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Chloroplast ultrastructure, photosynthesis and accumulation of secondary metabolites in *Glechoma longituba* in response to irradiance

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Abstract

Glechoma longituba (Nakai) Kupr. is a perennial shade plant with pharmaceutical importance. The aim of this study was to investigate the effects of light intensity on the growth, photosynthesis, and accumulation of secondary metabolites in *G. longituba* grown under six different light environments. The high light intensity decreased the leaf size, specific leaf area, and aboveground dry mass, the number of grana per chloroplast, the number of lamella per granum, the thickness of the grana, the apparent quantum efficiency, the chlorophyll (Chl) content, the concentrations of ursolic and oleanolic acid. The high light increased the stomatal density, the stoma size, the number of chloroplast per a cell, the chloroplast size, the dark respiration rate, the light saturation point, the light compensation point, and the Chl *a*/*b* ratio. With the reduction in the light intensity, the light-saturated net photosynthetic rate, the aerial dry mass per plant, and the yields of ursolic and oleanolic acid decreased after an initial increase, peaking at 16 and 33% of sunlight levels. Overall, the 16 and 33% irradiance levels were the most efficient in improving the yields and qualities of the medicinal plant. The lower light demand and growth characteristics suggest that *G. longituba* is an extremely shade-tolerant plant and that appropriate light intensity management might be feasible to obtain higher yields of secondary metabolites in agricultural management.

Additional key words: gas exchange; light adaptation; stomatal index; thylakoid; triterpene acids.

Introduction

Light is an important environmental factor, influencing many physiological processes, such as plant growth, metabolite production, and yields. In nature, light not only provides energy for plants, but affects detrimentally plants under excessive sunlight (Bilger *et al.* 1995, Wittmann *et al.* 2001). Excessive irradiance may inactivate or impair the photosynthetic reaction centres of the chloroplasts and cause photoinhibition (Gilmore 2004, Zhou *et al.* 2010). However, lack of sunlight may decrease the absorption of light energy and inhibit plant growth and yield by affecting net photosynthetic rate (P_N) (Wei *et al.* 2005, Gregoriou *et al.* 2007). Although excess or lack of sunlight may be harmful for the photosynthetic apparatus, plants have evolved some sophisticated mechanisms to avoid these effects through the adjustment of their morpho-anatomical, physiological, and biochemical status (Lichtenthaler and Burkart 1999, Kim *et al.* 2005, Guo *et al.* 2006). The morpho-anatomical strategies include greater leaf thickness and stomatal density, more starch grains, and a reduced leaf area, specific leaf area (SLA), number of grana and grana lamella in the chloroplast, *etc*. (Meier and Lichtenthaler 1981, Niinemets 2001, Temesgen and Weiskittel 2006, Gregoriou *et al.* 2007). In addition, the physiological and biochemical strategies reduce the Chl/protein ratio and apparent quantum yields, increasing the Chl *a*/*b* ratio in sun leaves (Sims and Pearcy 1989, Lichtenthaler *et al.* 2007, Dai *et al.* 2009). These strategies could greatly enhance the overall adaptation abilities of plants to particular environmental conditions in their ecological niche (Guo *et al.* 2006, Bussotti 2008).

Sunlight is an important environmental factor and

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Abbreviations: AQE – apparent quantum efficiency; Chl – chlorophyll; LCP – light compensation point; LSP – light saturation point; OA – oleanolic acid; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; R_D – dark respiration rate; SLA – specific leaf area; UA – ursolic acid.
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plays a key role in secondary metabolite accumulation and morphological structure changes in plants (Theis and Lerdau 2003). Secondary metabolites are usually considered to protect plants against herbivores and microbial infection (Mooney *et al.* 1983, Coley and Barone 1996, Theis and Lerdau 2003) and are used as therapeutic agents in traditional medicine. The carbon/nitrogen balance theory proposes that the photosynthetic rates might affect the biosynthesis of carbohydrates and C-based defense compounds (such as terpenoids or phenols, *etc*.) if light became a limiting factor in nutrient-rich environments (Bryant *et al.* 1983). In general, N-containing secondary metabolites in plants would increase with decreasing light intensity (Coelho *et al.* 2007). However, the extensive accumulation of N-based secondary metabolites in weak light was observed in shade-tolerant plants, such as *Tabernaemontana pachysiphon* (Höft *et al.* 1998), but not in high-light demanding species, such as *Rauvolfia vomitoria* (Cai *et al*. 2009) when photosynthesis decreased under low light intensity. At present, it is still unknown whether plant growth and photosynthesis would be affected by secondary metabolites (Herms and Mattson 1992, Almeida-Cortez *et al.* 1999). Therefore, understanding the relationship between growth, photosynthetic characteristics, and the production of secondary metabolites under different light environments is significant in managing medicinal plants and optimizing field growth conditions to acquire the maximal yield of phytomedicinal compounds. Much attention has been paid to increasing secondary metabolite production through some special techniques, such as plant tissue culture technologies, *etc*. However, the contents and accumulation rate of secondary metabolites are relatively low in general (Aoyagi *et al.* 2001). Therefore, unravelling the physiological mechanism of secondary metabolite biosynthesis in response to environmental stress is indispensable for their field production. In the recent decades, physiological changes have been intensively investigated in plants grown under different light intensities, while little research has been performed on medicinal plants (Guo *et al.* 2006, Dai *et al.* 2009).

G. longituba belongs to the Labiatae family, and wild plants usually grow in shady and humid environments, such as forests, roadsides, or nearby creeks (Wu *et al*. 1977). The dried aboveground parts have a long history as a widely used traditional Chinese medicine (TCM). This species is recorded as "Lianqiancao" in Chinese traditional medicinal recipes. *G. longituba* possesses a wide range of effective applications as an antipyretic, diuretic, and choleretic agent for jaundice disease, as well as for the treatment of febrile symptoms, cholagogue, diuretic, swelling, detoxification, traumatic injury, eliminating concretion, and anti-diarrhoea (Xiao *et al*. 2010, Ni *et al.* 2010). Moreover, two major bioactive components of *G. longituba*, ursolic acid (UA) and oleanolic acid (OA), have recently attracted considerable interest because of their various pharmacological activities, including antioxidant activities in leukemic cells (Ovesná *et al.* 2006), effective anti-AIDS (Kashiwada *et al.* 2000, Ma *et al.* 2000), anti-inflammation (Ryu *et al.* 2000, Giner-Larza *et al.* 2001), and anti-tumour (Ohigashi *et al.* 1986, Li *et al.* 2002, Ikeda *et al.* 2006) activities. In addition to its pharmaceutical uses, this plant has been also used as an ornamental plant for garden landscaping (Li *et al.* 1999, Liu *et al*. 2008).

Because wild resources of *G. longituba* cannot meet the increasing commercial demands in China, promoting its agricultural cultivation has been considered an alternative strategy to satisfy market demands. However, knowledge of the growth, photosynthesis, and ecological adaptability of *G. longituba* is missing. Because agroforestry has been successfully used as an integrated approach in the cultivation and management of some medicinal plants (Cai *et al.* 2009, Hou *et al.* 2010), it could be effective in ameliorating the quality and trait of interest for other crops, such as *G. longituba* (Eibl *et al.* 2000).

G. longituba cannot grow normally in either full sunlight or overshade conditions in practical production. Full sunlight irradiance leads always to leaf yellowing and early senescence. Under overshade conditions, *G. longituba* grows poorly and cannot bloom normally. At present, the effects of different irradiance intensities on the morphological, biochemical, and photosynthetic properties of *G. longituba* have not been investigated. In this paper, we studied the growth, chloroplast ultrastructure, stomatal indexes, gas exchange, and contents of secondary metabolites of *G. longituba* in response to different light environments using a range of light regimes, including high and low light intensities. The aim was to provide an in-depth understanding of the photoacclimation mechanisms of *G. longituba* under different light environments. These results might be used to determine whether appropriate light control can improve the yield and medicinal quality of *G. longituba* in agricultural management and conservation of cultivated *G. longituba*.

Materials and methods

Plants and experimental conditions: *G. longituba* was collected from the Chinese Traditional Medicine Germplasm Resource Centre, Nanjing Agricultural University, China. In May 2010, *G. longituba* clonal fragments with two oppositifolious leaves were planted in fertile sandy soil and were maintained for approximately one month in a growth chamber (25/18°C day/night temperature, maximum irradiance of 1,000–1,200 μmol(photon) m^{-2} s^{-1}, and relative humidity of approximately 70%). After their recovery from transplantation, uniform and healthy seedlings were selected and subjected to different irradiance treatments. Twenty seedlings were randomly sampled in

each treatment (n = 20).

The plants were sheltered with shade nets (1.6 m above the ground) and grew at different irradiance levels: 100% (the control without the shade net, L1), 75% (L2), 58% (L3), 33% (L4), 16% (L5), and 9% (L6) of full sunlight. The diurnal light intensity changes of all shade levels were determined in July and September using a light meter (*QRT1, Hansatech*, Norfolk, UK), and the mean values are shown in Fig. 1*A*. The diurnal changes in the leaf temperature were measured on fully expanded leaves with a portable *Li-6400XT* photosynthesis system (*LI-COR*, Lincoln, NE, USA), and the results are shown in Fig. 1*B*. The plants were irrigated daily to saturation at 18:00; they were irrigated twice a week with full strength Hoagland's solution during the growth period. The plant growth was evaluated by the indexes that are related to growth, ultrastructure, photosynthesis, and secondary metabolism after five months of growth under different irradiance.

Analysis of plant growth indexes and morphology: The morphology change and growth indexes [leaf size, SLA, leaf number, and aboveground dry mass (DM) per plant] were determined at the end of the experiment on the October 31, 2010. The fully expanded leaves were sampled from the medial part of the stem. The leaf size was measured with a *LI-3000* portable area meter (*LI-COR*, Lincoln, NE, USA). The leaf DM was determined after incubating the leaves at 110°C for 10 min and drying at 60°C until the constant mass. Finally, the leaf area was calculated and expressed as the ratio of leaf area to leaf DM. Each treatment contained five individual plants (n = 5).

Chloroplast ultrastructure: Small pieces (approx. 4 mm^2) of healthy tissue were excised from the middle part of the leaf, excluding the midrib to ensure uniformity of the sample material. The leaf samples were fixed in 2.5% (w/v) glutaraldehyde with 0.1 mol L^{-1} of phosphate buffer (pH 7.4) for 6 h at 4°C. After being washed, the samples were post-fixed with 1% osmium tetroxide for 4 h at 4°C, dehydrated in an ethanol series, and embedded in the *Epon-812* resin. Ultrathin sections of the embedded samples were cut on a *Reichert* Ultratome (*Leica*, *Reichert*, Germany) and then post-stained with uranyl acetate and lead citrate following the method of Reinolds (1963). The parameters of the transmission electron microscopy (*H-7650*, *Hitachi*, Tokyo, Japan) were set as 75 kV according to the procedure of Kutík (1998). The leaf cross sections in the leaf cells were analyzed using an *Axio Imager A1m* optical microscope (*Zeiss*, Jena, Germany). Ten replicates were used for each experiment (n = 10).

Determination of the stomatal indexes: The lower epidermal stomata were counted on fully expanded leaves. The dry imprints of the abaxial epidermis were obtained from the leaves by smearing with colourless

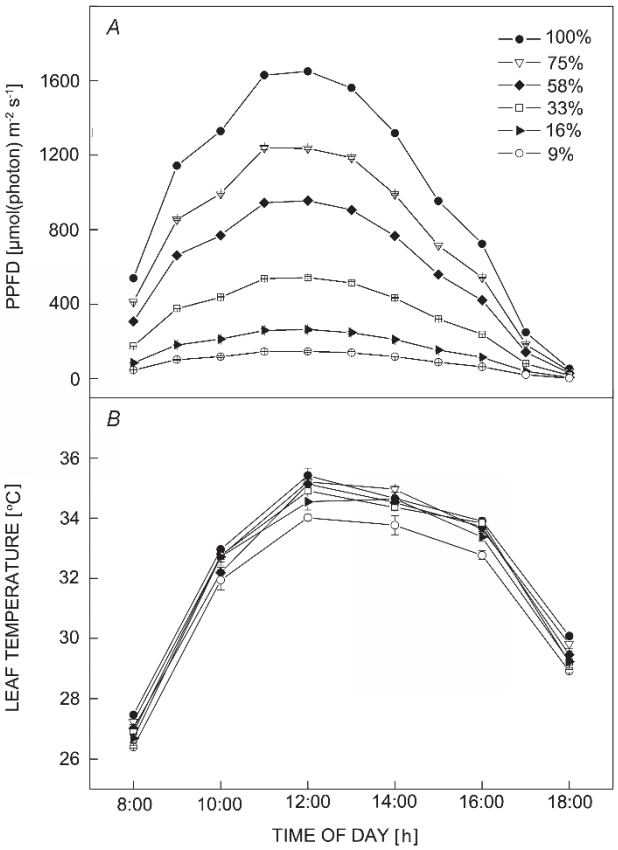

Fig. 1. Diurnal changes in the photosynthetic photon flux density (PPFD) (*A*) and leaf temperature (*B*) under 100, 75, 58, 33, 16, and 9% of sunlight irradiance. Each value of PPFD is the mean of twenty replicates and that of leaf temperature is the mean of ten replicates.

adhesive nail polish according to Chen *et al*. (2001), photographed with an *Axio Imager A1m* optical microscope (*Zeiss*, Jena, Germany), and measured with *Motic Images Plus* software. The imprints of five individual leaves were used to count the lower epidermal stomata in each treatment (n = 5). Four areas of 0.1555 mm^2 were selected and analyzed on each leaf sample. The stomatal density was measured according to the method of Ceulemans *et al*. (1995).

The Chl concentrations were determined according to Lichtenthaler (1987). A sample of fresh leaf was extracted in 80% (v/v) acetone in the dark for 48 h at room temperature (approximately 25°C). The absorbance of the extract was measured at 645 and 663 nm using a UV/visible spectrophotometer (*Lambda 25*, *Perkin Elme*r, CT, USA).

Gas-exchange measurements: The gas exchange was measured with a portable *Li-6400XT* photosynthesis system (*LI-COR*, Lincoln, NE, USA) using an open system mode in the morning between 09:00 and 11:00 on clear days in October 2010. The relative humidity, leaf

temperature, and vapour pressure deficit (VPD) were 70 ± 0.5%, 25 ± 0.3°C, and less than 1 kPa, respectively. The seedlings were watered to avoid drought stress before all of the leaves were marked and measured. Three replicates from different plants were used for photosynthetic measurements in each treatment (n = 3). The leaf light-response curves (P_N/PPFD) were determined at irradiances between 1,500 and 0 μmol(photon) m^{-2} s^{-1} by a LED-B built-in light source and were fitted according to the model of Ye (2007). Based on the given environmental conditions (CO_2 concentration, humidity, temperature, and oxygen concentration), the leaf light-response curves were expressed as follows (Ye 2007, 2008):

$P_N(I) = \alpha \times (1 - \beta I) \times (I - I_C)/(1 + \gamma I)$, where I_C is a light compensation point (LCP), which can be directly obtained from this equation, and α, β, and γ are coefficients which are independent of the light intensity (I).

The light saturation point (LSP) was calculated as: $I_M = [\sqrt{(\beta+\gamma)\times(1+\gamma I_C)/\beta} - 1]/\gamma$; the light-saturated photosynthetic rate (P_{Nmax}) was calculated as: $P_N(I_M) = \alpha \times (1 - \beta I_M) \times (I_M - I_C)/(1 + \gamma I_M)$; the dark respiration rate (R_D) was calculated as: $R_D = -\alpha I_C$; and the apparent quantum efficiency (AQE) was calculated as: $P_N(I_C) = \alpha \times [1 + (\gamma - \beta) \times I_C - \beta \gamma I_C^2]/(1 + \gamma I_C)^2$. The measurements were carried out under a reference CO_2 concentration of 380 μmol(CO_2) mol^{-1}. At each PAR level, the minimum waiting time for reading stabilization was set at 120 s and the maximum at 150 s during the measurements.

Determination of UA and OA concentrations: The samples for HPLC quantification were prepared by the method of Fang *et al*. (2010). Briefly, the plant tissues were ground in a commercial blender, and the powder (0.100 g) was ultrasonically extracted for 30 min after a 30-min immersion in 20 mL of 75% ethanol solution containing 1% formic acid. After centrifugation at 10,000 × g for 10 min, the supernatant was filtered through a 0.45-μm water solute nylon membrane (*Waters*, Massachusetts, USA). HPLC analyses for the UA and OA were conducted at 30°C using an HPLC system consisting of an *LC-20AT* Liquid Chromatograph (*Shimadzu*, Kyoto, Japan). The samples (10 μL) were separated by an *Agilent ZORBAX SB-Aq* C18 (250 × 4.6 mm, particle size 5 μm) reverse-phase column at a 0.6 mL min^{-1} flow rate with methanol-0.5% ammonium acetate (88:12, v/v) as the mobile phase. Two bioactive component concentrations were determined by a UV detector at wavelength of 210 nm. The UA and OA concentrations in the aboveground parts were calculated by their regression equations with reference to the external standard (Wang *et al.* 2008).

Statistical analyses: The statistical analyses were performed by a one-way analysis of variance (*ANOVA*), and the least significant difference (LSD) test was used to assess the differences among the treatments. To meet the assumptions of normality and homoscedasticity, the data were transformed by common logarithm or square root. The confidence level was set at 95% ($P \leq 0.05$), and the data were displayed as the means ± standard errors (SE). *Pearson*'s correlation analyses and statistical analyses were performed using *SPSS 16.0* software (*SPSS*, Chicago, IL, USA).

Results

Significant changes were observed in the leaf number and leaf size of the plants grown under shade conditions compared with those of the L1 treatment (Table 1). With the decreasing light intensity, the leaf number in the L2, L3, L4, L5, and L6 treatments increased by 1.31-, 1.34-, 1.51-, 1.68-, and 1.04-fold, respectively, compared with L1. The changes in the leaf size (leaf length, leaf width, and leaf area) displayed similar patterns as those in the leaf number. The aboveground DM increased continuously with the decreasing sunlight before reaching the maximum mass in the L4 treatment, after which the masses decreased. These data indicated that shade showed a positive effect on the accumulation of the aboveground DM (Fig. 2).

Stomatal density is closely positively correlated with plant photochemical efficiency. Under different shade treatments, the stomatal density of *G. longituba* leaves declined significantly with the decreasing irradiance (Table 1). Compared to the L1 treatment, the stomatal density was significantly reduced by 16.7, 30.3, 37.2, 41.4, and 49.0% in the L2, L3, L4, L5, and L6 treatments, respectively. The shade treatments reduced significantly

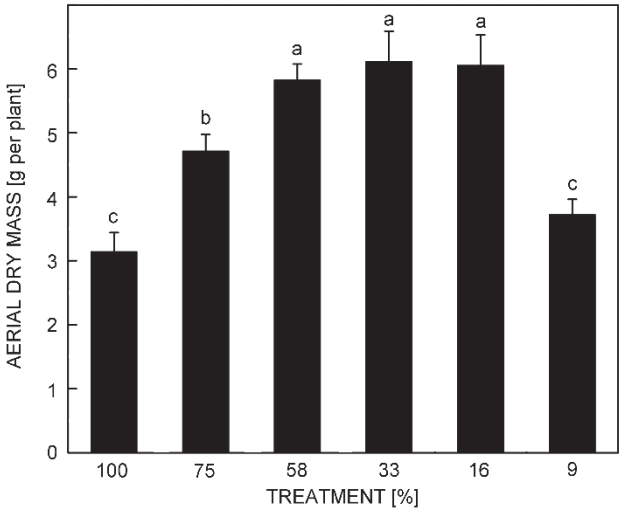

Fig. 2. Effects of different sunlight levels on the aerial dry mass of *Glechoma longituba* (n = 5). The different *small letters* indicate significance at $P<0.05$. The bars above the means indicate standard error (SE). The x-axis indicates the sunlight irradiance in percentage.

Table 1. The growth indexes, stomatal density, and size of *Glechoma longituba* leaves under different sunlight irradiance. Mean ± SE, n = 15. Different *small letters* indicate significant differences for the same index at P<0.05 by the least significant difference (LSD) test. L1, L2, L3, L4, L5, and L6 mean 100, 75, 58, 33, 16, and 9% of sunlight irradiance, respectively.

Parameters	L1	L2	L3	L4	L5	L6
Leaf length [cm]	2.51 ± 0.03^{d}	2.53 ± 0.06^{d}	3.22 ± 0.12^{c}	3.66±0.18^{b}	4.07±0.20^{a}	3.33±0.14^{bc}
Leaf width [cm]	2.88 ± 0.06^{c}	3.11 ± 0.14^{c}	3.58 ± 0.16^{b}	4.33±0.20^{a}	4.55±0.19^{a}	3.84±0.16^{b}
Leaf area [cm^2]	4.86 ± 0.17^{e}	5.68 ± 0.44^{de}	7.82 ± 0.66^{cd}	11.31±1.19^{ab}	13.48±1.36^{a}	8.87±0.94^{bc}
Specific leaf area [cm^2 mg^{-1}]	0.27 ± 0.01^{c}	0.28 ± 0.02^{c}	0.29 ± 0.00^{c}	0.35 ± 0.03^{b}	0.37 ± 0.03^{b}	0.69 ± 0.05^{a}
Leaf number per plant	67.00 ± 1.85^{d}	87.80 ± 2.64^{c}	90.11 ± 1.62^{c}	101.11 ± 3.72^{b}	112.83 ± 4.81^{a}	69.90 ± 1.42^{d}
Stomatal density [number mm^{-2}]	333.06 ± 5.91^{a}	277.44 ± 5.81^{b}	232.11 ± 4.35^{c}	209.29 ± 4.00^{d}	195.14 ± 3.91^{e}	169.74 ± 2.93^{f}
Stomatal length [μm]	27.270 ± 0.272^{a}	25.599 ± 0.269^{b}	25.442 ± 0.326^{b}	24.537 ± 0.288^{c}	23.436 ± 0.352^{d}	17.992 ± 0.261^{e}
Stomatal width [μm]	13.518 ± 0.250^{a}	11.899 ± 0.237^{b}	12.134 ± 0.217^{b}	11.632 ± 0.165^{b}	10.927 ± 0.302^{c}	9.286 ± 0.157^{d}
Stomatal length-to-width ratio	2.042 ± 0.020^{c}	2.169 ± 0.018^{a}	2.109 ± 0.011^{b}	2.114 ± 0.006^{b}	2.180 ± 0.031^{a}	1.944 ± 0.010^{d}

the stomatal density, length, and width.

Moreover, the different irradiance treatments also affected significantly the stomatal length-to-width ratio (Table 1), which was 2.042, 2.169, 2.109, 2.114, 2.180, and 1.944 in L1, L2, L3, L4, L5, and L6, respectively.

The leaves of the control, L1 treatment possessed the representative sun-type chloroplasts, which were characterized by few lamellae in the stroma part of the chloroplast section, by less appressed thylakoid membranes, less lamellae per granum, and large starch grains (Fig. 3). With the increasing light intensity, an increasing trend was observed for the number of chloroplasts per cell (Table 2). Compared with the control, the shade-treated leaves displayed the smaller chloroplast size (length and width) and lower portion of starch per chloroplast (Fig. 3, Table 2). Furthermore, the shaded leaves contained smaller starch grains, but more grana per chloroplast and lamellae per granum than that of the control chloroplasts (Table 2, Fig. 3). As a result, the thickness of the grana was obviously greater in the shade-type chloroplasts than that in the control chloroplasts (Table 2).

The P_N/PPFD curves of the six irradiance treatments are shown in Fig. 4. In all the treatments, the P_N values increased sharply with the PPFD from 0 to 200 μmol(photon) m^{-2} s^{-1}, and then increased slowly with the PPFD until the maximum value. However, the values declined significantly as the light intensity reached 1,500 μmol(photon) m^{-2} s^{-1}. The maximum P_{Nmax} was observed in the L4 and L5 treatments (Fig. 4, Table 3); it was 1.47- and 1.42-fold higher than in the control, respectively. The P_N/PPFD curves of the L4 and L5 treatments were almost coincident, and both decreased after peaking. All of the AQE were significantly higher in L3–L6 treatments than those in the L1 and L2 treatments (Table 3). Therefore, a decrease in the sunlight irradiance led to a lower R_D, LSP, and LCP in *G. longituba* (Table 3).

The data in Table 3 indicate that *G. longituba* responded to the decrease in irradiance by enhancing the concentrations of Chl; the Chl *a*, Chl *b*, and total Chl concentrations were significantly higher under low sunlight levels than those under full sunlight. The plant leaves that were grown under shade treatments showed a lower Chl *a*/*b* ratio compared to the leaves in full sunlight. Moreover, the ratio gradually decreased as the irradiance decreased.

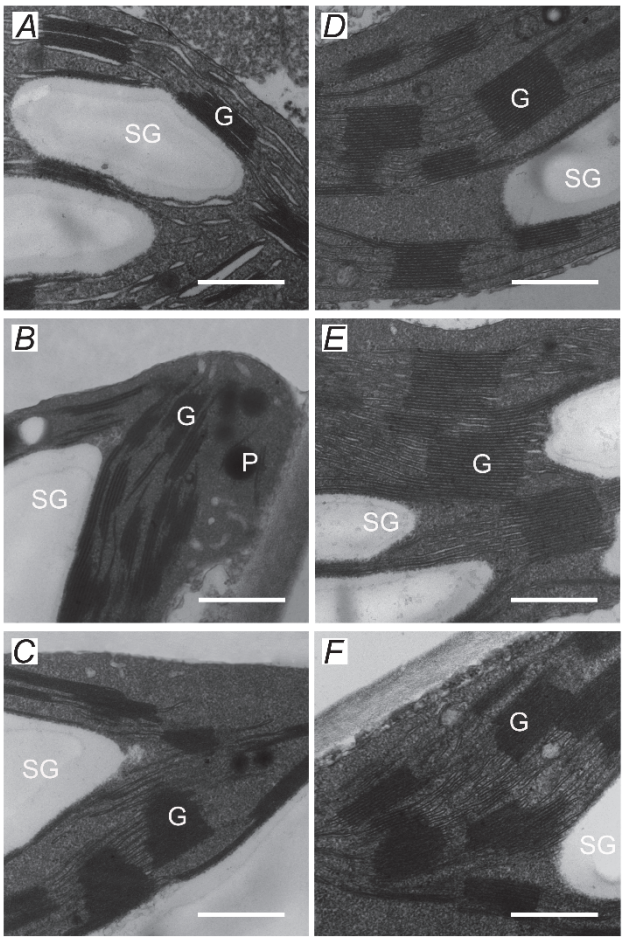

Fig. 3. Transmission electron micrograph images of the chloroplasts of *Glechoma longituba* leaves under different sunlight irradiance. Leaf chloroplasts of plants grown at 100% (*A*), 75% (*B*), 58% (*C*), 33% (*D*), 16% (*E*), and 9% (*F*) of full sunlight. Bar 0.5 μm. G – granum; P – plastoglobuli; SG – starch grain.

In response to the decrease in the irradiance, the

Table 2. Effects of different sunlight irradiance on the chloroplast ultrastructure in *Glechoma longituba* leaves. Mean ± SE. The different *small letters* indicate significant differences for the same index at $P<0.05$ by the least significant difference (LSD) test. The chloroplast ultrastructure data were calculated from 10 replicate sections from five plants. L1, L2, L3, L4, L5, and L6 indicate 100, 75, 58, 33, 16, and 9% sunlight irradiance, respectively.

Treatment	Number of chloroplasts per cell	Chloroplast length [μm]	Chloroplast width [μm]	Portion of starch per area of chloroplast section [%]	Number of grana per chloroplast	Number of lamellae per granum	Thickness of grana [μm]
L1	5.60 ± 0.24^{a}	7.79 ± 0.32^{a}	3.54 ± 0.16^{a}	58.75 ± 2.71^{a}	18.40 ± 0.84^{d}	4.77 ± 0.35^{b}	0.082 ± 0.006^{c}
L2	5.40 ± 0.24^{a}	7.15 ± 0.37^{ab}	3.42 ± 0.15^{ab}	47.61 ± 6.64^{ab}	19.80 ± 1.45^{cd}	7.14 ± 0.87^{b}	0.125 ± 0.009^{c}
L3	5.00 ± 0.22^{ab}	6.93 ± 0.51^{ab}	3.17 ± 0.22^{ab}	38.11 ± 7.35^{bc}	23.00 ± 0.58^{bc}	14.30 ± 0.95^{a}	0.213 ± 0.027^{b}
L4	4.50 ± 0.29^{bc}	6.96 ± 0.23^{ab}	2.93 ± 0.13^{bc}	25.62 ± 6.79^{c}	25.67 ± 0.85^{ab}	14.83 ± 1.30^{a}	0.325 ± 0.031^{a}
L5	4.25 ± 0.25^{bc}	6.70 ± 0.31^{b}	2.61 ± 0.18^{cd}	25.61 ± 1.72^{c}	27.25 ± 1.62^{a}	15.44 ± 1.71^{a}	0.376 ± 0.041^{a}
L6	4.00 ± 0.32^{c}	5.51 ± 0.17^{c}	2.20 ± 0.06^{d}	24.93 ± 2.59^{c}	26.50 ± 0.99^{a}	16.63 ± 1.55^{a}	0.381 ± 0.029^{a}

concentrations of UA and OA increased dramatically in the aboveground parts of *G. longituba* (Fig. 5*A*). Compared to those in the control (L1), the concentrations of UA increased by 10.5, 10.3, and 18.0% in the L4, L5, and L6 treatments, respectively, and no significant differences were detected among the L1, L2, and L3 treatments. The change in the OA concentration showed a similar pattern; the contents increased by 9.1, 9.6, and 14.4% in the L4, L5, and L6 treatments compared to those of the control, respectively. No significant differences were detected among the L1, L2, and L3 treatments.

With the sunlight levels decreased, the aboveground DM first increased to 1.94-fold in the L4 treatment compared with L1, and then decreased (Fig. 5*B*). The aboveground DM showed a significantly positive correlation with P_{Nmax} ($r^2 = 0.81$, $P<0.05$). The yields of UA and OA displayed similar trends; their contents increased by 1.53- and 1.50-fold in the L2 treatment, 1.85- and 1.81-fold in the L3 treatment, 2.15- and 2.11-fold in the L4 treatment compared to the L1, respectively, and then decreased drastically (Fig. 5*B*).

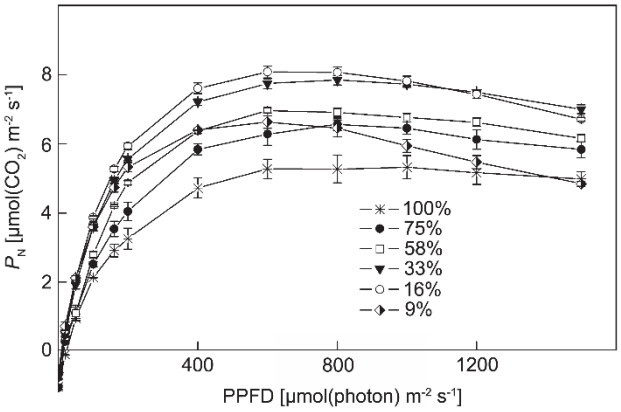

Fig. 4. Photosynthetic light-response (P_N/PPFD) curves from the leaves of *Glechoma longituba* grown under 100, 75, 58, 33, 16, and 9% of sunlight irradiance. The values represent the means ± SE ($n = 3$).

Discussion

The leaf area, leaf size, and SLA of plants are closely related to the light conditions under which plants grow (Rosati *et al.* 2001). An increase in the leaf area and SLA is a typical morphological characteristic to adapt to weak light environments (Niinemets *et al*. 1998). In this study, the light was limited; therefore, *G. longituba* adopted strategies by allocating additional biomass to leaves and increasing SLA to acclimatize to weak light environments. These adaption strategies could enhance its capacity of capturing light energy and increase the relative proportion of assimilation tissue in the leaf tissue, improving the net accumulation of carbon (Wang and Feng 2005). The results agree with those of a previous study in which the leaves of *Rauwolfia vomitoria* Afzel exposed to low sunlight treatments showed higher SLA compared to those exposed to full sunlight (Cai *et al.* 2009). The aerial dry mass of *G. longituba* was mostly composed of leaf dry matter production (Tao and Zhong 2000). The plants grew poorly with fewer and smaller leaves at the full sunlight. As a result, the aerial dry mass was lower than that under the shade treatments.

Chl *a* is directly involved in determining the photosynthetic activity (Šesták 1966). The decreases in the Chl *b* concentrations have been considered an indication of Chl disorganisation by excess sunlight irradiance (Griffin *et al.* 2004). Compared to the control, the significant increases in the total Chl concentrations in shading treatments confirmed the ability of plants to maximize the light-harvesting capacity in low-light environments (Lei *et al.* 1996). In our experiments, the shade treatments greatly increased the photosynthetic rates in *G. longituba*. The higher Chl *a* concentrations that were observed under shade conditions might in part explain the higher rates of photosynthesis. However, the differences in the

Table 3. Photosynthetic parameters and leaf chlorophyll (Chl) concentrations in *Glechoma longituba* seedlings growing under different sunlight irradiance. Mean ± SE, $n = 3$. Different *small letters* indicate significant differences for the same index at $P<0.05$ by the least significant difference (LSD) test. L1, L2, L3, L4, L5 and L6 indicate 100, 75, 58, 33, 16, and 9% of sunlight irradiance, respectively. AQE – apparent quantum efficiency; LCP – light compensation point; LSP – light saturation point; P_{Nmax} – light-saturated net photosynthetic rate; R_D – respiration rate.

Parameters	L1	L2	L3	L4	L5	L6
P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	5.58 ± 0.26^{d}	6.63 ± 0.16^{bc}	7.05 ± 0.06^{b}	7.95 ± 0.10^{a}	8.23 ± 0.12^{a}	6.42 ± 0.08^{c}
AQE	0.0454 ± 0.0011^{c}	0.0513 ± 0.0025^{bc}	0.0552 ± 0.0013^{b}	0.0637 ± 0.0045^{a}	0.0702 ± 0.0001^{a}	0.0718 ± 0.0024^{a}
R_D [μmol m^{-2} s^{-1}]	0.942 ± 0.015^{a}	0.861 ± 0.001^{b}	0.788 ± 0.024^{c}	0.703 ± 0.038^{d}	0.656 ± 0.003^{de}	0.615 ± 0.009^{e}
LSP [μmol m^{-2} s^{-1}]	874.63 ± 25.72^{a}	836.48 ± 6.06^{ab}	812.89 ± 3.98^{b}	761.05 ± 12.33^{c}	685.32 ± 2.99^{d}	579.31 ± 8.10^{e}
LCP [μmol m^{-2} s^{-1}]	23.10 ± 0.02^{a}	18.26 ± 0.89^{b}	15.33 ± 0.13^{c}	11.70 ± 0.13^{d}	9.82 ± 0.04^{e}	9.13 ± 0.16^{e}
Chl *a* [mg g^{-1}]	0.562 ± 0.045^{d}	0.663 ± 0.017^{c}	0.674 ± 0.013^{c}	0.851 ± 0.036^{b}	1.032 ± 0.015^{a}	0.978 ± 0.010^{a}
Chl *b* [mg g^{-1}]	0.160 ± 0.006^{d}	0.192 ± 0.004^{c}	0.204 ± 0.002^{c}	0.260 ± 0.009^{b}	0.344 ± 0.006^{a}	0.328 ± 0.006^{a}
Chl *a*/*b* ratio	3.505 ± 0.042^{a}	3.460 ± 0.064^{a}	3.303 ± 0.077^{b}	3.267 ± 0.048^{b}	2.995 ± 0.009^{c}	2.977 ± 0.024^{c}
Total Chl [mg g^{-1}]	0.722 ± 0.031^{d}	0.855 ± 0.020^{c}	0.878 ± 0.012^{c}	1.111 ± 0.044^{b}	1.376 ± 0.021^{a}	1.306 ± 0.016^{a}

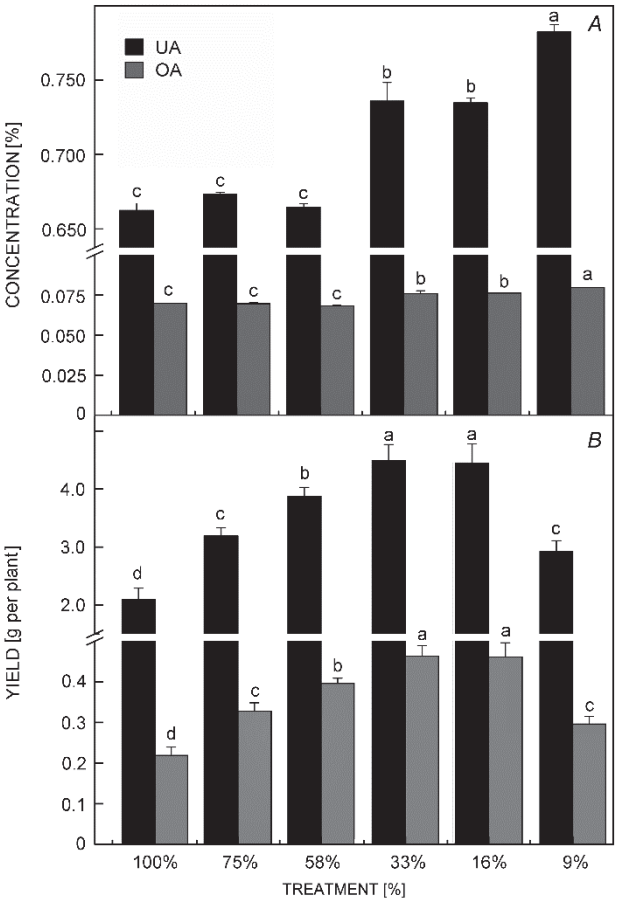

Fig. 5. Ursolic acid (UA) and oleanolic acid (OA) concentrations (*A*) and yields (*B*) in the aboveground parts of *Glechoma longituba* under different sunlight irradiance ($n = 4$). The different *small letters* indicate significance at $P<0.05$. The bars above the means indicate standard error (SE). The x-axis indicates the sunlight irradiance in percentage.

photosynthetic rates between full sunlight and under shading nets may be attributable to evapotranspiration and transpiration (Li *et al.* 2009). Plants grown under weaker light have much smaller and fewer stomata than those under stronger light (Wilson and Cooper 1969, Cai *et al.* 2004). This phenomenon is consistent with our study; the leaves showed reduced stomatal density and size when they were treated with lower irradiance. Concomitantly, a large stomatal density and size would have high transpiration, which is a typical characteristic when the leaves are exposed to full sunlight. The strong irradiance and evapotranspiration in turn make plants sensitive to drought under natural conditions. It is generally accepted that the performance of plants grown in full sunlight may be ameliorated by moderate shade under drought conditions (Rousset and Lepart 2000). Thus, shade is a suitable treatment to improve shade-tolerant plant yields.

Different sunlight intensities affected significantly *G. longituba* photosynthesis. More specifically, high irradiances determine a decline of P_{Nmax}, likely ascribed to the occurrence of photoinhibition (Griffin *et al*. 2004 Galmés *et al*. 2007). The plants were not able to acclimate and elevate P_{Nmax}, although they had higher LSP under high irradiance levels. The results suggest that plants might experience supraoptimal irradiances, and the energy dissipation mechanism could be somewhat damaged. The higher AQE indicated that *G. longituba* leaves utilized light energy better in the shade environments (Xu 2002, Park *et al.* 2010). A lower LCP in the shade environments suggests that *G. longituba* possesses the capacity to adapt to weak light environments and that it is a shade-tolerant species; this is related to the maximum value of P_{Nmax} under the shade environments.

Typically, chloroplasts show high plasticity in response to light and adjust their structure to help the leaf adapt to different light environments (Zhang and Gao 2001). The integrated chloroplast structure under shade treatments, especially at 16 and 33% sunlight, ensured the process of photosynthesis and facilitates the synthesis and accumulation of carbohydrates, including starch (Fig. 3). The chloroplast number per cell decreased with the decline of light intensity, which may be equal to that in

meristem cells (Butterfass 1995). The chloroplast number per cell is closely correlated with the size of the meristem cell and determined by the size of the cell (Pyke and Leech 1987). In our study, it was observed that the size of the cell became gradually smaller with the decline of light intensity (unpublished).

UA and OA are secondary metabolites with various pharmacological activities. In particular, OA has been marketed as an oral drug for human liver disorders in China (Liu 1995, Sohn *et al.* 1995). These metabolites are major active components in *G. longituba*, and their contents are used as bench markers to judge the quality of this medicinal plant (Liu *et al.* 2012). Our results demonstrated that the concentrations as well as the yields of these substances increased with the decreasing irradiance (except for the L6 treatment) (Fig. 5). The low light boosted the syntheses and accumulation of UA and OA, which was consistent with the previous studies, where reduced light availability increased the concentrations of specific secondary metabolic substances in some medicinal plants, such as glycyrrhizic acid and liquiritin in *Glycyrrhiza uralensis* (Hou *et al.* 2010), methylxanthines in *Ilex paraguariensis* (Coelho *et al*. 2007), and aloin (barbaloin) in *Aloe mutabilis* (Chauser-Volfson and Gutterman 1998). However, the yields of UA and OA were positively correlated with the changes in the aboveground dry mass, and they were correlated with the maximum photosynthetic rates across all sunlight levels. These results indicated that the appropriate shade treatment did not inhibit photosynthesis, on the contrary, increased the yields of the aboveground dry mass and improved the accumulation of UA and OA. Our findings were consistent with previous reports on suitable shade treatments in promoting the growth, photosynthesis, and secondary metabolites accumulation (Dai *et al.* 2009, Li *et al.* 2009). We suggest that beneficial light control could increase the yields of UA and OA in *G. longituba* possessing a shade-tolerant capacity to some extent and a strong ability to acclimate to low light intensity in the long term.

Conclusion: Significant changes in the growth, ultrastructure, photosynthesis, and dry mass allocation were found in seedlings of *G. longituba* under experimental sunlight levels. They indicated this species had high plasticity in morphological and physiological adaptation to light. Both the dry matter yield and the concentrations of UA and OA could be dramatically affected by environmental conditions. The results also indicated that *G. longituba* is an extreme shade-tolerant species that can produce high concentrations of UA and OA under lower light. This demonstrated that the appropriate lower irradiance is helpful for its growth and secondary metabolite production. Therefore, an agroforestry system that provides controlled light would be a practical alternative to the cultivation and management of *G. longituba* to satisfy the need of the growing market.

References

Almeida-Cortez J.S., Shipley B., Arnason J.T.: Do plant species with high relative growth rates have poorer chemical defenses? – Funct. Ecol. **16**: 819-827, 1999.

Aoyagi H., Kobayashi Y., Yamada K. *et al*.: Efficient production of saikosaponins in *Bupleurum falcatum* root fragments combined with signal transducers. – Appl. Microbiol. Biotechnol. **57**: 482-488, 2001.

Bilger W., Schreiber U., Bock M.: Determination of the quantum efficiency of photosystem II and of non-photochemical quanching of chlorophyll fluorescence in the field. – Oecologia **102**: 425-432, 1995.

Bryant J.P., Chapin F.S., Klein D.R.: Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. – Oikos **40**: 357-368, 1983.

Bussotti F.: Functional leaf traits, plant communities and acclimation processes in relation to oxidative stress in trees: a critical overview. – Global Change Biol. **14**: 2727-2739, 2008.

Butterfass T.H.: Reproduction and continuity of chloroplasts in spermatophytes. – Bot. Rev. **61**: 1-27, 1995.

Cai Z.Q., Qi X., Cao K.F.: [Response of stomatal characteristics and its plasticity to different light intensities in leaves of seven tropical woody seedlings.] – Chin. J. Appl. Ecol. **15**: 201-204, 2004. [In Chinese]

Cai Z.Q., Wang W.H., Yang J., Cai C.T.: Growth, photosynthesis and root reserpine concentrations of two *Rauvolfia* species in response to a light gradient. – Ind. Crop Prod. **30**: 220-226, 2009.

Ceulemans R., Praet L.V., Jiang X.N.: Effects of CO_2 enrichment leaf position and clone on stomatal index and epidermal cell density in poplar (*Populus*). – New Phytol. **131**: 99-107, 1995.

Chauser-Volfson E., Gutterman Y.: Content and distribution of anthrone C-glycosides in the South African arid plant species *Aloe mutabilis* growing in the direct sunlight and the shade in the Negev Desert of Israel. – J. Arid Environ. **40**: 441-451, 1998.

Chen L.Q., Li C.S., Chaloner W.G. *et al*.: Assessing the potential for the stomatal characters of extant and fossil *Ginkgo* leaves to signal atmospheric CO_2 change. – Am. J. Bot. **88**: 1309-1315, 2001.

Coelho G.C., Rachwal M.F.G., Dedecek N.A. *et al*.: Effect of light intensity on methylxanthine contents of *Ilex paraguariensis* A. St. Hil. – Biochem. Syst. Ecol. **35**: 75-80, 2007.

Coley P.D., Barone J.A.: Herbivory and plant defense in tropical forests. – Annu. Rev. Ecol. Syst. **27**: 305-335, 1996.

Dai Y.J., Shen Z.G., Liu Y. *et al*.: 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, 2009.

Eibl B., Fernandez R.A., Kozarik J.C. *et al*.: Agroforestry systems with *Ilex paraguariensis* (American holly or yerba mate) and native timber trees on small farms in Misiones. – Agroforest. Syst. **48**: 1-8, 2000.

Fang L., Lin N.M., Wu Y.J.: [Simultaneous determination of four active components in *Spica Prunellae* by HPLC.] – China. J. Chin. Materia. Med. **35**: 616-619, 2010. [In Chinese]

Galmés J., Medrano H., Flexas J.: Photosynthesis and photoinhibition in response to drought in a pubescent (var. *minor*) and a glabrous (var. *palaui*) variety of *Digitalis minor*. – Environ. Exp. Bot. **60**: 105-111, 2007.

Gilmore A.D.: Excess light stress: probing excitation dissipation mechanisms through global analysis of time- and wavelength-resolved cholorophyll *a* fluorescence. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 555-581. Springer, Dordrecht 2004.

Giner-Larza E.M., Máñez S., Recio M.C. *et al.*: Oleanonic acid, a 3-oxotriterpene from Pistacia, inhibits leukotriene synthesis and has anti-inflammatory activity. – Eur. J. Pharmacol. **428**: 137-143, 2001.

Gregoriou K., Pontikis K., Vemmos S.: Effects of reduced irradiance on leaf morphology, photosynthetic capacity, and fruit yield in olive (*Olea europaea* L.). – Photosynthetica **45**: 172-181, 2007.

Griffin J.J., Ranney T.G., Pharr D.M.: Photosynthesis, chlorophyll fluorescence, and carbohydrate content of *Illicium taxa* grown under varied irradiance. – J. Am. Soc. Hortic. Sci. **129**: 46-53, 2004.

Guo Y.P., Guo D.P., Zhou H.F. *et al.*: Photoinhibition and xanthophyll cycle activity in bayberry (*Myrica rubra*) leaves induced by high irradiance. – Photosynthetica **44**: 439-446, 2006.

Herms D.A., Mattson W.J.: The dilemma of plants: to grow or defend. – Q. Rev. Biol. **67**: 283-335, 1992.

Höft M., Verpoorte R., Beck E.: Growth and alkaloid patterns of roots of *Tabernaemontana pachysiphon* and *Rauvolfia mombasiana* as influenced by environmental factors. – Bot. Acta **111**: 222-230, 1998.

Hou J.L., Li W.D., Zheng Q.Y. *et al.*: Effect of low light intensity on growth and accumulation of secondary metabolites in roots of *Glycyrrhiza uralensis* Fisch. – Biochem. Syst. Ecol. **38**: 160-168, 2010.

Ikeda Y., Murakami A., Nishizawa T., Ohigashi H.: Ursolic acid enhances cyclooxygenases and tumor necrosis factor-alpha expression in mouse skin. – Biosci. Biotech. Bioch. **70**: 1033-1037, 2006.

Kashiwada Y., Nagao T., Hashimoto A. *et al.*: Anti-AIDS agents 38. Anti-HIV activity of 3-O-acyl ursolic acid derivatives. – J. Nat. Prod. **63**: 1619-1622, 2000.

Kim G.T., Yano S., Kozuka T., Tsukaya H.: Photomorphogenesis if leaves: shade-avoidance and differentiation of sun and shade leaves. – Photoch. Photobio. Sci. **4**: 770-774, 2005.

Kutík J.: The development of chloroplast structure during leaf ontogeny. – Photosynthetica **35**: 481-505, 1998.

Lei T.T., Tabuchi R., Kitao M., Koike T.: The functional relationship between chlorophyll content, leaf reflectance, and light capturing efficiency of Japanese forest species under natural shade and open light regimes. – Physiol. Plantarum **96**: 411-418, 1996.

Li J., Guo W.J., Yang Q.Y.: Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. – World J. Gastroentero. **8**: 493-495, 2002.

Li X.W., Dai Y., Chen S.L.: Growth and physiological characteristics of *Fritillaria cirrhosa* in response to high irradiance and shade in age-related growth phases. – Environ. Exp. Bot. **67**: 77-83, 2009.

Li Y.L., Liu Y., Huang L., Liang S.L.: [An Illustrated Book of Chinese Common Flowers.] Pp. 139. – Henan Science and Technology Press, Zhengzhou 1999. [In Chinese]

Lichtenthaler H.K., Burkart S.: Photosynthesis and high light stress. – Bulg. J. Plant Physiol. **25**: 3-16, 1999.

Lichtenthaler H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. – Methods Enzymol. **148**: 350-382, 1987.

Lichtenthaler H.K., Ac A., Marek M.V. *et al.*: Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. – Plant Physiol. Biochem. **45**: 577-588, 2007.

Liu J.: Pharmacology of oleanolic acid and ursolic acid. – J. Ethnopharmacol. **49**: 57-68, 1995.

Liu L., Zhu Z.B., Guo Q.S. *et al.*: Variation in contents of major bioactive compounds in *Glechoma longituba* related to harvesting time and geographic distribution. – J. Med. Plants Res. **6**: 122-128, 2012.

Liu Y.J., Wang H.L., Qu S.H.: [Application of Garden Ornamental Flowers.] Pp. 688. – Liaoning Science and Technology Press, Shenyang 2008. [In Chinese]

Ma C., Nakamura N., Hattori M. *et al.*: Inhibitory effects on HIV-1 protease of constituents from the wood of *Xanthoceras sorbifolia*. – J. Nat. Prod. **63**: 238-242, 2000.

Meier D., Lichtenthaler H.K.: Ultrastructural development of chloroplasts in radish seedlings grown at high- and low-light conditions and in the presence of the herbicide bentazon. – Protoplasma **107**: 195-207, 1981.

Mooney H.A., Gulmon S.L., Johnson N.D.: Physiological constraints on plant chemical defenses. – In: Hedin A. (ed.): Plant Resistance to Insects. Pp. 21-36. American Chemical Society, Washington 1983.

Ni S.M., Qian D.W., Duan J.A. *et al.*: UPLC-QTOF/MS-based screening and identification of the constituents and their metabolites in rat plasma and urine after oral administration of *Glechoma longituba* extract. – J. Chromatogr. B **878**: 2741-2750, 2010.

Niinemets Ü.: Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. – Ecology **82**: 453-469, 2001.

Niinemets Ü., Kull O., Tenhunen J.D.: An analysis of light effects on foliar morphology, physiology and light interception in temperate deciduous woody species of contrasting shade tolerance. – Tree Physiol. **18**: 681-696, 1998.

Ohigashi H., Takamura H., Koshimizu K. *et al.*: Search for possible antitumor promoters by inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus activation; ursolic acid and oleanolic acid from an antiinflammatory Chinese medicinal plant, *Glechoma hederacea* L. – Cancer Lett. **30**: 143-151, 1986.

Ovesná Z., Kozics K., Slameňová D.: Protective effects of ursolic acid and oleanolic acid in leukemic cells. – Mutat. Res-Fund. Mol. M. **600**: 131-137, 2006.

Park S.A., Kim M.G., Yoo M.H. *et al.*: Plant physiological responses in relation to temperature, light intensity, and CO_2 concentration for the selection of efficient foliage plants on the improvement of indoor environment. – Korean J. Hortic. Sci. **28**: 928-936, 2010.

Pyke K.A., Leech R.M.: The control of chloroplast number in wheat mesophyll cells. – Planta **170**: 416-420, 1987.

Reinolds E.S.: The use of lead citrate of high pH as an electron-opaque stain in electron microscopy. – J. Cell Biol. **17**: 208-212, 1963.

Rosati A., Badeck F.W., Dejong T.M.: Estimating canopy light interception and absorption using leaf mass per unit leaf area in *Solanum melongena*. – Ann. Bot.-London. **88**: 101-109, 2001.

Rousset O., Lepart J.: Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*). – J. Ecol. **88**: 401-412, 2000.

Ryu S.Y., Oak M.H., Yoon S.K. *et al*.: Antiallergic and antiinflammatory triterpenes from the herb of *Prunella vulgaris*. – Planta Med. **66**: 358-360, 2000.

Sims D.A., Pearcy R.W.: Photosynthetic characteristics of a tropical forest understory herb, *Alocasia macrorrhiza*, and a related crop species, *Colocasia esculenta* grown in contrasting light environments. – Oecologia **79**: 53-59, 1989.

Sohn K.H., Lee H.Y., Chung H.Y. *et al.*: Anti-angiogenic activity of triterpene acids. – Cancer Lett. **94**: 213-218, 1995.

Šesták Z.: Limitations for finding a linear relationship between chlorophyll content and photosynthetic activity. – Biol. Plantarum **8**: 336-346, 1966.

Tao J.P., Zhong Z.C.: [Morphological responses to different nutrient supply in the stoloniferous herb *Glechoma longituba*.] – Acta Ecol. Sin. **20**: 207-211, 2000. [In Chinese]

Temesgen H., Weiskittel A.R.: Leaf mass per area relationships across light gradients in hybrid spruce crowns. – Trees **20**: 522-530, 2006.

Theis N., Lerdau M.: The evolution of function in plant secondary metabolites. – Int. J. Plant Sci. **164**: 93-102, 2003.

Wang B.Y., Feng Y.L.: [Effects of growth light intensities on photosynthesis in seedlings of two tropical rain forest species.] – Acta Ecol. Sin. **25**: 23-30, 2005. [In Chinese]

Wang H.H., Wang Z.Z., Guo W.B.: Comparative determination of ursolic acid and oleanolic acid of *Macrocarpium officinalis* (Sieb. et Zucc.) Nakai by RP-HPLC. – Ind. Crop Prod. **28**: 328-332, 2008.

Wei S.L., Wang W.Q., Chen X.H. *et al.*: [Studies on the shade-endurance capacity of *Glycyrrhiza uralensis*.] – China J. Chin. Mater. Med. **30**: 100-104, 2005. [In Chinese]

Wilson D., Cooper J.P.: Effect of light intensity during grown on leaf anatomy and subsequent light-saturated photosynthesis among contrasting *Lolium* genotypes. – New Phytol. **68**: 1125-1135, 1969.

Wittmann C., Aschan G., Pfanz H.: Leaf and twig photosynthesis of young beech (*Fagus sylvatica*) and aspen (*Populus tremula*) trees grown under different light regime. – Basic Appl. Ecol. **2**: 145-154, 2001.

Wu Z.Y., Chen X.Q., Li X.W. *et al*.: [Flora of China. **65**]. Pp. 316-318, Science Press, Beijing 1977. [In Chinese]

Xiao P.G., Yu D.Q., Ding J. *et al.*: [Pharmacopoeia of People's Republic of China.] Pp. 158-159. – Chemical Industry Press, Beijing 2010. [In Chinese]

Xu D.Q.: [Photosynthetic Efficiency.] Pp. 124-125. Shanghai Scientific and Technical Publishers, Shanghai 2002. [In Chinese]

Ye Z.P.: A new model for relationship between irradiance and the rate of photosynthesis in *Oryza sativa*. – Photosynthetica **45**: 637-640, 2007.

Ye Z.P.: [A new model of light-response of photosynthesis and its application.] – J. Biomath. **23**: 710-716, 2008. [In Chinese]

Zhang S.R., Gao R.F.: [Light induces leaf orientation and chloroplast movements of hybrid poplar clones.] – Acta Ecol. Sin. **21**: 68-74, 2001. [In Chinese]

Zhou S.B., Liu K., Zhang D. *et al*.: Photosynthetic performance of *Lycoris radiata* var. radiata to shade treatments. – Photosynthetica **48**: 241-248, 2010.