Light Intensity Affects Growth, Photosynthetic Capability, and Total Flavonoid Accumulation of *Anoectochilus* Plants

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Abstract. *Anoectochilus formosanus*, a medicinal plant used to treat hypertension, lung disease, and liver disease, was grown to maximize biomass and secondary metabolite production in a controlled environment under four levels of photosynthetic photon flux (PPF), namely, 10, 30, 60, or 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, that is L₁₀, L₃₀, L₆₀, and L₉₀ treatments, respectively. On Day 45, all growth values were greatest for the L₃₀ plants. Dry weight was lowest for the L₁₀ plants. Leaf area, stem length, and fresh weight were lowest for the L₉₀ plants. The chlorophyll concentration was highest in the L₁₀ treatment and decreased with increasing PPF. Electron transport ratios of leaves were highest in the L₃₀ treatment and lowest in the L₉₀ for the second leaf (counted down from the apex) and in the L₁₀ for the third leaf. An increase in light intensity from 10 to 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased the superoxide dismutase activity and was associated with an increase in the total flavonoid concentration. The total flavonoid concentration (mg·g⁻¹ DW) was greatest in the L₆₀ and lowest in the L₉₀. However, the total flavonoid content (mg/plant) was highest in the L₃₀ plants as a result of great biomass. The results indicated that *A. formosanus* is a typical shade plant suitable to grow under low light intensity at PPF of 30 to 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for both growth and production of total flavonoid. A light intensity of 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ induced stress on plant growth and reduced photosynthetic capability and the flavonoid accumulation.

Anoectochilus, a perennial herb, belonging to the Orchidaceae family, which comprises more than 35 species that are widespread in the tropical regions, from India through the Himalayas and Southeast Asia to Hawaii (Asahishinbun, 1997). *Anoectochilus* is a short (10 to 15 cm in length) and typical shade plant and mainly distributed at an altitude of 400 to 1200 m of the wet zone. Several species have been used in Chinese medicine. Among

them, *Anoectochilus formosanus*, only found in Taiwan and Okinawa, has been used for hypertension, lung disease, and liver disease and underdeveloped children as a folk remedy (Kan, 1986). One of the most active components in *Anoectochilus* was flavonoid (Takatsuki et al., 1992). Many of flavonoid constituents have antioxidant properties (Shih et al., 2003).

The international trade of medicinal plants is becoming a major force in the global economy, and the demands of *A. formosanus* are rapidly increasing in Chinese medicine. However, overharvesting wild species is one of the serious issues for plant conservation. In addition, the explosive demand in plant materials for medicinal preparation has been accompanied by issues of quality and consistency, because the natural sources of *A. formosanus* are exhausted and growth of *A. formosanus* in the field has many problems, for instance, small seeds with slow germination rate, slow growth, and sensitive to stressful environments. To conserve wild species and to meet the demands

for medicinal plant materials, new technologies for *A. formosanus* plant production with great biomass and high quality are required.

In 2000, a new concept for the production of transplants in a controlled environment was introduced (Kozai et al., 2000). In this system, artificial light is the sole light source for plant growth; photosynthetic photon flux (PPF), CO₂ concentration, air temperature, relative humidity, and air speed are well controlled for optimizing plant productivity and quality. The major advantages of growing plants under controlled environments are maximization of plant biomass, consistency, and quality. Environmental factors also have remarkable effects on secondary metabolite biosynthesis. The secondary metabolites are defined as bioactive molecules, which provide the plant with defense mechanisms to survive from herbivores, environmental stresses, disease, or competition and may affect the growth and development of other organisms (Seigler, 1996). Quality of medicinal plants is determined by their superior genetic characteristics and great biomass with high and consistent secondary metabolite content (Kozai, 2005). High-quality medicinal plants can be produced only under carefully controlled environments (Afreen et al., 2005). Therefore, growing plants under a controlled environment can be considered an alternative way for medicinal plant production to ensure safety and efficacy.

A number of earlier investigations have reported that environmental factors such as light intensity can significantly improve growth and alter the metabolite concentrations. For example, the increasing light intensity at a PPF of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ significantly improved the growth and photosynthetic capability of in vitro *Momordica grosvenori* plantlets (Zhang et al., 2009). Briskin and Gawienowski (2001) reported that growing St. John's wort plants at a PPF of 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ significantly increased the hypericin concentration with enhanced photosynthetic activity. Light is also involved in regulating antioxidant enzymes and secondary metabolites. Mohammad et al. (2005) found that micropagated *Phalaenopsis* plantlets had higher superoxide dismutase (SOD) content to adapt the increasing light intensity. Zhong et al. (1991) successfully increased anthocyanin production in cell culture of *Perilla frutescens* (shiso) by increasing light intensity.

However, there is little information about the effects of light intensity on growth and secondary metabolite of *A. formosanus*. Therefore, the objective of this study was to determine the effects of light intensity on the growth, photosynthetic capability, and total flavonoid accumulation of *A. formosanus* plants in a controlled environment.

Materials and Methods

Plant material, treatments, and culture conditions. *Anoectochilus formosanus* plantlets were cultured in vitro for 30 d and acclimated ex vitro for 10 d. The acclimated plantlets, each with four or five unfolded leaves, were used in this study. The average leaf area,

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fresh and dry weight, and stem length per plant were $764 \pm 16 \text{ mm}^2$, $821 \pm 17.3 \text{ mg}$, $78 \pm 2.6 \text{ cm}$, and $6.98 \pm 0.09 \text{ cm}$, respectively. The plants were transplanted into a plastic tray ($540 \times 270 \times 50 \text{ mm}$) containing vermiculite and peatmoss (Growing Mix I; Fafard Co., Ltd., Inkerman, Canada) in a 1:2 ratio by volume and placed in a growth chamber (2.3 m in length, 1.8 m in width, 1.4 m in height, Zhejiang Qunxin Biological Industry Development Co., Ltd., Jiaxing, China). The experiment included four treatments, L_{10} , L_{30} , L_{60} , and L_{90} , in which L indicates light intensity and subscripts denote PPF. Light intensity was measured on the surface of the empty shelves with a light meter (Model LI-250A; LI-COR, Lincoln, NE).

The air temperature and relative humidity in the growth chamber were maintained at $25 \pm 1^\circ\text{C}$ and $80\% \pm 5\%$, respectively. The photoperiod was 14 h per day supplied with cool-white fluorescent lamps. The CO_2 concentration was maintained at $1000 \mu\text{mol}\cdot\text{mol}^{-1}$ in the growth chamber. The CO_2 concentration was measured and controlled by a CO_2 controller with a nondispersive infrared detector CO_2 sensor (T6004; Telaire Co., Ltd., Shoreview, MN). Seventy-two replications (72 plants) were used for each treatment. The experiment was conducted twice.

Measurements, calculation, and statistical analysis. The plants were harvested on Day 45 for destructive measurements and analysis. Plant growth, chlorophyll concentration and fluorescence, SOD activity, and total flavonoid concentration were measured. Leaf area was determined using image and quantify analysis software (LIA32 for Windows 95; K. Yamamoto, Nagoya, Japan) with an image scanner. Dry weight of the plants was determined after oven-drying at 80°C for 72 h.

For chlorophyll measurement, the second and third fully expanded leaves from the apex were cut into pieces, soaked in 5 mL N, N-dimethylformamide solution, and shaken once every 10 min for 2 h at 4°C (Moran and Porath, 1980). Absorbance was measured with an ultraviolet-vis spectrophotometer (Helios Gamma; Thermo Spectronic Co., Ltd., Cambridge, UK) at wavelengths of 664 nm, 647 nm, and 603 nm. The chlorophyll concentration, including chlorophyll a and chlorophyll b, was determined on a fresh weight basis ($\mu\text{g}\cdot\text{g}^{-1}$) and calculated using the formula of Moran (1982).

The ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) and electron transport rate (ETR) were measured and calculated with a Maxi-Imaging-PAM chlorophyll fluorescence measuring system (Walz, Effeltrich, Germany). After 43 d of culture, the second and third fully expanded leaves (counted down from apex) were used for the measurement. Six replications or six leaves were measured in each treatment. For chlorophyll fluorescence measurement, the plants were placed in a dark room to adapt for 15 min. Next, randomly chosen intact leaves from four treatments were placed in the measuring head ($10 \times 13 \text{ cm}$) to measure the F_v/F_m . The leaves without dark adaptation were used to evaluate ETRs. Each ETR value was obtained using actinic irradi-

ance for 10 s. All relevant fluorescence parameters, including actinic irradiance and leaf temperature, were recorded and ETRs were calculated in the Maxi-Imaging-PAM system.

The extraction and activity analysis of SOD were performed by using the method described by Rao and Sresty (2000). In brief, the 0.5 g leaves were ground with 5 mL ice-cold 0.05 mol/L phosphate buffer (pH 7.8). The homogenates were centrifuged at 4°C for 20 min at $12,000 \times g$ and the resulting supernatants were used for determination of enzyme activity. SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The 3-mL reaction mixture contained 1.5 mL phosphate buffer (pH 7.8), $13 \mu\text{M}$ methionine, $75 \mu\text{M}$ NBT, $2 \mu\text{M}$ riboflavin, $10 \mu\text{M}$ EDTA- Na_2 , and $50 \mu\text{L}$ of enzyme extract. The reaction was initiated by exposing the tubes to 4000 lx light intensity for 20 min. A nonirradiated complete reaction mixture served as a blank and another complete reaction mixture without enzyme, which gave the maximal blue color, served as a control. The reaction was stopped by quenching the irradiation light, and then the tubes were covered with a black cloth waiting for testing. The absorbance was recorded at 560 nm by the ultraviolet-vis spectrophotometer and one unit of SOD was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction.

Total flavonoid concentration in the leaves of plants grown at different light intensities

was measured following the method of Sakanaka et al. (2005). The reaction contained 0.25 mL of the methanolic plant extract or (+)-catechin standard solution and 1.25 mL of distilled water followed by the addition of 0.75 mL 5% sodium nitrite solution. After 6 min, 0.15 mL of 10% aluminum chloride solution was added and the mixture was allowed to stand 5 min and then 0.5 mL of 1 M sodium hydroxide was added. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately at 510 nm. The data were calculated using (+)-catechin for total flavonoid concentration.

Statistical significance was determined by one-way analysis of variance using Sigma Stat (SigmaStat™ for Windows Version 2.03; SPSS Inc., Chicago, IL). Differences among means were assessed with the Holm-Sidak test and a significance level of $P \leq 0.05$.

Results

Plant growth. The results of plant growth with different light intensities on Day 45 are summarized in Table 1 and Figure 1. Among all treatments, the plant growth was greatest in the L_{30} treatment followed by L_{60} . Leaf area, stem length, and fresh weight were 1.4, 1.1, and 1.3 times greater, respectively, in the L_{30} than those in the L_{90} . Dry weight was 1.4 times greater in the L_{30} than that in the L_{10} . There was no significant difference in dry weight between L_{60} and L_{90} treatments. An increase

Table 1. Effects of four levels of light intensity on the growth of *Anoectochilus formosanus* per plant grown for 45 d under a controlled environment.

Treatment	Leaf area (mm^2)	Stem length (cm)	Plant weight (mg)		
			Fresh	Dry	DW/FW (%)
L_{10}^z	$963 \pm 16 \text{ b}^y$	$8.26 \pm 0.10 \text{ b}$	$995 \pm 13 \text{ c}$	$103 \pm 1.3 \text{ c}$	10.4
L_{30}	$1167 \pm 22 \text{ a}$	$8.57 \pm 0.06 \text{ a}$	$1184 \pm 22 \text{ a}$	$140 \pm 2.7 \text{ a}$	11.8
L_{60}	$986 \pm 15 \text{ b}$	$7.97 \pm 0.08 \text{ c}$	$1043 \pm 20 \text{ b}$	$126 \pm 2.4 \text{ b}$	12.1
L_{90}	$850 \pm 19 \text{ c}$	$7.90 \pm 0.07 \text{ c}$	$936 \pm 13 \text{ d}$	$121 \pm 1.5 \text{ b}$	12.9

^z L_{10} , L_{30} , L_{60} , and L_{90} were four light intensity treatments, in which L indicates light intensity and subscripts denote photosynthetic photon flux density.

^yData are mean $\pm$ SE, and means denoted by the same letter in a column were not significantly different ($P \leq 0.05$).

DW/FW = dry weight/fresh weight.

Fig. 1. *Anoectochilus formosanus* grown under controlled environment with four levels of light intensity, 10, 30, 60, or $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, for 45 d. L_{10} , L_{30} , L_{60} , and L_{90} were four light intensity treatments, in which L indicates light intensity and subscripts denote photosynthetic photon flux (PPF).

in light intensity increased the ratio of dry weight to fresh weight of plant (DW/FW).

The plants cultured in the L_{10} treatment not only had lowest dry weight, but also had lowest ratio of DW/FW. The plants cultured in the L_{90} treatment had the highest ratio of DW/FW, but the thickset stem, and small, thick, and yellow leaves were observed in the L_{90} treatments (Fig. 1).

Chlorophyll concentration and chlorophyll a/b ratio. The chlorophyll concentration of plants was significantly influenced by the different light intensities. Among all treatments, the chlorophyll concentration was greatest in the L_{10} treatment and then decreased consistently as the light intensified, lowest in the L_{90} treatment (Fig. 2). In contrast, the ratio of chlorophyll a to chlorophyll b increased to a maximum (3.72) as the light intensified to a PPF of $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and then decreased to 3.67 at a PPF of $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, creating a parabola.

Ratio of Variable to Maximum Chlorophyll Fluorescence and Electron Transport Rate. The F_v/F_m ratio, reflecting the maximal photochemical efficiency of photosystem II (PSII), is usually steady with fluctuations from 0.75 to 0.85 for nonstressed plants (Bolhar-Nordenkamp et al., 1989). In our present study, the F_v/F_m (determined using the second or third leaf from the apex) ranged from 0.75 to 0.81, except for the L_{90} , which had an abnormal ratio (0.36) in the second leaf. Probably, photoinhibition had occurred in the second leaf taken from the L_{90} treatment and consequently it had the lowest ETR (Fig. 3A).

The ETRs of leaves including the second and third leaves were highest in the L_{30} treatment followed by the L_{60} . The ETRs were, respectively, lowest in the L_{90} treatment for the second leaf and the L_{10} for third leaf (Fig. 3). Moreover, the maximum ETR for all treatments was at a PPF of $35 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the second leaf and a PPF of $55 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the third leaf.

Superoxide dismutase activity and total flavonoid content. Increase in light intensity from 10 to $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ resulted in a significant increasing SOD activity (Fig. 4). SOD activity in the L_{60} treatment was 2.3 times higher than that in the L_{10} . However, SOD activity decreased as light intensity increased to L_{90} .

Fig. 2. Effects of four levels of light intensity, 10, 30, 60, or $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, on the chlorophyll concentration and chlorophyll a/b ratio of *Anoectochilus formosanus* plants. L_{10} , L_{30} , L_{60} , and L_{90} were four light intensity treatments, in which L indicates light intensity and subscripts denote photosynthetic photon flux (PPF). Vertical bars represent SE.

Similarly, as the light intensity increased from 10 to $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the total flavonoid concentration of the plant increased to the highest in the L_{60} treatment, but then significantly decreased to the lowest in the L_{90} (Fig. 5). The total flavonoid concentration of the L_{60} plant was 1.3 times greater than that of the L_{90} plant.

Discussion

Plant growth and photosynthetic capability. Light intensity is one of the key environmental factors influencing plant growth. The results clearly showed that *A. formosanus* plants grown under L_{30} achieved a great biomass with largest leaf area and longest stem length among all treatments (Table 1; Fig. 5). Chen et al. (1994) reported that the light saturation point of *Anoectochilus* was $\approx 60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, indicating *Anoectochilus* is a typical shade plant. Moreover, a light intensity either too low or too high would not be suitable to growth of *A. formosanus* plants. In the present study, the plant grown under L_{10} and L_{90} had lower biomass and smaller leaf area. The low biomass of plants has frequently been explained by low photosynthetic ability. The low photosynthesis can result in low growth rate. ETRs allow for the rapid and noninvasive assessment of light response of PSII and are used to study the photosynthetic performance and photoacclimation of photosynthetic organisms. The maximum ETR was related to the maximum photosynthetic capability, which was obtained when the photosynthetic rate was limited by the

Fig. 3. The rapid light curves of *Anoectochilus formosanus* plants (A) for the second leaves and (B) for the third leaves from the apex of the shoot at four levels of light intensity, 10, 30, 60, or $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ on Day 45. L_{10} , L_{30} , L_{60} , and L_{90} were four light intensity treatments, in which L indicates light intensity and subscripts denote photosynthetic photon flux (PPF). Vertical bars represent SE.

activity of the electron transport chain or Calvin cycle enzymes (Behrenfeld et al., 2004; Ralph et al., 2005). As shown in Figure 3, the L_{30} plants had the highest ETRs, which contributed to a great dry weight. The L_{10} and L_{90} plants, however, had the lowest ETRs in the third and second leaves, respectively, which contributed to low dry weight in both treatments.

Photoinhibition may occur when light intensity exceeds what is required for the saturation of photosynthesis. The decline in F_v/F_m represents the accumulation of a photodamaged PSII center (Rosenqvist and Kooten, 2003). In our study, the decline in F_v/F_m was observed in the second leaves of the L_{90} plants ($F_v/F_m = 0.36$). The $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (L_{90}) probably exceeded the photoprotective capacity of the plants and resulted in photodamage. This adverse phenomenon can manifest as a loss of PSII activity (Masuda et al., 2002). A loss in the reaction-center content of PSII was associated with a slower ETR (Pell et al., 1994). Thus, the photoinhibition could account for the lower capacity of ETR as shown in the leaf from the L_{90} treatment in our study. Vasilikiotis and Melis (1994) also reported that in photoinhibition, the chloroplasts contained much of the same amount of PSII as they did under low light intensity. However,

Fig. 4. Effects of four levels of light intensity, 10, 30, 60, or $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, on the superoxide dismutase (SOD) activity of *Anoectochilus formosanus* plants. L_{10} , L_{30} , L_{60} , and L_{90} were four light intensity treatments, in which L indicates light intensity and subscripts denote photosynthetic photon flux (PPF). Vertical bars represent SE.

Fig. 5. The total flavonoid concentration of *Anoectochilus formosanus* plants at four levels of light intensity, 10, 30, 60, or $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, on Day 45. L_{10} , L_{30} , L_{60} , and L_{90} were four light intensity treatments, in which L indicates light intensity and subscripts denote photosynthetic photon flux (PPF) density. Vertical bars represent SE.

up to $\approx 80\%$ of PSII were photochemically inactive because of photodamage. This is the reason why the L_{10} plant with low light intensity and the L_{90} plant with photoinhibition had low ETRs and resulted in low carbohydrate accumulation in both treatments. In the present study, we also found that the ETRs in the second leaves from the L_{90} treatment were lower than those from the L_{10} . In contrast, the ETRs in the third leaves from the L_{90} were higher than those from the L_{10} . The phenomenon can be explained by the fact that the upper leaves may create a dense canopy to protect lower ones from photodamage during plant growth. Thus, the dry weight of plants from the L_{90} treatment was greater than that from L_{10} .

Although the decline in F_v/F_m was not observed in the leaves of the L_{60} plants, it could be assumed that the L_{60} plants also probably had little light stress resulting from lower ETRs compared with the L_{30} plants. Moreover, as shown in Figure 3, the *PPF* for the maximum photosynthetic capability of *A. formosanus* plants in all the treatments was $35 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the second leaf and $55 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the third leaf. We therefore suggest that *PPF* of 30 to $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ would be suitable for growth of *A. formosanus* plants.

Chlorophyll concentration. The chlorophyll concentration of plants plays an important role in the absorption of light during photosynthesis. In the present study, low light intensity significantly enhanced chlorophyll concentration of plants, and the chlorophyll concentration decreased as the light intensity increased. The greatest chlorophyll concentration was estimated at a lowest *PPF* of $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The results agree with the finding of Walters et al. (2003) that leaves of plants growing at low *PPF* have relatively higher contents of chloroplastic pigments, electron carriers, and increased number of chloroplast. The result also demonstrates that plants could balance light absorption and translation by regulating chlorophyll synthesis (Bailey et al., 2001). The change of chlorophyll concentration was possibly an acclimation of plants to different light intensities.

For many plants, changes in light intensity may elicit physiological responses at the level of leaf and chloroplast (Bailey et al., 2001). In our study, the leaf responded to the light intensity by adjusting or reducing the chlorophyll concentration. Chloroplast responded to light intensity by changing chlorophyll a/b and adjusting to the ETR. As shown in Figure 2, an increase in light intensity from L_{10} to L_{60} was associated with an increase in chlorophyll a/b and a decrease in the size of the PSII light-harvesting antenna (Bailey et al., 2001; Masuda et al., 2002). Thus, interconversion of chlorophyll a and chlorophyll b is significant for the establishment of required chlorophyll a/b ratio during the adaption of leaves to high and low light intensity (Ito et al., 1993).

Superoxide dismutase activity. SOD is an important enzyme, which contributed to clear up superoxide in plants in the case of light stress, and SOD activity can give us some

valuable information whether the plant was stressed by high light. Plants can fix carbon and photosynthesize by absorbing light energy at a proper light intensity. Excess light absorption resulted in acidic chloroplast lumen, reduced electron transport chain, and excess excitation energy (EEE) within chloroplast (Powles, 1984). EEE is toxic for plants by producing singlet oxygen, which can cause photoinhibition mainly resulting from oxidative damage to the PSII. Depletion of the NADP^+ pool under EEE causes an increase in the rate of electron flow from the donor side of PSI to oxygen, generating reactive oxygen species (ROS) such as superoxide and hydrogen peroxide (Asada, 1999). If these ROS are not removed immediately, they can easily cause damage to the cellular and molecular machinery, protein modification, and lipid peroxidation. Plants have evolved various antioxidative enzymes to avoid damage. SOD is an important antioxidative enzyme, which contributes to clear up superoxide in plants. In the present study, increasing light intensity from 10 to $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased SOD activity, which suggested SOD plays an important role against the ROS caused by light stress. However, when light intensity reached $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, SOD activity was found inversely, which probably related to the susceptibility of the plant to photodamage or to a higher temperature around the plants under high light intensity. Similarly, Rabinowitch (1980) found that in scorching conditions, SOD activity decreased; without scorching, SOD activity should increase.

Total flavonoid content. The production of total flavonoid content can be explained by the interaction of oxidative stress and photosynthesis. It is widely believed that the synthesis of secondary metabolites in plants is part of the defense responses of plants to oxidative stress. A number of earlier investigations have suggested that oxidative stress plays an important role for the synthesis of secondary metabolite in plant growth (Wojtaszek, 2002). In our study, as the light intensity increased from 10 to $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the SOD activity increased along with an increase in the total flavonoid concentration. The greatest total flavonoid concentration found in the L_{60} plants may be the result of the increased ROS induced by oxidative stress. The secondary metabolite synthesis of plants requires endogenous signal components such as ROS (Menke et al., 1999). ROS can function as a signal for the induction of defense systems and could enhance secondary metabolite production (Berglund and Ohlsson, 1995). This enables the plants to protect them against oxidative stress (Scalet et al., 1995).

Meanwhile, for many carbon-based metabolites, several hypotheses have been developed to explain phenotypic and evolutionary patterns in the distribution of plant secondary metabolites (Heyworth et al., 1998). For example, according to the "overflow metabolism" concept, when carbon production exceeds the carbon demand associated with plant growth, the excess carbon is channeled into biosynthesis of secondary metabolites (Matsuki, 1996). In

the present study, the total flavonoid concentration ($\text{mg}\cdot\text{g}^{-1}$ DW) in the L_{30} plants was lower than that of the L_{60} plants. However, the total flavonoid content (mg/plant) in the L_{30} plants was higher than that of the L_{60} plants as a result of a higher dry mass or enhanced photosynthesis. The total flavonoid concentration significantly decreased in the L_{90} plants can be explained by low photosynthesis resulting from photoinhibition and synthesis mechanical damage. Further study is required to better understand the relationship among photosynthesis, oxidative stress, and secondary metabolite contents in *A. formosanus* plants.

Conclusion

To summarize, *A. formosanus* is a typical shade plant suitable to grow under lower intensity of *PPF* ranging from 30 to $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for both growth and production of total flavonoid content. Higher light intensity such as a *PPF* of $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ induced stress on plant growth and reduced photosynthetic capability and the flavonoid accumulation. The production of *A. formosanus* plants with high total flavonoid content under a controlled environment can be further increased by optimizing other environmental parameters such as light quality, photoperiod, temperature, and relative humidity and by optimizing the nutrient composition. Such studies are currently underway in our laboratory. Therefore, growing plants in a controlled environment can be considered a good alternative way of producing high-quality *A. formosanus* to meet the demand for its medicinal use. We are hopeful that application of a controlled environment with the optimization of environmental factors will be an efficient method for producing *A. formosanus* plants with great biomass and high secondary metabolite.

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