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GROWTH, PHYSIOLOGICAL RESPONSES AND SECONDARY METABOLITE PRODUCTION IN *PINELLIA TERNATA* UNDER DIFFERENT LIGHT INTENSITIES

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Abstract

Pinellia ternate (Thunb.) Breit (Araceae) is a shade-tolerant perennial medicinal plant, but its optimum light intensity is not sufficiently known. Hence, the influence of light intensity on the physiological responses, growth and secondary metabolic products in *P. ternata* under four light conditions (15%, 25%, 45% and 100% of full sunlight) in a greenhouse were to be investigated. The results show that the fresh and dry tuber weight, leaf area, tuber number and propagation index were highest in *P. ternata* grown under the intermediate light level (45% sunlight). In contrast, 15% sunlight induced the lowest growth parameters but the highest plant height. Sunlight from 15% to 45% significantly increased the chlorophyll concentration compared to that under full sunlight conditions. Moreover, 100% sunlight enhanced the POD and CAT activities and induced MDA, total alkaloid, guanosineand succinic acid accumulation. The maximum yields of total alkaloids, guanosineand succinic acid occurred under 45% sunlight. Overall, 45% sunlight was the most efficient way of improving the tuber yields and qualities of *P. ternata*. These results suggest that *P. ternata* is suitable for growth in a moderate-low-light environment and an appropriate light control can produce higher tuber and secondary metabolite yields within agroforestry systems.

Key words: *Pinellia ternata*, Light intensity, Growth, Physiological responses, Secondary metabolites.

Introduction

Light is one of the crucial environmental factors that significantly influences plant development and growth and, eventually, plant yield and quality (Naoya *et al*., 2008). Light intensity affects various plant characteristics. In general, plants grown under high light intensity have been shown to be less susceptible to photoinhibition than plants grown under low light intensity (Long *et al*., 1994). Low light may increase the plant height, chlorophyll content and specific leaf area (Gregoriou *et al*., 2007; Hou *et al*., 2010). The photosynthesis demand can be met by these adaptations which maximize the capture of available light (Steinger *et al*., 2003). In contrast, high light acclimating many morpho-physiological traits, such as reduction in the specific leaf area to protect the plant under high light and increase the plant leaf reactive oxygen species (ROS) scavenging enzymes (e.g., peroxidases (POD), catalase (CAT) and superoxide dismutase (SOD)) due to the formation of ROS caused by photooxidative stress (Asada & Takahashi, 1987; Ali *et al*., 2005; Wu *et al*., 2015). These measures ensure that the photosynthesis is proceeding at its normal rate through preventing damage caused by excessive light energy (Wentworth *et al*., 2006; Matos *et al*., 2009).

Light intensity affects the biosynthesis and metabolism of bioactive compounds (Zavala & Ravetta, 2001; Coelho *et al*., 2007; Cai *et al*., 2009). The theory of the carbon/nutrient balance, which suggests that plants grown in nutrient-rich environments with low light (i.e., shade) availability show the decline in growth, concentration of carbohydrates and carbon-based defenses compounds, besides the an increase in the concentration of nitrogen-based defense compounds, for instance cyanogenic glycosides and alkaloids (Bryant *et al*., 1983). For instance, some nitrogen-based defense compounds are increased (Coelho *et al*., 2007) while carbon-based defenses compounds decreased (e.g., terpenes, tannins, phenols, etc.) in some medicinal plants leaves under a decreased light intensity (Briskin & Gawienowski, 2001; Wang *et al*., 2007; Zhang *et al*., 2015). Nevertheless, high irradiance enhanced the production of nitrogen-based secondary metabolites (e.g., purine alkaloid and reserpine) in *Coffea arabica* non-photosynthetic tissues (Kurata *et al*., 1997) and in *Rauvolfia vomitoria* root tissues (Cai *et al*., 2009). Understanding how different light environments impact the secondary metabolites production will be of great consequence for optimizing field growth conditions for maximal yield of phytochemicals and for the sustainable use and conservation of medicinal plants.

Pinellia ternata (Thunb.) Breit (Araceae) is a shade-tolerant perennial medicinal plant that grows in humid and shady environments, such as roadsides, forests and stream sides (Flora of China, 1977). *P. ternata* is widely distributed throughout East Asia, particular in China, mainly in the eastern and southern provinces and regions of China, including Guizhou, Sichuan, Hubei, Henan, Jiangsu and Jiangxi (Zhang *et al*., 2013). The dried tuber of*P. ternata*, Pinelliae Rhizoma, called "Banxia" in Chinese, is a traditional Chinese herbal medicine that is valued for its therapeutic effects, including treating coughs, dispelling phlegm, reducing vomiting, terminating early pregnancy, and fighting infection and inflammation (Chen *et al*., 2003; Kim *et al*., 2006). Moreover, modern pharmacological studies suggest that *P. ternata* possesses multiple activities, such as antitussive, antiemetic, antitumor, expectorant, antibacterial, anti-inflammatory, antioxidant and sedative-hypnotic activities (Wang *et al*., 2008).

The market demand for *P. ternata* has been raising in these few years because of these discoveries mentioned above.The enormous demand in China cannot be met by wild resources, hence, it has been suggested since the 1970s that *P. ternata* be cultivated in small-scale plantations in eastern China (Guo *et al*., 1993). The cultivation of medicinal plants could also curtail the excessive harvesting of wild resources, which leads to a loss of genetic diversity, resource depletion and habitat destruction (Canter *et al*., 2005). In recent years, some well known medicinal plants that have been successfully employed for the cultivation and management within agroforestry systems, such as *Ilex paraguariensis* (Coelho *et al*., 2007), *Rauvolfia verticillata* (Cai *et al*., 2009), *Glycyrrhiza uralensis* Fisch (Hou *et al*., 2010), and *Glechoma longituba* (Zhang *et al*., 2015). Previous studies have reported that *P. ternata* plants possess some degree of shade tolerance and thus could be suitable for growth in agroforestry systems (Zhang *et al*., 2009). However, knowledge of the growth properties, physiological responses and secondary metabolite production of this species under different light intensities is very limited.

The present study was to examine in a greenhouse the results of different light intensities irradiation on the growth traits, chlorophyll concentration, antioxidant enzyme activities, secondary metabolites contents and yields of *P. ternata*, including its total alkaloid, succinic acid and guanosine contents, which are recognized as standards for the quality control of Pinelliae Rhizoma under different natural light levels. This study was to provide a deep understanding of *P. ternata* photoacclimation mechanisms under four light conditions. Our results should be applied to determine whether suitable light control can facilitate the tuber yields and enhance the medicinal qualities of *P. ternata* in cultivation.

Materials and Methods

Plant materials: The experiments were carried out from June to July 2012 at the Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou Province, P.R. China. *P. ternata* tubers were collected from the same population in Weining County, Guizhou Province, and identified by Prof. Qiaosheng Guo of Nanjing Agricultural University. Uniform and healthy tubers (1.5 ± 0.2 cm in diameter and 2.0 ± 0.1 g in weight) were selected and were cultured in a plastic pot (18 cm in diameter and 15 cm in height) containing fertile sandy soil under natural day/night conditions in a greenhouse of the Guizhou Academy of Agricultural Sciences. The field capacity (FC) of the sandy soil mixture was 27.79%. Each pot contained 7 tubers, which were submerged and maintained under a 3 cm layer of soil.

Experimental design: After being cultured for 5 days, 9 randomly selected pots for each treatment were sheltered with shade nets (2.0 m aboveground) and moved to one of four irradiance treatments: 100% irradiance (maximum illumination 1000 ± 20 μmol m^{-2} s^{-1}), 45% irradiance (55% shaded), 25% irradiance (75% shaded), and 15% irradiance (85% shaded). Each treatment with 9 replications (pots), a total of 36 pots. On a clear day, the photosynthetic photon flux density (PPFD) was measured at 12:00 and 13:00 using a TES-1335 Digital Luxmeter (Rotronic, Taipei, Taiwan) that was positioned 20 cm aboveground. All of *P. ternata* plants were well-watered to FC, the plants were fertilized with 10 g of organic compound fertilizer through the experiment. The plants were collected to determine plant growth indicators (plant height and leaf area), antioxidative enzyme activities, malondialdehyde content and chlorophyll contents at 25 days after shade treatment. Plant biomass parameters (tuber fresh and dry weight) and bioactive component contents (total alkaloids, succinic acid and guanosine) in the dry tubers were investigated at 35 days after treatment, at harvest.

Growth and biomass analysis: Plant growth parameters, including the fresh tuber weight, dry tuber weight, plant height and leaf area, were measured. The *P. ternata* leaf area was measured using a portable area meter (LI-COR LI-3100, Lincoln, Neb), and the plant height was measured by a ruler. The tubers per plant were dried at 60°C until a constant weight and measured using an electronic balance (Sartorius Bp221S, Germany). The tubers of each *P. ternata* plant were collected and counted. The morphology and growth experiment was repeated 3 times for each treatment.

The propagation index was calculated using the following equation:

Propagation index = Tuber number after harvest/Planting tuber number

Chlorophyll concentrations: Fresh leaf tissue (0.2 g) was extracted in 80% acetone (15 mL) in the shade for 24 h. The extract was centrifuged at 5000 rpm (Allegra X-22R, Beckman Coulter, Fullerton, CA, USA) for 15 min and then used for measurement with an UV-visible spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA, USA) at 663 nm (OD_{663}) for Chl a and 645 nm (OD_{645}) for Chl b.

Antioxidant enzyme activities: Fresh leaf tissue (0.5 g) were homogenized in 10 mL of 50 mM sodium phosphate buffer containing 1% polyvinylpyrrolidone (w/v). The homogenate was centrifuged for 10 min at 4°C at 15,000 rpm, and then collect supernatant to determination the antioxidative enzyme activities.

POD activity was measured according to the method of Shannon *et al*. (1966) with some modification. The POD reaction solution contained 40 mM H_2O_2, enzyme extract (0.1 mL), 50 mM phosphate buffer (pH 6.0) and 50 mM guaiacol. Changes in the absorbance were determined every 30 s for 4 min at 470 nm. One unit of POD activity was defined as 1 mg substrate catalysised 0.01 μmol H_2O_2 for 1 min in the reaction system.

CAT activity was measured according to Pukacka & Ratajczak (2005). The reaction mixture was 3 mL contained 0.2 mL of 200 mmol·L^{-1} H_2O_2, 1 mL of 30 mmol·L^{-1} Tris-HCl buffer (pH 7.0) and enzyme extract (0.1 mL). Decrease in absorbance of the reaction solution at 240 nm determined at an interval of 20 s. An absorbance change of 0.01 unit min^{-1} was defined as one unit CAT activity. The activities of POD and CAT were expressed on the basis of fresh weight (U·g^{-1} FW·min^{-1}).

Malondialdehyde content: The malondialdehyde (MDA) was measured according to the method of Stewart & Bewley with minor modifications (Stewart & Bewley, 1980). Briefly, a 0.5 g fresh leaves were homogenated in 20 mL 5% trichloracetic acid (TCA). The homogenate was centrifuged for 10 min at 5000 rpm. Then, the collected supernatant (1 mL) was added to 3 mL 5% thiobarbituric acid (TBA) in a 95°C boiling water bath for 10 min, and then rapidly cooled . The supernatant was obtained by centrifuging at 3000 rpm for 15 min. The absorbance of the supernatant was recorded at 532 nm. The MDA concentration was calculated by using an extinction coefficient of 155 mmol cm^{-1}, corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The MDA content was expressed in micromoles per gram of fresh weight (μmol·g^{-1} FW).

Total alkaloid content: Dried tuber powder (0.5 g) was extracted with ammonia water (0.5 mL) and chloroform (10 mL) in an ultrasoniccleaners for 50 min at a cool temperature and then filtered through filter paper. Distilled water (5 mL), citric acid buffer (pH 5.4, 5 mL) and the sample extract (10 mL) were added to 1 mL of bromothymol blue (BTB) reagent. After mixing, the ion-pair complex that formed was extracted with chloroform (10 mL) by shaking vigorously for 1 min. The absorbance was recorded at 414 nm against a reagent blank. The total alkaloid content was calculated in milligrams of pseudoephedrine hydrochloride equivalents per 100 mg of dry weight (%). To calculate the total yield from the dry weight of the tuber mass multiplied by the total alkaloid concentration divided by the dry weight per plant tuber.

Succinic acid content: Succinic acid was measured using the potentiometric titration method (Jie *et al*., 2015). In this method, powdered tuber (5.0 g) was mixed with ethyl alcohol (50 mL) and then extracted with normal heating reflux for 1 h. After cooling, the solution was added to 10 mL of NaOH solution (0.1 mol·L^{-1}) and extracted ultrasonically for 30 min. According to the Chinese Pharmacopoeia (Committee for the Pharmacopoeia of P.R. China, 2015), a standard HCl solution (0.1 mol·L^{-1}) was used for titration, and the results were corrected by blank titration. An aliquot of 1.0 mL of NaOH titration fluid (0.1 mol·L^{-1}) was equivalent to succinic acid (5.904 mg). The succinic acid standard was purchased from the Institute of Chemical Technology, SYQT (Beijing, China). The total yield was calculated from the dry weight of the tuber mass multiplied by the succinic acid concentration divided by the dry weight per plant tuber.

Guanosine content: The tuber powder (1.0 g) was mixed with 15 mL of 10% methanol in an ultrasonic bath at normol temperature for 35 min, and the extracted solution was then centrifuged at 12,000 rpm for 15 min. The supernatant was filtered through a organic membrane filter (0.45 μm) before HPLC injection. Each extract sample was analyzed on an Agilent 1200 series HPLC (Agilent Technologies Inc., Santa Clara, CA, USA). C_{18} column (150 × 4.6 mm, 5 μm, Agilent Technologies, Palo Alto, CA, USA) at 25°C with a sample injection volume of 20 μL. Detection wavelength operated at 254 nm, the flow rate was 1.0 mL min^{-1}, and a mixed methanol-0.1% acetic acid solution (5:95, v/v) as the mobile phase. The guanosine standard was purchased from Lancaster Synthesis (Lancaster, United Kingdom) and used to prepare the calibration curves. Samples analytes were identified by the comparison of retention time with that of the authentic standard, and the guanosine concentration was determined from a standard curve of peak area vs concentration. The total yield was calculated from the dry weight of tuber mass multiplied by the guanosine concentration divided by the dry weight per plant tuber.

Statistical analyses: All data was analyzed using a one-way ANOVA. The differences among treatments were calculated using Duncan's multiple range tests (p<0.05) of SPSS 17.0 software (SPSS, Chicago, IL, USA) confirme.

Results

Growth, morphology and biomass: The morphology of *P. ternata* plants was significantly different under different light intensities (Table 1). The 15% sunlight treatment induced the lowest fresh and dry tuber weights, leaf area, tuber number and propagation index but the highest plant height, while 45% sunlight induced the highest tuber fresh and dry tuber weights, leaf area, tuber number and propagation index compared to the control treatment. When sunlight was increased from 15% to 45%, an increase in the fresh and dry tuber weights, leaf area, tuber number and propagation index and a decrease in the plant height were observed. Under the 45% sunlight, these five growth parameters (except for plant height) were the highest, with a significant difference from greater than the other three groups.

Chlorophyll content: Chlorophyll content was increased as the light intensity decreased (Fig. 1). The chlorophyll (a + b) and chlorophyll b (Chl b) concentrations were significantly higher under the 15% to 45% sunlight treatments than in the control. The chlorophyll a (Chl a) concentration was significantly higher under the 25% to 45% sunlight treatments than in the control. There were no differences in chlorophyll a between the 15% sunlight treatment and the control. The plants that were grown under low light intensity showed a low Chl a/b ratio compared to the control. However, Chl a/b remained unchanged with increased sunlight intensity from 15% to 45%.

Table 1. Effects of different light intensities on the morphology of *P. ternata* plants

Light treatment	Tuber fresh weight (g)	Tuber dry weight (g)	Plant height (cm)	Leaf area (cm^2)	Tuber number	Propagation index
15%	2.19 ± 0.01 c	0.72 ± 0.03 c	22.25 ± 0.59 a	13.35 ± 0.25 d	7.5 ± 0.29 d	0.84 ± 0.03 d
25%	2.37 ± 0.02 c	0.90 ± 0.01 b	18.66 ± 0.54 b	18.25 ± 1.42 b	11.5 ± 0.65 b	1.06 ± 0.07 b
45%	2.69 ± 0.02 a	1.08 ± 0.11 a	17.28 ± 0.45 b	21.28 ± 0.74 a	13.0 ± 0.91 a	1.44 ± 0.10 a
100%	2.50 ± 0.03 b	0.91 ± 0.04 b	15.02 ± 0.28 c	15.91 ± 0.89 c	8.5 ± 0.29 c	0.93 ± 0.03 c

Each value is presented as the mean ± SD (n=3). Different letters in columns indicate statistically significant differences (p<0.05)

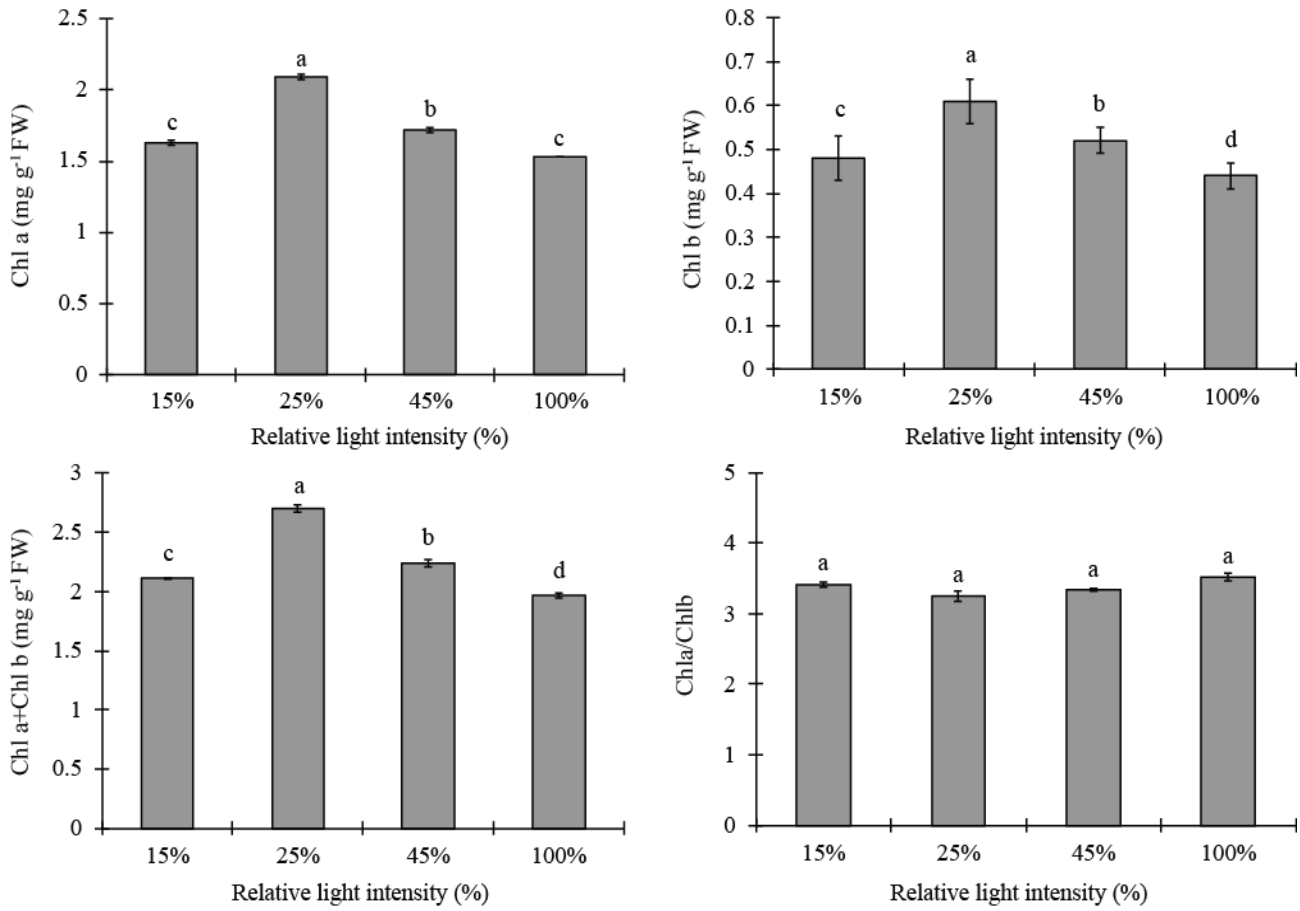

Fig. 1. Effects of different light intensities on the leaf chlorophyll contents of *P. ternata* after 25 days of treatment. The values are expressed as the mean ± SD (n = 3). Bars carrying different letters are significantly different at $p<0.05$ among at the different light intensities.

Table 2. Effects of different light intensities on the total alkaloid, succinic acid and guanosine contents of *P. ternata* tubers.

Light treatment	Total alkaloids (%)	Succinic acid (%)	Guanosine (%)
15%	0.0232 ± 0.0003 d	0.252 ± 0.0012 d	0.0188 ± 0.0016 b
25%	0.0244 ± 0.0002 c	0.260 ± 0.0013 c	0.0163 ± 0.0016 c
45%	0.0273 ± 0.0001 b	0.264 ± 0.0013 b	0.0197 ± 0.0014 b
100%	0.0295 ± 0.0003 a	0.284 ± 0.0019 a	0.0232 ± 0.0012 a

Each value is presented as the mean ± SD (n=3). Different letters in columns indicate statistically significant differences ($p<0.05$)

Table 3. Effects of different light intensities on the total alkaloid, succinic acid and guanosine yields of *P. ternata* tubers.

Light treatment	Total alkaloids (mg plant^{-1})	Succinic acid (mg plant^{-1})	Guanosine (mg plant^{-1})
15%	0.17 ± 0.00 d	1.82 ± 0.05 d	0.14 ± 0.00 b
25%	0.22 ± 0.01 c	2.32 ± 0.08 c	0.15 ± 0.01 b
45%	0.29 ± 0.02 a	2.85 ± 0.20 a	0.21 ± 0.01 a
100%	0.27 ± 0.01 b	2.56 ± 0.08 b	0.21 ± 0.01 a

Each value is presented as the mean ± SD (n=3). Different letters in columns indicate statistically significant differences ($p< 0.05$)

Antioxidant enzyme activity and malonaldehyde accumulation: There were significantly different CAT and POD activities and MDA content in *P. ternata* plants under different light intensities (Fig. 2). The highest MDA content and CAT and POD activities were observed under the 100% sunlight treatment, whereas the lowest activities of CAT and POD were observed under the 15% sunlight treatment. Meanwhile, the lowest MDA content was found in the 45% sunlight treatment. When sunlight decreased from 25% to 15% or increased from 25% to 45%, a gradual decrease in the POD and CAT activities was observed. There was no significant difference in the CAT activity among the 15%, 25% and 45% sunlight treatments. When sunlight was increased from 15% to 45%, a significant decrease in the MDA content was observed.

Total alkaloid, succinic acid and guanosine contents: The total alkaloid and succinic acid contents of *P. ternata* tubers were highest under 100% sunlight and lowest under 15% sunlight. When the sunlight was increased from 15% to 45%, a significant increase in the total alkaloid and succinic acid contents was observed. Meanwhile, the highest and lowest guanosine contents were found under 100% sunlight and 25% sunlight, respectively. When sunlight was decreased from 25% to 15% or increased from 25% to 45%, a gradual increase in the guanosine content was observed (Table 2).

Total alkaloid, succinic acid and guanosine yields: The total alkaloid, succinic acid and guanosine yields of *P. ternata* tubers were highest under 45% sunlight and lowest under 15% sunlight. When the sunlight increased from 15% to 45%, a significant increase in the total alkaloid, succinic acid and guanosine yields was observed. However, when sunlight exceed 45%, the total alkaloid and succinic acid yields (except for guanosine) significantly decreased (Table 3).

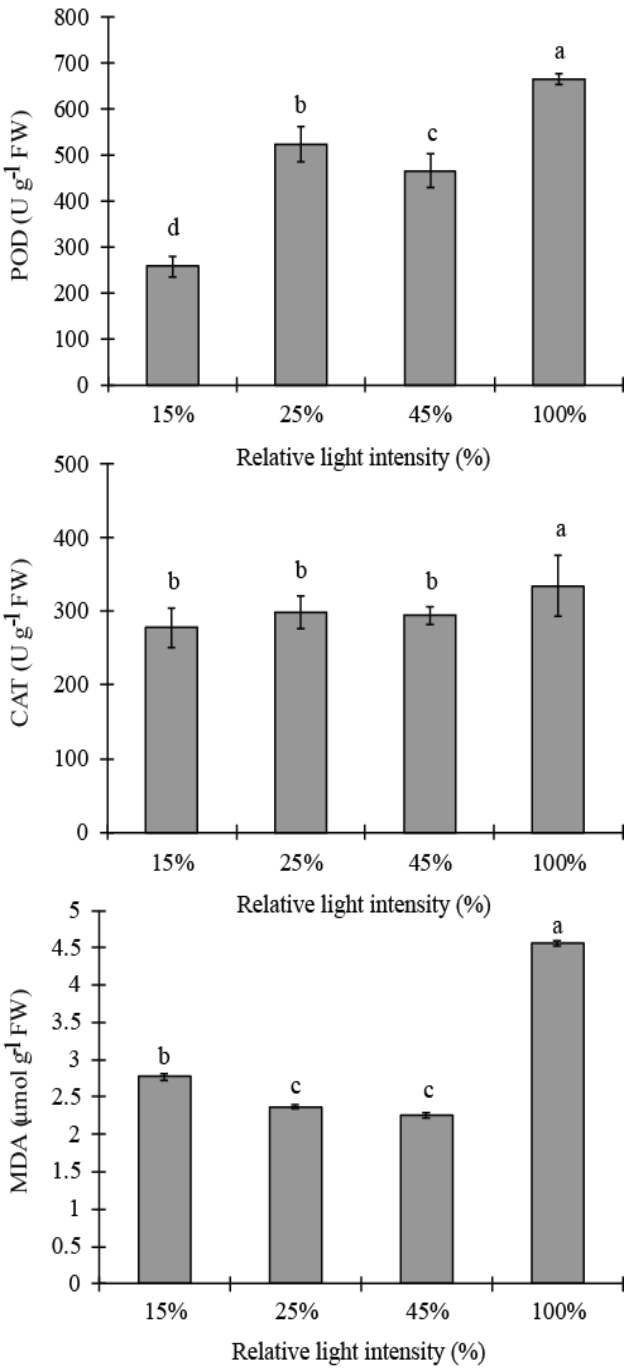

Fig. 2. Effects of different light intensities on the peroxidase (POD) and catalase (CAT) activities and malonaldehyde (MDA) content in the leaves of *P. ternata* after 25 days of treatment. Bars are expressed as the mean ± SD (n = 3). Bars carrying different letters are significantly different at $p<0.05$ among the different light intensities.

Discussion

The growth, physiological responses and secondary metabolite production in plants are regulated by environment light signals (Huang *et al*., 2004; Coelho *et al*., 2007; Zhang *et al*., 2015). Previous studies have indicated that increased shading drastically reduces the biomass of plant (Zavala & Ravetta, 2001; Huang *et al*., 2004; Gregoriou *et al*., 2007). Contrast with it, the addition in chlorophyll concentrations and leaf area has been shown to occur under those conditions (Huang *et al*., 2004; Gregoriou *et al*., 2007; Zhang *et al*., 2015). In the present study, *P. ternata* plants under low light intensity were taller, indicating that plants under low light intensity may allocate more biomass to the shoot for the growth of leaves and for the full absorption of limited energy to meet the demand for plant photosynthesis (Walters & Reich, 1999). Meanwhile, the increased leaf area of *P. ternata* grown under low light treatment indicates that plants increase their photosynthetic surface to contribute to a more efficient absorption of light radiation (Lambers *et al*., 2008). In our study, the low sunlight levels of 15% and 25% induced lower fresh and dry weight, tuber number, and propagation index in *P. ternata*, indicating that low light levels are not suitable for the growth of this species (Table 1). The intermediate light level (45% sunlight) produced the highest fresh and dry weight, tuber number and propagation index of *P. ternata* plants, suggesting that 45% sunlight increased root allocation to favor an increase in water uptake and is considered a preferable light irradiance for the tuber growth of *P. ternata* plants. However, at greater than 45% sunlight, these four growth parameters gradually decreased, showing that high light probably decreases the photosynthetic efficiency, results in photoinhibition and strongly inhibits *P. ternata* growth.

The pigment compositions of *P. ternata* leaves have demonstrated significant variation under different sunlight intensities (Table 2). In our study, the lower light level (25% sunlight) promoted higher Chl a and Chl a+b concentrations that might be associated with maximizing the light absorption capacity under low light treatments (Lei *et al*., 1996; Zhang *et al*., 2015). A similar study showed that high light condition could negatively affect chlorophyll contents in *P. ternata* (Meng *et al*., 2007). However, the light levels of 15% or 100% decreased Chl b content irradiance, which might be related to Chl disorganization by low or excess sunlight (Griffin *et al*., 2004; Zhang *et al*., 2015). The results suggested that the photooxidative stress damage to the photosystems Ⅰ and Ⅱ in the leaves of *P. ternata* was similar due to the similar reduction of the Chl a and Chl b contents and the Chl a/b ratio almost unchanged.

The activities of POD and CAT increased significantly under full sunlight conditions compared to the low light level (15%-45% sunlight) (Table 3). The POD activity increased markedly may have resulted because of the H_2O_2 formation (Ali *et al*., 2005), enabling plants to defend themselves against oxidative stress (Scalet *et al*., 1995). Some previous studies have shown that an increase of POD, SOD and CAT activities induced by higher light inleaves of *P. ternata* and other plants (Logan *et al*., 1998; Mittler, 2002; Meng *et al*., 2007; Xu *et al*., 2010). Thus, these results suggest that enhanced antioxidant defense system in the *P. ternata* can be alleviated photoinhibition by detoxifying excess ROS under higher light environment.

MDA is an end product of lipid peroxidation in membrane, and its level can be increased when exposed to stress conditions (Liu *et al*., 2009). ROS excessive outpit in plants under oxidative stress conditions and will accordingly boost MDA content (Alscher *et al*., 2002). In our study, the highest MDA content in *P. ternata* leaves was observed under full sunlight treatment, indicating that high light intensity induces oxidative stress (Sielewiesiuk,

2002). Our result is similar to previously reported findings (Meng *et al*., 2007). In addition, a remarkable increase in the MDA content was observed in plant leaves, indicating more serious peroxidation in the membrane (Xu *et al*., 2010; Dong *et al*., 2014).

The increase in POD and CAT activities mainly suggests that *P. ternata* grown under a high light intensity causes photooxidative stress owing to the becoming of ROS (e.g., H_2O_2 and $O_2^{\bullet-}$). Secondary metabolite accumulation is a defense system that can help plants to face various challenges by altering cellular metabolism to respond and adapt to oxidative stress (Gulen & Eris, 2004).In this study, we found that the total alkaloid, guanosineand succinic acid contents were the highest in the full sunlight treatment. Similar results were obtained for the highest total alkaloid and guanosine contents in *P. ternata* under full sunlight conditions (Zhang *et al*., 2009). Similarly, previous investigations have shown that purine alkaloids actually exhibit better antioxidant capacity in cellular and animal assays (Tsoi *et al*., 2015). Additionally, a pharmacological experiment showed that total alkaloids improved the activity of antioxidases (e.g., SOD) against ROS and inhibited peroxidation in rat lung homogenate (Zhao *et al*., 2016). Therefore, the free-radical-scavenging and membrane-stabilizing capacity of total alkaloids and guanosine (a component of purine alkaloids) induce oxidative stress when exposed to full sunlight stress.

High light intensity accelerated the production of neutral lipids (Brown *et al*., 1996; Yakovleva, 2005). This result showed that adequate chlorophyll and light were conducive for the NADPH production, which could increase the ACCase activity, thus resulting in the conformation of more lipids and fatty acids (Livne & Sukenik, 1992; Thelen & Ohlrogge, 2002). This result is consistent with our research and thus explains why more fatty acids (e.g., succinic acid) were generated under the higher light intensity.

The hypothesis of the balance of carbon/nutrient (Bryant *et al*., 1983) proposes an augment in nitrogen-based defense compounds because carbohydrate synthesis decreases due to inhibition of photosynthesis. In our study, an increase in the nitrogen-based secondary metabolites (e.g., total alkaloids and guanosine) was observed in *P. ternata* when photosynthesis was inhibited due to full sunlight irradiance. However, an add in the nitrogen-based defense compounds was not observed in *P. ternata* plants when photosynthesis was inhibited due to low light. This phenomenon is consistent with previous studies (Ralphs *et al*., 1998; Hägele & Rowell-Rahier, 1999). It is possible that the increase in nitrogen-based secondary metabolites under low light environments is very typical of shade-tolerant plants (Hoft *et al*., 1998).

The yields of total alkaloids, guanosineand succinic acid were correlated with the maximum photosynthetic rates across all of the sunlight levels and were positively correlated with the changes in the *P. ternata* tuber dry mass. These results indicate that the intermediate light level (45% sunlight) did not inhibit photosynthesis but rather increased the yields of the *P. ternata* tuber dry mass and maintained a critical concentration of total alkaloids, guanosineand succinic acid. Consequently, the maximum total alkaloid, guanosineand succinic acid yields were measured under 45% sunlight, while they decreased sharply in all of the other conditions.

Conclusion

Compared to those of the other light treatments, our results demonstrate that the fresh and dry tuber weights, leaf area, tuber number and propagation index were highest in *P. ternata* plants grown under the intermediate light level (45% sunlight). In contrast, 15% sunlight induced the lowest values of these growth parameters but the highest plant height. Sunlight from 15% to 45% increased chlorophyll concentrations compared to those under full sunlight conditions. Moreover, 100% sunlight induced excess ROS generation by affecting PSII reaction center normalization, thereby changing the physiological and biochemical strategies of *P. ternata*, including increasing the POD and CAT activities and MDA and secondary metabolite contents. The maximum total alkaloid, guanosineand succinic acid yields were obtained under 45% sunlight. Therefore, our findings suggest that appropriate light intensity is helpful for *P. ternata* growth and secondary metabolite production. Furthermore, *P. ternata* could be used for cultivation in agroforestry systems to meet the growing market need.

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