[3], Hongfei Lu[1*]

[1]College of Chemistry and Life Science, Zhejiang Normal University, Jinhua, Zhejiang, 321004, China
[2]Biochemistry and Biology Molecular Department, Laboratory of Plant Metabolism, Federal University of Ceará, CP 6004, CEP 60451-970, Fortaleza, Ceará, Brazil
[3]Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331-3002, USA

***Corresponding author. Luhongfei63@yahoo.com.cn**

Abstract

To meet the increasing demand for *Dendrobium candidum* plants and its bioactive products, the optimal shade condition and the best harvest time was determined. Gas exchange of *D. candidum* plants under various shade treatments (93%, 88%, 85% and 75% shading) was measured in four seasons to determine the optimal light intensity for plant growth. Diurnal variations of the net CO_2 assimilation rate showed that *D. candidum* plants under the 85% and 88% shading had the highest value of P_N. Also, the 85% and 88% shading treatments resulted in higher values of areas enclosed by diurnal various curves of net CO_2 assimilation rate and x axis (AECX) than other treatments in the first two seasons (spring and summer) and the last two seasons (autumn and winter), respectively. To obtain highest levels of bioactive products, the optimal harvest time of *D. candidum* was determined to be of two-year-old stems grown under 88% shading treatments which had the highest contents of polysaccharides and alkaloids. The relationships among AECX, increased stem volume, leaf area, leaf perimeter, polysaccharide content and alkaloid content showed that biomass and bioactive substance contents were directly proportional to photosynthesis. Therefore, approximately 88% shading and harvesting two-year-old stems in the autumn season can be recommended for this species as photosynthesis, biomass and bioactive products were comprehensively considered.

Keywords: alkaloids; *Dendrobium candidum*; harvest time; polysaccharides; shade treatment.
Abbreviations: P_N - net CO_2 assimilation rate; E - transpiration rate; AECX - area enclosed by diurnal variation curves of net CO_2 assimilation rate and x axis; PAR - photosynthetically active radiation; $\boldsymbol{T}_{\mathbf{leaf}}$ - leaf temperature.

Introduction

Dendrobium candidum is an ornamental and traditional Chinese medicinal plant belonging to the Orchidaceae family. Its dried stems are used as a Yin tonic to nourish the stomach, promote the production of necessary bodily fluids, prevent the development of cataracts, relieve throat inflammation and fatigue, reduce peripheral vascular obstructions, and enhance the body's immune system (Biao et al., 2006). Its bioactive substances are mainly polysaccharides and alkaloids, especially dendrobine and dendrowardine (Chen and Guo, 2001). It is well documented that polysaccharides isolated from *D. candidum* have immunological activity (Huang et al., 1996) and the ability to inhibit the growth of tumor cells (Luo et al., 2000). Moreover, the horticultural production of *D. candidum* has developed rapidly in China and abroad. The sale in China amounts to seventy million dollars and the planted area of *D. candidum* is now about 700 hectares, of which 85% is in Zhejiang Province. Because of over exploitation and a low rate of propagation in nature, the natural resources of this species are in a steady decline. Fortunately, propagation through stem cuttings and tissue culture are well established for this endangered orchid species. However, plant materials for medicinal purposes derived from the same species can show significant differences in quality when cultivated at different sites, owing to the influence of season, growth environment and other factors (Leung et al., 2006). Therefore, strategies for the cultivation of *D. candidum* should be developed with the aim to increase the production of a specific bioactive compound. Abiotic agents such as climate, soil nutrients, and water have long been understood to be primary environmental factors influencing indoor and outdoor plant cultivation (Boyer, 1982; Jaidee et al., 2012; Novoa and Loomis, 1981; Zheng et al., 2012). It is relatively easy to control water and nutrient supplies through irrigation and fertilization, and light is also easy to be regulated indoors. In contrast, light intensities are more difficult to control in the field. It is widely understood that through the process of photosynthesis, light energy is used to produce ATP and NADPH in the light reaction. Subsequently, in the light-independent pathway, carbon is fixed as carbohydrates and oxygen is produced (Dai et al., 2009a). Some researchers used exogenous growth regulators to change the photosynthetic characteristics, and the light intensity was studied to optimize photosynthesis. Under high irradiance, the photosynthetic apparatus absorbed excessive light energy, resulting in the inactivation or impairment of reaction centers of PS II (Bertaminia et al., 2006). As a consequence of this photoinhibition, photosynthetic activity decreased. In contrast, under low irradiance, insufficient ATP is produced to allow carbon fixation and carbohydrate biosynthesis, which lead to a reduced the plant growth and lower total content of bioactive compounds. Although *D. candidum* has been reported to be a shade-preferring plant (Xu et al., 1993), no studies have determined the optimal light levels for its growth and production of polysaccharides and alkaloids. Furthermore, in many commercial *Dendrobium* hybrids, the juvenile periods varies between 2 and 4 years (Sim et al., 2007), and the concentration of biologically active constituents varies with growth and developmental stage of medicinal herbs (Leung et al., 2006). However, current methods simply harvest at maximum dry matter production, without considering the concentrations of these bioactive substances (Carr, 1972). The present study was conducted to determine the optimal light conditions and best harvest time of *D. candidum* with view to polysaccharide and alkaloid contents.

Results

Effects of shade on leaf gas exchange in D. candidum plants

In this study we observed that shade treatments caused significant changes in physiological parameters of *D. candidum* plants in all seasons observed. For example, in spring the photosynthetically active radiation (PAR) in the morning was higher in the treatment with 75% shade and reached the smallest values in the 93% shade treatment (Fig. 1A). It is important to note that during this period there was no significant difference between the treatments of 85% and 88% shade. In the afternoon, all treatments had similar effects. On the other hand, the leaf temperature (T_{leaf}) showed a similar pattern in all treatments studied (Fig. 1B). The net CO_2 assimilation and transpiration rate were strongly reduced over time in all treatments analyzed (Figs. 1C, D). In fact, all the shade treatments showed a two-peak pattern of diurnal photosynthetic changes in spring (Fig. 1C). Plants submitted to 75% shade showed an initial large peak at 8: 30 h and a smaller peak at 16: 00 h. However, the other shade treatments showed an initial P_N peak at about 9: 30 h and a second smaller peak at about 13: 00 h, except for the 93% shade treatment whose secondary peak occurred at about 13: 30 h. P_N values were highest under the 88% shade treatment. Plants grown under 93%, 88% and 85% shade treatments showed a midday depression at 11: 00 h, while plants under 75% shade treatment showed the depression at 13: 30 h. Diurnal patterns of *E* were remarkably similar to those observed in P_N in all shade treatments (Fig. 1D). In summer (Fig. 2), the PAR and T_{leaf} values increased from early morning and then decreased in all shade treatments (Fig. 2A and B). Compared with the data of spring, PAR and T_{leaf} reached higher values in summer. During this period, the first peak value of P_N also corresponded to $P_{N\max}$. These values were found at about 8: 30 h for 88%, 85% and 75% shade treatments, with the 88% treatment showing the highest value. However, plants grown under 93% shade treatment required more time to reach $P_{N\max}$ (Fig. 2C). In addition, P_N values suffered a depression at about 11: 00 h in all treatments. Furthermore, P_N in summer reached much more negative values than those observed in spring. As shown in Figs. 2C and 2D, the parameters P_N and *E* were often positively correlated. In autumn, the PAR (Fig. 3A) and T_{leaf} (Fig. 3B) values changed in a similar pattern with those observed in summer, although the values were much lower. The P_N response showed only one peak, except for the 75% shade treatment. Plants exposed to 88%, 85% and 75% shade reached their peaks at about 9: 30 h, while plants submitted to 93% shade reached their peak one hour later (Fig. 3C). Negative values of P_N were observed in plants under 93%, 88% and 85% shade treatments after 12: 30 h, while the P_N of 75% shade treatment reached its second peak at 14: 00 h. Diurnal changes of *E* during autumn showed a double-peak pattern under all treatments (Fig. 3D). Before the midday depression, the patterns of *E* were remarkably similar to those of P_N under each shade treatment. On the other hand, after the midday depression, the patterns of *E* and P_N were negatively correlated. In winter, sunrise was around 8: 00 h am local time and radiation increased rapidly (Fig. 4A). After reaching the peak, the values decreased slowly. The T_{leaf} reached its maximum at about 14: 00 h for all shade treatments (Fig. 4B). P_N values in four shade treatments fluctuated and were negative most of the time (Fig. 4C). Regarding transpiration, plants grown under 75% shade treatment showed significantly higher values of *E* than those of any other treatment before 13: 30 h. The highest *E* value in winter was found in plants exposed to 75% of shade at about 10: 00 h (Fig. 4D).

Effects of shade on polysaccharide and alkaloid content in D. candidum plants

The polysaccharide content in one-year-old stems was higher in autumn and winter than in spring and summer in all shade treatments (Fig. 5A). In fact, in spring and summer, the content remained low, and there was no significant difference among the four shade treatments. However, the polysaccharide level increased rapidly in autumn with mean values 2-fold higher than those in spring. Subsequently, in winter, the concentration was reduced except in the 88% shade treatment which reached the highest value among all treatments. Two-year-old stems showed similar values of polysaccharide content in 93%, 85% and 75% shade treatments in all seasons. However, the sugar concentration in the plants exposed to 88% of shade reached a higher level in summer and autumn than in spring and winter (Fig. 5B). In the third year, the stems showed similar patterns in polysaccharide accumulation in all the four treatments analyzed. It is important to note that the 88% shade treatment during the spring showed the highest levels of sugars when compared to other treatments in all seasons (Fig. 5C). In one-year-old stems, the alkaloid content increased progressively from spring to autumn in all treatments. However, in winter these concentrations fell (Fig. 6A). It is important to note that the highest level of alkaloids was observed in autumn irrespective of shading level. Similarly, two-year-old stems showed a progressive increase in alkaloid content from spring to autumn. However, unlike the first year, their level was maintained in winter (Fig. 6B). Similar to one-year-old stems, treatments with 88% and 85% shade had the highest alkaloid content. In the third year, their levels were significantly decreased in all seasons in all four treatments analyzed when compared to the previous two years (Fig. 6C). In this year, the highest contents of these compounds were reached in the 88% shade treatment, independently of the season.

Determination of AECX and its relationship with biomass and bioactive substances

Areas enclosed by diurnal variation curves of the net CO_2 assimilation rate and x axis (AECX) are showed in Table 1. Under any shade treatment, these areas were higher in spring and autumn than those in the two other seasons (summer and winter). Furthermore, the 85% and 88% shading treatments resulted in higher values of AECX than other treatments in the first two seasons (spring and summer) and the last two seasons (autumn and winter), respectively. Correlations among stem volume, leaf area, leaf perimeter, polysaccharide content,

Table 1. The areas enclosed by diurnal variation curves of net CO_2 assimilation rate and x axis (AECX).

Season	Shade treatments			
	93%	88%	85%	75%
Spring	479.730	956.443	821.700	511.650
Summer	109.315	465.681	300.933	142.954
Autumn	203.355	477.872	749.646	540.675
Winter	-481.977	-185.816	6.165	-34.511
Average value	77.606	428.545	469.611	290.192

Table 2. Pearson's correlation coefficients among increased stem volume (SV), increased leaf area (LA), increased leaf perimeter (LP), increased polysaccharide content (PC), increased alkaloid content (AC), and area enclosed by diurnal variation curve of net CO_2 assimilation rate and x axis (AECX).

	LA	LP	PC	AC	AECX
SV	0.696**[a]	0.683**	0.691**	0.725**	0.740**
LA		0.904***	0.426	0.507*	0.620*
LP			0.581*	0.626**	0.626**
PC				0.788***	0.738**
AC					0.707**

[a] * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

alkaloid content and AECX are given in Table 2. The Pearson's correlation indicated that AECX was significantly correlated with stem volume, leaf area, leaf perimeter, polysaccharide and alkaloid contents. A strong correlation was also observed between stem volume and the other parameters. In addition, the contents of polysaccharides and alkaloids were also significantly correlated with the other parameters, except leaf area.

Discussion

Diurnal changes in photosynthetic parameters were measured to assess the response of *D. candidum* plants to environmental conditions. On the basis of gas exchange data from the experiments (Figs. 1, 2, 3 and 4), shade treatments of 85% and 88% applied in this study increased P_N, which is in accordance with Loach et al. (1970) in that the productivity of many plants would benefit from suitable shading. It is well known that irradiance levels above the light saturation point reduce photosystem II efficiency, i.e. cause photoinhibition (Demmig-Adams et al., 1995). At super-optimal levels of irradiance, the photosynthetic apparatus absorbing excessive light energy will be injured (Melis, 1994; Dai et al., 2009b). This results in photoinhibition and photooxidation (Vasil'ev et al., 2004), which leads ultimately to a decrease of light energy conversion efficiency (Aro et al., 1993). This was also confirmed by other aspects of the diurnal variation of photosynthesis here. Low light levels in the morning and afternoon (shown by decreased PAR measurements) correspond to peak values of P_N illustrating the effects of reduced light on leaf photosynthesis (Fig. 1C, Fig. 2C and Fig. 3C). On the other hand, the P_N of plants in summer suffered midday depression because of the high irradiance at noon (Mohotti and Lawlor, 2002). Some other factors must also be involved in winter, since P_N decreased and remained low, not returning to the value of the morning as PAR decreased in the afternoon, even though the absolute levels of PAR would have been sufficient for higher P_N values. The relationships between PAR, T_{leaf}, P_N and E suggested that the capacity for CO_2 assimilation was decreased when the plants were exposed to high irradiance and temperature. The patterns of E were similar to those observed in P_N. Thus, it can be concluded that *D. candidum* plants grown under high light condition closed stomata to decrease water loss and adapted to high light irradiance (Dai et al., 2009a). Since *D. candidum* is a species of medicinal importance, knowledge of their polysaccharide and alkaloid contents are essential to determine the best harvest time. Therefore, this plant should be harvested when the production and the contents of bioactive substances are all relatively high. As the volume of one-year-old stems do not increase further, the content of polysaccharides and alkaloids are much more important. In this study, two-year-old stems grown under 88% shade showed the highest contents of the two bioactive substances in autumn (Figs. 5 and 6). These results are similar to those found by Lin (2009), who suggested that the best harvest time of *D. candidum* was the period from December to March, before flowering of 1.5-2 year old stems. In addition, the AECX of 85% and 88% shade treated plants were higher than those of 93% and 75% shade treatments. Therefore, it may be concluded that proper shading is necessary to optimize P_N, which is consistent with the results of gas exchange. Due to its physiological and growth responses to irradiance, *D. candidum* could be best described as a shade-tolerant species. Kumar and Tieszen (1980) reported that the net photosynthetic rates of shade-tolerant plants provided with shading are nearly twice as high than those of unshaded ones. Many studies have reported that shade-tolerant species have high P_N values when grown under shading (Fischer et al., 2000; Regnier et al., 1988; Stoller and Myers, 1989). Furthermore, Zhang et al. (2004) suggested that under full sunlight, photosynthesis is largely restricted by low stomatal conductance in shade-tolerant species, which acts as a protective response to excess transpiration. The significant positive correlation between AECX and increased stem volume, leaf area, leaf perimeter, polysaccharide content, and alkaloid content suggested that biomass and bioactive substance contents were directly proportional to AECX. A similar conclusion was reported by Kumar et al. (1998) in that the yield of crop plants ultimately depends on the size and efficiency of their photosynthetic system. Thereby, the higher values of biomass and bioactive substance contents were observed when higher AECX values appeared. Thus, the 85% and 88% shade treatments were recommended due to their higher AECX values as showed in Table 1.

Materials and methods

Plants and growth conditions

The experiments were carried out in a nursery localized in Yiwu Senyu Co. Ltd, Zhejiang Province in China. *Dendrobium candidum* plants were planted in the field with an area of approximate 40 hectares of which about 500 m^2 was subjected

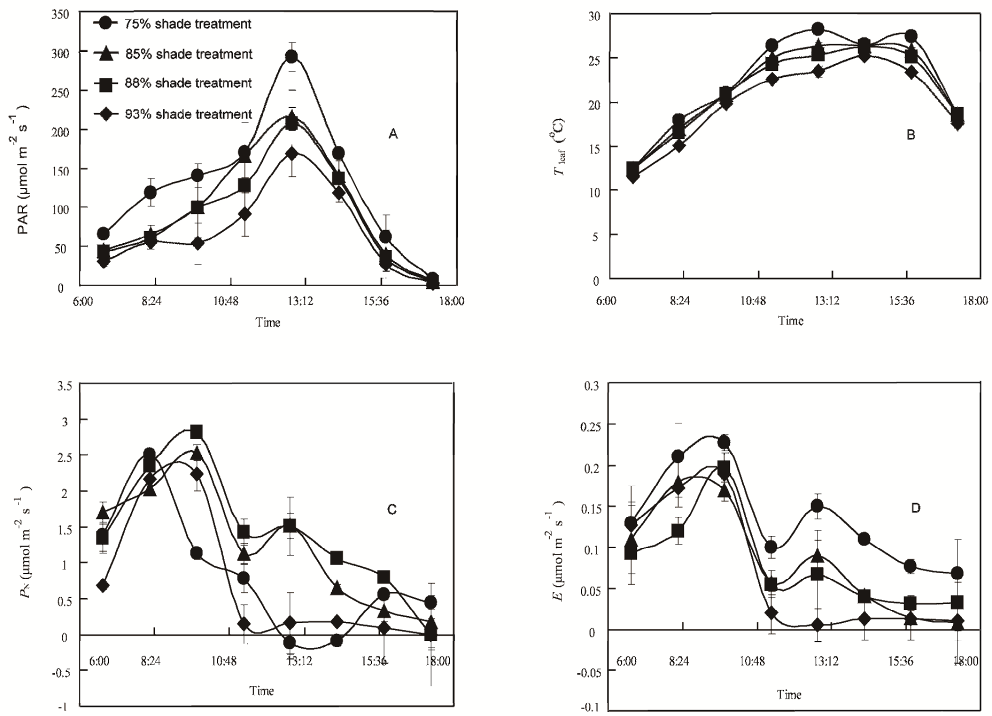

Fig 1. Diurnal change of PAR (A), T_{leaf} (B), P_N (C) and E (D) in spring under 93% (◆), 88% (■), 85% (▲) and 75% (●) shade treatments in *Dendrobium candidum* plants.

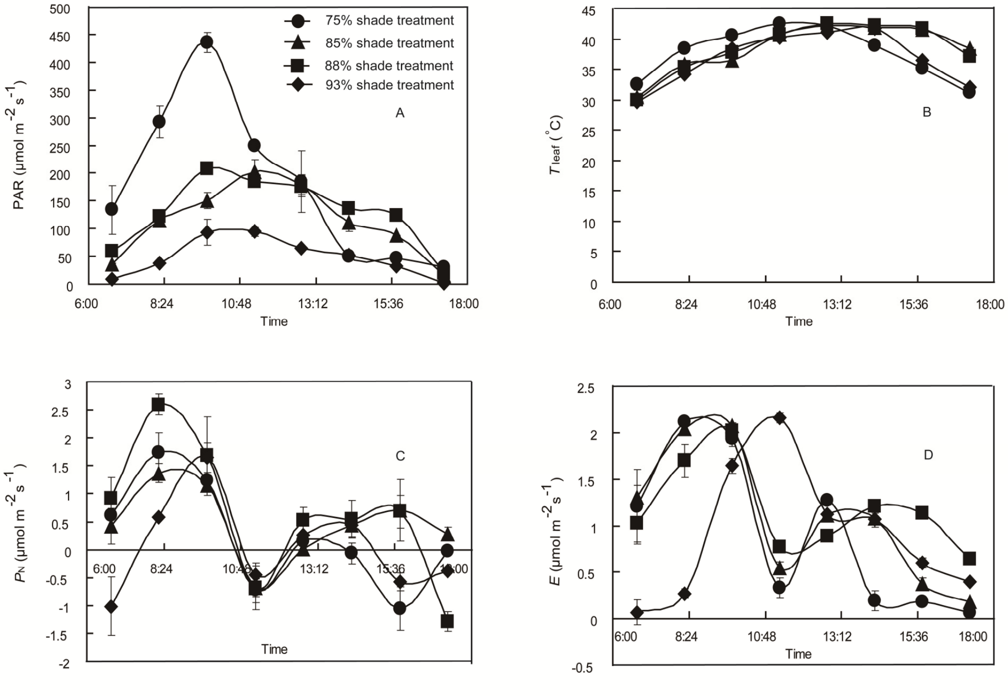

Fig 2. Diurnal change of PAR (A), T_{leaf} (B), P_N (C) and E (D) in summer under 93% (◆), 88% (■), 85% (▲) and 75% (●) shade treatments in *Dendrobium candidum* plants.

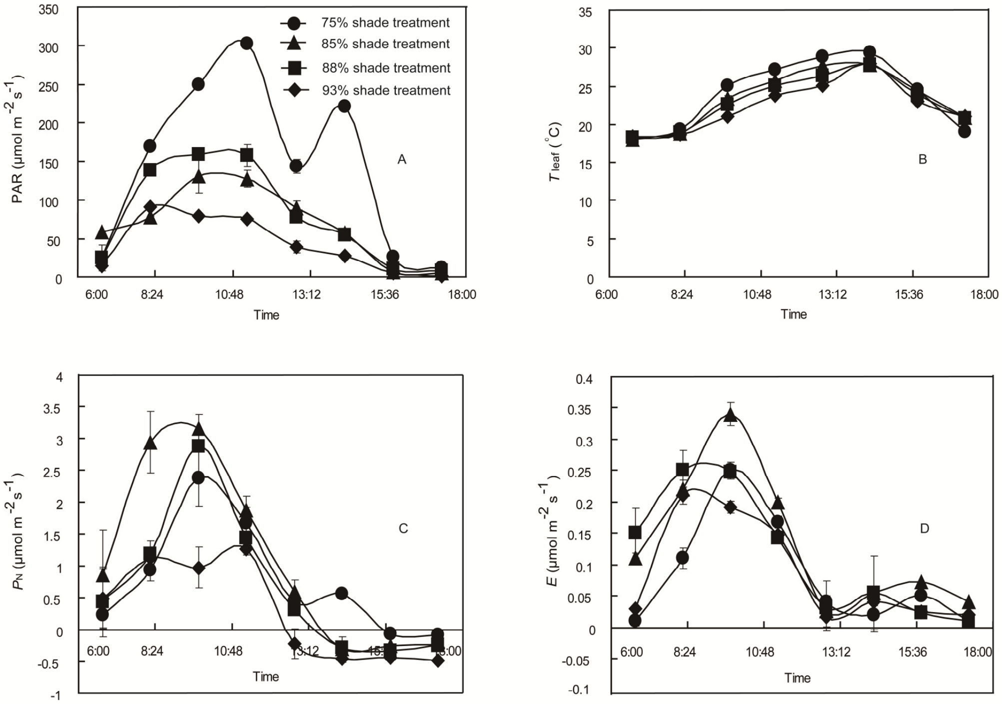

Fig 3. Diurnal change of PAR (A), T_{leaf} (B), P_{N} (C) and E (D) in autumn under 93% (◆), 88% (■), 85% (▲) and 75% (●) shade treatments in *Dendrobium candidum* plants.

to four shade treatments, 93%, 88%, 85%, and 75% shade. All measurements were conducted in spring (March), summer (July), autumn (November), and winter (January) over three years, respectively. The light intensities of each treatment was measured at 8:00 h, 13:00 h, and 16:00 h every day. Irrigation and fertilization were applied according to local production practices.

Leaf gas exchange

Through the process of photosynthesis, light changed into ATP and NADPH, with the help of which carbon is fixed into carbohydrates and oxygen. However, high light intensity could damage the photosynthetic apparatus and low light intensity produces too little ATP to fix carbon and biosynthesize carbohydrate (Xu et al., 2006). Therefore, whether the light intensity is suitable for the plant growth can be determined by detection of photosynthesis. The net CO_2 assimilation rates (P_N) and transpiration rates (*E*) were determined according to the methods of Mao et al. (2007) using the Portable Gas Exchange Fluorescence System LCA-4 (Analytical Development Company, Ltd., Hoddesdon, England). Measurements were taken in uppermost fully expanded leaves from 7:00 h to 18:00 h in bright sunlight on clear, cloudless days in each of the four seasons. The areas enclosed by diurnal variation curves of net CO_2 assimilation rate and x axis (AECX) (as shown in the shaded part of Fig. 7) were calculated using OriginLab (OriginPro, version 8.0). The CO_2 assimilation capacity of plants over a whole day was well quantified by this value. Therefore, the value is negative when respiration capacity is higher than CO_2 assimilative capacity.

Biomass measurements

Biomass allocation was measured according to the methods described by Shen et al. (2007), using WinFOLIA and WinRHIZO (Regent Instruments Inc., Quebec, Canada). Leaves were scanned with a Xatbed graphics scanner, and the images analyzed with WinFOLIA. The stems were spread out in water, scanned and the digitized images processed using WinRHIZO. Five plants were collected from each treatment in each season (spring, summer, autumn, and winter), taking care to keep the root systems intact for accurate measurements.

Polysaccharide and alkaloid contents determination

The quantity of polysaccharides and alkaloids, the two main bioactive substances in *D. candidum*, is influenced by many factors (Leung et al., 2006). They change especially with increased time of cultivation and seasons in a year. To determine the best harvest time for *D. candidum* plants, when the plants have the highest contents of bioactive substances, contents of polysaccharide and alkaloid were measured in one, two, and three years old stems under different seasonal conditions. According to the method described by Dubios (1956), polysaccharides were extracted from dried, ground plant material (0.1 g samples ground to power with 300μm in diameter) using 10 mL of 80% ethanol (v/v), followed by addition of 3 mL distilled water and a 30 min ultrasonic treatment. After filtration and triplicate extraction with 10 mL of chloroform, supernatants were concentrated and ethanol was added to a final concentration of 80% (v/v). Samples were kept overnight at 4 °C. The precipitated polysaccharides were collected and washed with absolute ethyl alcohol, acetone, and

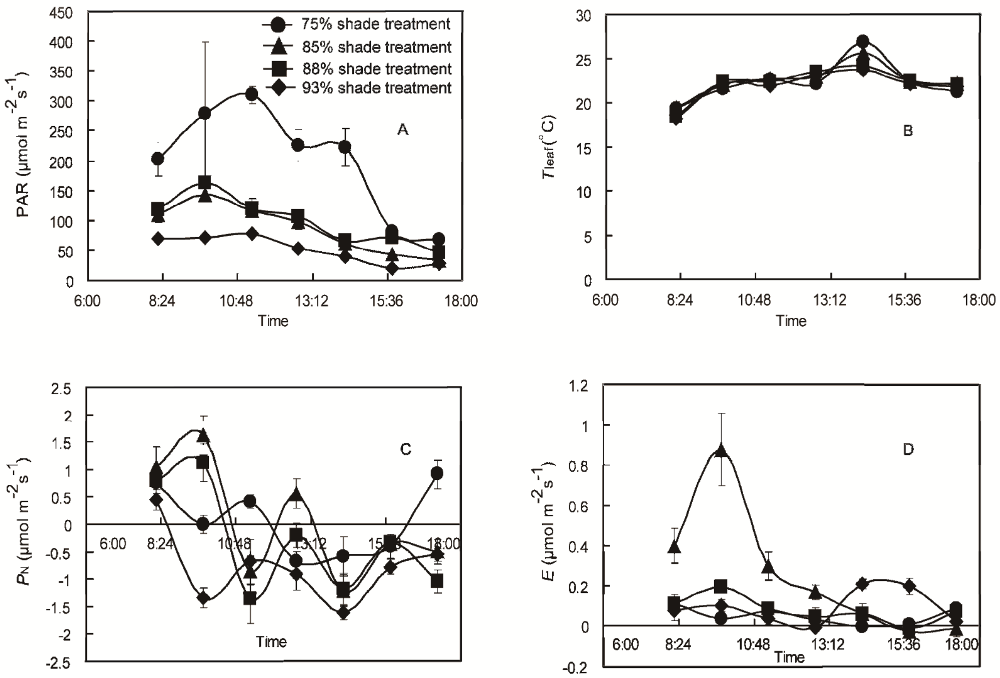

Fig 4. Diurnal change of PAR (A), T_{leaf} (B), P_N (C) and E (D) in winter under 93% (◆), 88% (■), 85% (▲) and 75% (●) shade treatments in *Dendrobium candidum* plants.

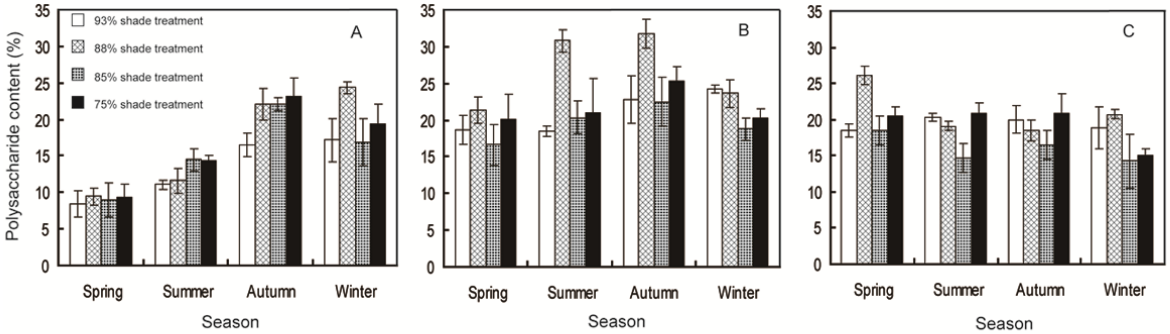

Fig 5. Polysaccharide content of one (A) -, two (B) - and three (C) -year-old stems in four seasons under different shade treatments in *Dendrobium candidum* plants.

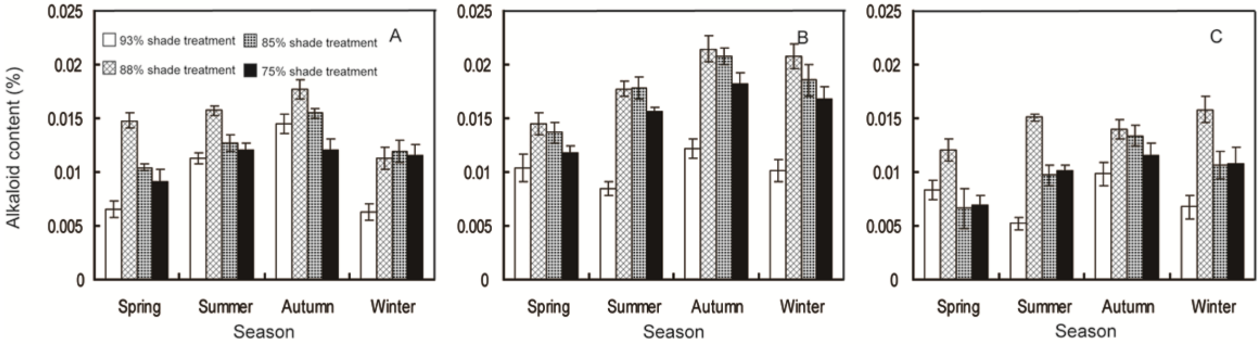

Fig 6. Alkaloid contents of one (A) -, two (B) - and three (C) -year-old stems in four seasons under different shade treatments in *Dendrobium candidum* plants.

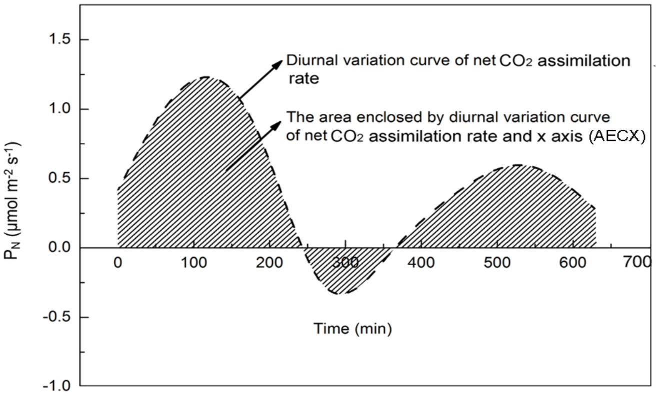

Fig 7. The schematic diagram of the area enclosed by diurnal variation curve of net CO_2 assimilation rate and x axis.

ether and then vacuum dried. Polysaccharide content was determined by the phenol-sulphuric acid method (Dubios, 1956). Plant samples were oven dried at 105 °C for 30 min, and then at 60 °C until reaching a constant weight. Samples of 0.4 g that had been ground to powder with 300 µm diameter were used for alkaloid content determination using the method of Bush et al. (1997). Samples were suspended in 10 mL of 28% ammonia solution and extracted with 10 mL of chloroform in a 100 mL conical flask. The flasks were weighed and placed in a 65 °C water bath for 2 h, then allowed to cool at room temperature, and chloroform added to their original weight. Then, 2 mL of the above filtered samples were mixed with 8 mL of chloroform, and 4 mL diluted solution was added to a separatory funnel and mixed with 6 ml chloroform. Afterward, 5 mL of pH 4.6 buffer, and 1 mL of 0.04% bromocresol green solution, and 1 mL of 0.01 mol/L NaOH were added to 6 mL of the filtered upper fractions from the separatory funnel for analysis. The absorbance was measured at 630 nm with a UV-VIS spectrophotometer (Lambda 5, Perkin-Elmer, USA).

Data analysis

The experiment was arranged in a completely randomized design with four treatments (93%, 88%, 85% and 75% shade treatments) with three replicates. Correlation analysis and Duncan's test were performed using the statistical analysis systems software package (SAS Institute, Cary, NC, USA). Differences at $p < 0.05$ were considered significant. The standard deviation (SD) is plotted in all figures.

Conclusion

In summary, light intensities significantly affected the growth of *D. candidum* plants. Photosynthetic activity of plants under 75% shading was depressed due to photoinhibition. Light irradiance in the treatment of 93% shading is too low to maintain the plant growth. Therefore, 85% or 88% shading is suggested the optimum light intensity. Two-year-old stems under 88% shading had the highest contents of polysaccharide and alkaloid when harvest in the autumn. Thus, approximately 88% shading and harvesting two-year-old stems in autumn can be suggested as the optimum light irradiance condition and best harvest time, respectively, for *D. candidum* cultivation. However, other cultivation conditions, such as soil water content and soil nutrients should be optimized in the future experiments.

Acknowledgement

This study was supported by the Key Program for Science and Technology of the Chinese Ministry of Education (Project 206061) and the Jinhua Science Technology Foundation of China (Project 2005-1-233). Thanks are extended to L.Y. Xu, D.Fang and R.R. Wang for their assistance in the measurements of alkaloid and polysaccharide contents. We acknowledge the workers of the lab of Yiwu Senyu Co. Ltd in Zhejiang Province for their help in raising the plants used in this study. The authors also thank Michael Möller for assistance with English language edition.

References

Aro EM, Virgin I, Andersson B (1993) Photoinhibition of photosystem II inactivation, protein damage and turnover. BBA-Bioenergetics 1143:113-134.

Bertaminia M, Muthuchelianb K, Rubinigga M, Zorera R, Velascoa R, Nedunchezhiana N (2006) Low-night temperature increased the photoinhibition of photosynthesis in grapevine (*Vitis vinifera* L. cv. Riesling) leaves. Environ Exp Bot 57:25-31.

Biao L, Wang BC, Tang K, Liang YL, Wang J, Wei JM (2006) A simple and convenient approach for isolating RNA from highly viscous plant tissue rich in polysaccharides. Colloid Surface B 49:101-105.

Boyer JS (1982) Plant productivity and environment. Science 218:443-448.

Bush LP, Wilkinson HH, Schardl CL (1997) Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol 114:1-7.

Carr MKV (1972) The climatic requirements of the tea plant: a review. Exp Agr 8:1-14.

Chen XM, Guo SX (2001) Advances in the research of constituents and pharmacology of *Dendrobium*. Nat Prod Res Dev 13:70-75. (In Chinese)

Dai YJ, Shen ZG, Liu Y, Wang LL, Hannaway D, Lu HF (2009a) Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. Environ Exp Bot 65:177-182.

Dai YJ, Shao MM, Hannaway DB, Wang LL, Liang JP, Hu LN, Lu HF (2009b) Effect of *Thrips tabaci* on anatomical features, photosynthetic characteristics and chlorophyll fluorescence of *Hypericum sampsonii* leaves. Crop Prot 28:327-332.

Demmig-Adams B, Adams III WW, Logan BA, Verhoeven AS (1995) Xantophyll cycle-dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. Aust J Plant Physiol 22:249-260.

Dubios M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350-356.

Fischer AJ, Messersmith CG, Nalewaja JD, Duysen ME (2000) Interference between spring cereals and *Kochia scoparia* related to environment and photosynthetic pathways. Agron J 92:173-181.

Huang MQ, Cai TY, Liu QL (1996) Effects of polysaccharides from *Dendrobium candidium* on white blood cells and lymph cell moving inhibition factor of mice. Nat Prod Res Dev 8:39-41. (In Chinese)

Jaidee R, Polthanee A, Saenjan P (2012) Growth and yield of soybean cultivars as affected by ground water levels and phosphorus rates grown under greenhouse and field conditions. Aust J Crop Sci 6:81-92.

Kumar RR, Manivel L, Marimuthu S (1998) Longevity and factors influencing photosynthesis in tea leaves. Photosynthetica 35:41-46.

Kumar D, Tieszen LL (1980) Photosynthesis in *Coffea arabica*. I. Effects of light and temperature. Exp Agr 16:13-19.

Leung PC (2006) Good agricultural practice-GAP: Does it ensure a perfect supply of medicinal herbs for research and drug development? In: Leung PC, Fong HHS, Xue CC (eds.) Current review of Chinese medicine: quality control of herbs and herbal materials, World Scientific Publishing Co. Pte. Ltd, Singapore, p 27-55.

Lin YF (2009) Study on quality standards and the best harvest time of *Dendrobium candidum* Wall. ex Lindl. MSc thesis, Zhejiang University, Hangzhou, p 65.

Loach K (1970) Shade tolerance in tree seedlings. II. Growth analysis of plants raised under artificial shade. New Phytol 69:273-286.

Luo HL, Cai TY, Cheng QL (2000) Enhancement of *Dendrobium candidium* polysaccharide on kolling effect of LAK cells of umbilical cord and peripheral cancer patients in vitro. Chin J Cancer 19:1124-1126. (In Chinese)

Mao LZ, Lu HF, Wang Q (2007) Comparative photosynthesis characteristics of *Calycanthus chinensis* and *Chimonanthus praecox*. Photosynthetica 45:601-605.

Melis A (1994) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage *in vivo*? Trends Plant Sci 4:130-135.

Mohotti AJ, Lawlor DW (2002) Diurnal variation of photosynthesis and photoinhibition in tea: effects of irradiance and nitrogen supply during growth in the field. J Exp Bot 53:313-322.

Novoa R, Loomis RS (1981) Nitrogen and plant production. Plant Soil 58:177-204.

Regnier EE, Salvucci ME, Stoller EW (1988) Photosynthesis and growth responses to irradiance in soybean and three broadleaf weeds. Weed Sci 36:487-496.

Shen JB, Li X, Wang DD, Lu HF (2007) In vitro culture of croftonweed (*Ageratina adenophora*): Considerable potential for fast and convenient plantlet production. Weed Technol 21:445-452.

Sim GE, Loh CS, Goh CJ (2007) High frequency early *in vitro* flowering of *Dendrobium* Madame Thong-In (Orchidaceae). Plant Cell Rep 26:383-393.

Stoller EW, Myers RA (1989) Response of soybeans (*Glycine max*) and four broadleaf weeds to reduced irradiance. Weed Sci 37:570-574.

Vasil'ev S, Shen JR, Kamiya N, Bruce D (2004) The orientations of core antenna chlorophylls in photosystem II are optimized to maximize the quantum yield of photosynthesis. FEBS Lett 561:111-116.

Xu YJ, Yu LW, Wu QS, Yang DL, Zhai LY, Cheng F.J (1993) Photosynthetic characteristics of three species of *Dendrobium* in Huoshan county of Anhui province. Chin J Appl Ecol 4:18-21. (In Chinese)

Zhang JZ, Shi L, Shi AP, Zhang QX (2004) Photosynthetic responses of four *Hosta* cultivars to shade treatments. Photosynthetica 42:213-218.

Zheng YP, Jiang W, Liao FL, Lu HF (2012) Optimization of light quality for production of alkaloid and polysaccharide in *Dendrobium candidum* Wall. ex Lindl. J Med Plants Res 6: 560-565.