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[1] State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou, China
[2] Guangdong Technology Research Center for Traditional Chinese Veterinary Medicine and Natural Medicine, South China Agricultural University, Guangzhou, China
[3] Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, South China Agricultural University, Guangzhou, China
[4] Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, China

Alkaloid content and essential oil composition of *Mahonia breviracema* cultivated under different light environments

Yanqun Li[1,2,3], Dexin Kong[1], Hui-Ling Liang[4], Hong Wu[1,2,3,*]

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Summary

Light can affect the yields of alkaloid and essential oil in the synthesis of secondary metabolites directly or indirectly through plant growth. Despite *Mahonia breviracema* being an endemic medicinal species in China, research on the influence of light on production of alkaloid and essential oil is scarce. Thus, this research evaluated the influence of various lighting conditions on alkaloid yields and the composition and yields of the essential oils of *M. breviracema*. The results revealed significant differences in alkaloid yields, oil yields and chemical characteristics of *M. breviracema* grown in four different light intensities from 10 to 100% full sun shine. The total amount of alkaloids in plants under I_{30} and I_{50} was higher than that under I_{10} and I_{100} due to the higher biomass of plants. Oil yield *of M. breviracema* leaf increased linearly with the increase of light incidence. Plants grown under I_{10} had less plastoglobuli, which coincided with the lowest oil yield (1.91 g kg^{-1}). The plastoglobuli in chloroplasts increased when the irradiance levels increased, resulting in the highest oil yields under I_{100} (4.53 g kg^{-1}). The principal components in the leaves of *M. breviracema* were hexadecanoic acid (10.54-72.19%) and α-ionone (1.25-42.39%). The highest hexadecanoic acid content was obtained under I_{50}, followed by I_{30}, and the highest α-ionone content was obtained under I_{100}. Therefore, it is necessary to control the light environment to obtain raw materials with high quality.

Keywords: *Mahonia breviracema*; Irradiance; Chloroplast; Alkaloids; Essential oil

Introduction

Mahonia is a genus of about 60 species which are distributed in Southeast and East Asia. A total of 35 species of *Mahonia* have been found in China, 20 of which are regarded as medicinal plants (He and Mu, 2015). The roots and stems are used as raw materials "GongLaoMu" recorded in the *Chinese Pharmacopoeia* (China Pharmacopeia Commission, 2010). It is a medicinal plant traditionally used to treat aridity, clear heat, and relieve cough and phlegm (China Pharmacopeia Commission, 2010). The leaves of *Mahonia* plants are the raw material "GongLaoYe" (Ye, 2009), which has curative effects of clearing heat, relieving cough, and reducing sputum antioxidant and anti-malignant tumor. The major bioactive components of "GongLaoMu" are alkaloids, including jatrorrhizine, palmatine and berberine. "GongLaoYe" contain abundant essential oil (Liu et al., 2010a, 2010b; Zeng et al., 2006).

Medicinal plants have been increasingly used, which promotes the loss and exploitation of species. About 40% flora faces the danger of extinction because of human activities, such as excessive collection and extraction of certain species (Oliveira, 2010). *Mahonia breviracema* Y.S. Wang & P.G. Xiao. is genus *Mahonia* (*Berberidaceae*.), and it is an endemic genus to Southwest China. *M. breviracema* is a short shrub mainly distributed in Guangxi, and grows in, coniferous forests, deciduous and evergreen forests. It has been reported that *M. breviracema* contains high amounts of alkaloids (Kong et al., 2011). In recent years, the number of *M. breviracema* in wild distribution areas has sharply decreased because *M. breviracema* is not only sold as "GongLaoMu" and *M. breviracema* but also used as ornamental plants. Plants in the environment different from their natural habitat may show quantitative and/or qualitative changes in their composition in terms of special metabolites. Therefore, exploring the influence of irradiation intensity on growth and development of plants, particularly on the accumulation of secondary metabolites, is important to cultivate medicinal plants.

Irradiance is an important environmental factor influencing normal physiological functions, plant growth and secondary metabolic product accumulation (Ma et al., 2010). High light intensity enhances the accumulation and synthesis of total flavonoids and phenolics in young *Ginger* (Ghasemzadeh et al., 2010). However, excessively high light intensity leads to low flavonoid contents and weak photosynthetic capability in *Anoectochilus* (Ma et al., 2010), whereas a decrease in the content of essential oil at low light intensity has been reported in *Ocimum basilicum* L. (Chang et al., 2008). The content of sabinene in *Origanum vulgare* L. ssp. *vulgare* essential oil is decreased by lower light intensity (De Falco et al., 2013). Kong et al. (2016) reported that there were obvious differences in the plastoglobules of *Mahonia bodinieri* (Gagnep.) Laferr. leaves at different light intensity conditions, and *M. bodinieri* leaf oils are complex systems with varying compositions (Dong et al., 2008). The influence of light intensity on the composition of essential oils and the content of alkaloid in *M. breviracema* has not been reported. Therefore, *M. breviracema* is used as the material to analyze the content changes of volatile oil and alkaloids under different light conditions through botanical microtechnique, HPLC and GC-MS. Moreover, the optimal cultivating condition of *M. breviracema* is discussed in the present study. The study result provides technical support and theoretical basis for the breeding and cultivation of *M. breviracema*.

Materials and methods

Plant material and growth conditions

The experiment was carried out at Guangxi Institute of Botany in Yanshan, Guilin, China. On Marth 15th, 2011, the seeds of *M. breviracema* were sown. Experimental design was performed according to the methods of Kong et al. (2016).

On May 15th, 2014, seedlings of *M. breviracema* with uniform size (40-50 cm in height) were transferred to pots containing peat soil

* Corresponding author

and limestone mountain soil (1:1, v/v; pH at 5.8). After one month, the plants were subjected to four treatments of light irradiance (10% (I_{10}), 30% (I_{30}), 50% (I_{50}) and 100% (I_{100}) light-full sun) for 6 months. The full sun treatment in an experiment conducted on the farm was about 2000 ± 20 μmol photons m^{-2} s^{-1} at noon, which is determined by a Li-6400 portable photosynthesis system (Li-6400, LICoR, Lincoln, NE, USA). Light intensity was controlled through the modified method proposed by Liao et al. (2005). A total of 20 pots included in each treatment were arranged from the north-west to the south-east every day.

Biomass

Five samples of plants were weighted after being separated into leaves, stems and roots to calculate their total dry biomass. The dry biomass was obtained by using an oven with air ventilation at 60 °C, until constant weight.

HPLC analysis

Five plants were collected randomly in each treatment to determine the content of alkaloid. The leaves were dried in oven for 20 min at 105 °C to deactivate enzymes and then cooled to 55 °C quickly. Afterwards, stems, roots and leaves were dried for at least 48 h in a oven at 55 °C. The samples were further ground (60 mesh) and preserved in a drying oven. Each sample was accurately weighed as 0.50 g and placed into a 250 mL conical flask; 100 mL of a hydrochloric acid-methanol solution (1:100, v/v) was added and the mixture was heated by reflux for 15 min at 55 °C. After reflux, the mixture was extracted with ultrasonication for 45 min; the mixtures were then shaken and cooled to room temperature and filtered. The residue was adjusted to 100 mL of hydrochloric acid-methanol (1:100, v/v), and the above procedure was repeated. Finally, the two filtrates were combined and the mixture was filtered. The filtered fluid was then dried, re-dissolved, and filtered through a 0.22 μm syringe filter prior to HPLC-DAD analysis.

An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, USA) consisting of a vacuum degasser, a G1311A quaternary pump, a G1315A diode array detector (DAD) and a G1329A autosampler was utilized to obtain HPLC chromatograms. The system was controlled by Agilent Chemstation software (Agilent Technologies, Palo Alto, USA). Chromatography was operated on a Gemini C_{18} reversed-phase column (4.6 mm × 250 mm, 5 μm) maintained at 25 °C. The mobile phase was performed by using solvent A (0.05 mol L^{-1} KH_2PO_4 buffer solution, pH 3.0 adjusted with H_3PO_4) and B (acetonitrile) (72:28, v/v) at a flow rate of 1.0 mL min^{-1}. The pump of the HPLC equipment was carefully washed by 10 % isopropyl alcohol for 10 min before determining the samples, and 10 min was required to equilibrate the column by mobile phase after each sample. The UV spectra were recorded at 265 nm. Identification of jatrorrhizine, palmatine and berberine was performed by comparing the retention times of chromatographic signals between detected samples and those of the three alkaloids standards under the same experimental conditions. Twenty μL of each sample was injected and HPLC analyses were performed in triplicate for statistical analysis.

Preparation of samples and extraction of essential oil

Fresh leaves were randomly collected from five plants of *M. breviracema* in each sample. Leaves were dried in shade for 15 days at room temperature. The dried samples were also ground (60 meshes) and preserved in a drying oven. The essential oil was extracted through hydrodistillation according to the methods recorded in the *Pharmacopoeia of the People's Republic of China* (China Pharmacopeia Commission, 2010). The leaves (15 g each) were conducted with hydrodistillation in a sealed vessel for 5 hours (Li et al., 2013). We determined the yield of essential oils in triplicate, and the mean values were expressed as the results. The volatile oils were kept at 4 °C for further analysis. The average concentration of essential oil was calculated as the weight of oil (g) per 1 kg of dry leaf biomass and essential oil yield per plant (mg $plant^{-1}$). The plots were analyzed through Origin software.

GC–MS analysis

The essential oil was analyzed by gas chromatograph (Finnigan Trace GC-2000)-mass spectrometer (Thermo Finnigan, American). A 30 m × 0.25 mm IDHP-1 bonded-phase fused-silica capillary column with a film thickness of 1 μm was used. The temperature of the injector was 150 °C. The initial temperature was maintained at 100 °C for 4 min and then increased to 130 °C at the rate of 5 °C/min; Afterwards, the temperature was maintained at 130 °C for 20 min. Linear velocity of the helium carrier gas was 1.2 mL.min^{-1} at the split ratio of 30:1; EI was used as the ion source at 230 °C. Sector mass analyzer scanned from 30 amu to 550 amu. Diluted samples (25 μg/mL) were prepared through methylene dichloride, and 0.4 μL samples were injected. The chemical constituents were identified by comparing with the National Institute of Standards and Technology (NIST05.LIB) library spectra and the literature (Adams, 2001). The retention indices were calculated by injecting a series of n-alkanes in the same conditions.

Light microscopy and transmission electron microscopy

The tissue of leaves was cut into pieces with the size of 1 mm × 1 mm × 0.5 mm. The specimens were processed through the method proposed by Liu et al. (2012). Sections (1 μm thick) were stained with 0.5% Toluidine Blue O and photographed through a Leica EM UC6 ultramicrotome (Leica, Germany). The chloroplasts number per mm^2 was counted and averaged within 15 1000 × 1000 mm^2 squares randomly selected from the cross sections. For the ultrastructural observations, ultrathin sections (70-90 nm in thickness) were stained with lead citrate and uranyl acetate. A Philips FEI-Technai 12 microscope was used to observe the sections.

Statistical analysis

Data were reported as mean ± standard deviation of at least three experiments. One-way analysis of variance (ANOVA) was subjected to using SPSS version 18.0 (SPSS Inc., Chicago, USA). Duncan's multiple range test was performed to detect differences between treatments on each variable ($p < 0.05$).

Results

Biomass

The results show that light conditions can significantly influence the biomass of root, stem and leaf and total biomass (Fig. 1), which were obviously higher under I_{30}, followed by I_{50}. There was no significant difference between I_{30} and I_{50}. However, the total biomass under both I_{30} and I_{50} was statistically higher than that under I_{10} and I_{100}.

Alkaloids analyses

In roots and stems, higher contents of jatrorrhizine and palmatine in *M. breviracema* were observed under moderate light intensity. Plants grown under I_{30} and I_{50} had higher concentrations of jatrorrhizine and palmatine in roots, i.e., 10.83 and 10.84 mg·g^{-1}, and 2.04 and 1.86 mg·g^{-1}, respectively. Higher concentrations of jatrorrhizine and palmatine in the stems were observed under I_{50}, (15.71 and 10.58 mg·g^{-1}, respectively), followed by I_{30} (13.49 and 10.40 mg·g^{-1}) (Fig. 2,

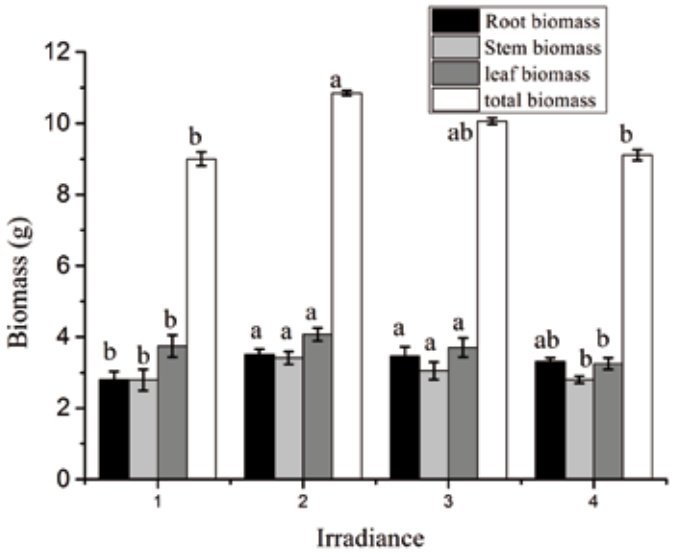

Fig. 1: Dry biomass of *M. breviracema* plant grown under different light intensities. The values are the average ± SE, and different letters indicate that there are obvious differences in the shade treatments (P<0.05).

A and C). The highest concentration of berberine were obtained under I_{10} (11.80 mg·g^{-1}) followed by I_{50} (11.21 mg·g^{-1}) in the roots (Fig. 2A). While the concentration of berberine in the stems decreased with the increase of light irradiance, the highest values of berberine were up to 10.83 mg·g^{-1} under I_{10} (Fig. 2C). We estimated the changes in total amount of alkaloid in root, stem, leaf and each plant further study the data. The variation of total amount of alkaloids with higher production in the roots and stems was obtained under the conditions of intermediate irradiance (I_{30} and I_{50}), which showed low values under I_{10} and I_{100} (Fig. 2, B and D), although it was not statistically significant among the various light intensities for roots. Moreover, the concentrations of jatrorrhizine and berberine were obviously higher than palmatine in the roots, while palmatine content in the stems was significantly higher than that in the roots. In particular, the concentrations of palmatine under I_{30} and I_{50} were higher than berberine (Fig. 2, A and C).

The concentrations of jatrorrhizine (0.65-0.35 mg·g^{-1}), palmatine (0.66-0.36 mg·g^{-1}), and berberine in leaves were significantly lower compared to those in the roots and stems (Fig. 2E). Jatrorrhizine was only detected under I_{50} and I_{100} (0.65 and 0.35 mg·g^{-1}, respectively), and berberine was not detected under all light treatments (Fig. 2E).

Leaf structure

There were notable changes taking place in leaf anatomical characteristics induced by light intensity. The results showed that the thickness of the entire lamina, palisade parenchyma and spongy parenchyma increased with the increase of light incidence (Tab. 1; Fig. 3). At the same time, chloroplast sizes and numbers in the palisade parenchyma decreased with increasing light intensity (Tab. 2).

To study the distribution and accumulation of oil in *M. breviracema* leaves, we investigated the microstructure of the leaves under different light intensities. Chloroplast structure of *M. breviracema* were obviously influenced by light intensity (Fig. 4). Most chloroplasts in leaves grown under I_{10} and I_{30} had large size and showed normal ultrastructural organization, with a typical arrangement of stroma and grana thylakoids (Fig. 4, C and F). Few plastoglobules were observed under I_{10} (Fig. 4, A and B). There were more electron-transparent plastoglobules in the chloroplasts under I_{30} (Fig. 4, D and E). Abnormal chloroplast structure with irregular and blurring grana lamellae arrangement was found under I_{50} (Fig. 4I). Electron-dense plastoglobules was significantly increased for I_{50} (Fig. 4, G and H). Under I_{100}, the grana were totally ruptured (Fig. 4, K and L), but the chloroplasts were filled with abundant plastoglobules (Fig. 4, J and K).

Yield of essential oil

The content of essential oil in leaves of *M. breviracema* grown under different light conditions changed significantly. The contents and yields of essential oil in *M. breviracema* leaves were sensitive to irradiance with a rising linear behavior, with the highest values found in plants grown under I_{100} (4.53 g kg^{-1}), followed by I_{50} (3.12 g kg^{-1}). Plants grown under I_{10} had the lowest essential oil yield (1.91 g kg^{-1}) (Fig. 5A). The total amount of essential oil also followed a similar trend (Fig. 5B).

Composition of essential oils

The irradiance affected the essential oil composition of *M. breviracema* eliciting a variation of 29, 31, 28 and 28 identified components at I_{10}, I_{30}, I_{50} and I_{100}, respectively, representing 91.97-97.34% of total essential oils and 26 common peaks (Tab. 3). The study showed that hexadecanoic acid (72.19-10.54%) and α-ionone (1.25-42.39%) were found in greater quantities in *M. breviracema* leaf oils. The content of hexadecanoic acid was the largest under I_{50}, followed by I_{30}, and the lowest content was found under I_{100}, i.e., only 10.54% of hexadecanoic acid. Interestingly, the α-ionone constituents were significantly increased under I_{100}, leading to a significant increase in the α-ionone content (42.39%); the other treatments only showed 1.25-2.32%. The contents of hexadecanoic acid, methyl ester and hexadecanoic acid, ethyl ester under I_{10} and I_{100} (1.48 and 1.83%, 1.20 and 1.77%, respectively) were higher than that of I_{30} and I_{50} (0.46 and 0.60%, 0.72 and 0.79%, respectively). Moreover, the changes in the contents of octadecanoic acid, ethyl ester and methyl linolenate with a higher production under intermediate irradiance (I_{30} and I_{50}) demonstrated that this component was also influenced by irradiance. Nerolidol increased in the treatments with 50% and 100% light. These results showed that the 4 samples mainly contained large levels of monoterpenes (2.69-42.95%) and oxygenated sesquiterpenes (13.27-73.33%). Interestingly, the result showed that the variation of monoterpenes and oxygenated sesquiterpenes were inversely related, i.e. a rise of the monoterpenes content accompanied the decrease in the content of oxygenated sesquiterpenes. The leaf oils had the highest content of monoterpenes (42.95 %) under I_{100}, while the highest oxygenated sesquiterpenes content (73.33 %) was observed under I_{50}.

Discussion

Irradiance is essential for plant growth, since it affects the primary metabolism providing energy for photosynthesis and generating signals that regulate their development and even interfere with the translocation of assimilates among different plant organs (Lima et al., 2010). Plants grown in full light conditions absorb much more light energy in the leaves than the plants require and utilize for photosynthetic CO_2 fixation (Wilhelm and Selmar, 2011). High irradiation often is co-occurring with water deficiencies (Kleinwächter and Selmar, 2015). Therefore, high light levels may reduce plant growth (Tang et al., 2015). The foliar thickness, the ratio of palisade and spongy tissue thickness, as well as palisade and spongy tissue thickness increased in *M. breviracema* with increasing irradiance to cope with these light stresses (Aleric and Kirkman, 2005). The adaptation of plant morphology, anatomy to the changes in light intensity may influence the accumulation of secondary metabolites (Ma et al., 2010). Similarly, irradiance can influence the production of alkaloids

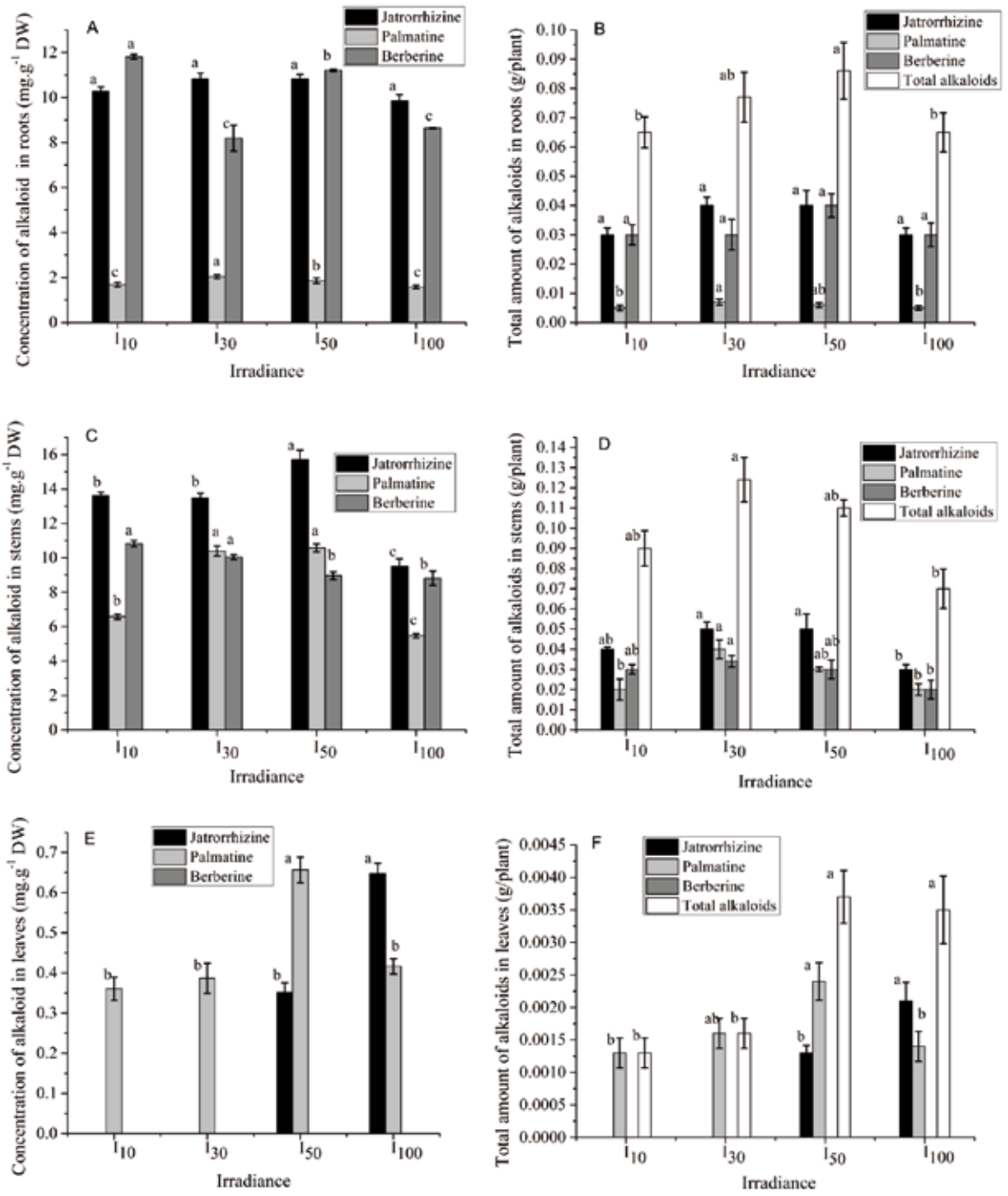

Fig. 2: Levels of three alkaloids in *M. breviracema* roots (A), stems (C) and leaves (E) at various light levels. Estimated total amounts of alkaloid in the roots (B), stems (D) and leaves (F) of a plant under different light intensities (g per plant). The values are the average ± SE. Different letters demonstrate that there are obvious differences in the shade treatments (P<0.05)

Tab. 1: The changes of anatomical characteristics of *M. breviracema* leaves under different light intensity.

Light intensity	Lamina thickness (μm)	Palisade tissue (μm)	Spongy parenchyma (μm)	Palisade/spongy
I_{10}	525.39±16.05^{d}	65.09±2.05^{d}	413.50±2.11^{d}	0.157±0.02^{c}
I_{30}	576.78±9.61^{c}	76.09±2.13^{c}	459.05±10.45^{c}	0.166±0.01b^{c}
I_{50}	682.56±4.53^{b}	91.50±0.91^{b}	503.09±4.49^{b}	0.182±0.01^{b}
I_{100}	742.90±2.14^{a}	131.02±2.57^{a}	571.50±9.87^{a}	0.229±0.01^{a}

The values represent the mean ± SE, and the same letters indicated no significant differences in four shade treatment (P<0.05)

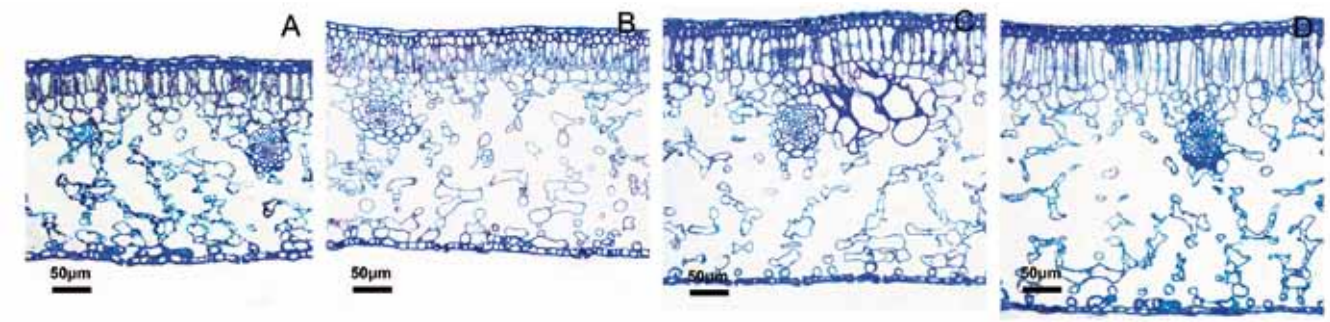

Fig. 3: Light micrographs of characteristic semi-thin cross-sections in the leaves of *M. breviracema* at different light intensities. I_{10} (A), I_{30} (B), I_{50} (C), I_{100} (D).

Tab. 2: Changes in number of mesophyll chloroplast (n mm^{-2}) and structural characteristics of chloroplast in leaves of *M. breviracema* in various light conditions.

Light intensity	Chloroplast length (μm)	Chloroplast width (μm)	Chloroplast number (n/mm^2)
I_{10}	7.14±0.23^a	4.27±0.26^b	5293±109.41^a
I_{30}	7.96±0.14^a	3.68±0.29^b	4403±232.38^b
I_{50}	7.80±0.47^a	3.44±0.68^b	3227±186.19^c
I_{100}	8.18±0.30^a	2.62±0.46^b	2796±172.05^c

The values indicate the average ± SE. The same letters show there is no obvious difference in the four shade treatments (P<0.05)

both directly and indirectly by increasing plant biomass (Kong et al., 2016). Li et al. (2009) reported that the contents of berberine, jatrorrhizine and palmatine increased with the light incidence for *Amur* corktree, while the production of biomass was the highest at 75% full-sunlight treatment. In this study, we reveled that the irradiance levels significantly affected the plant biomass and alkaloid content in different plant organs of *M. breviracema*. Notably, roots and stems showed higher contents of jatrorrhizine, palmatine and berberine, which were very low or even undetected in the leaves. Jatrorrhizine, palmatine and berberine showed similar trends in roots and stems. However, the content of palmatine in stems was much higher than that in roots. In addition, jatrorrhizine was not detected under I_{10} and I_{30} in leaves, which indicated that higher light intensity promotes the synthesis of alkaloids (Wilhelm and Selmar, 2011). The highest contents of jatrorrhizine and palmatine in roots and stems were all observed under I_{30} and I_{50}, and the content of berberine in roots and stems under I_{10} was the highest. However, the plant biomass was significantly higher under I_{30} and I_{50} than that under I_{10} and I_{100}. Thus, the total amount of jatrorrhizine, palmatine and berberine, and the total alkaloid yields in stems, roots and each plant under I_{30} and I_{50} were much higher compared with other treatments.

In many cases excessive light under nutrient or water limitation stimulates the synthesis of secondary metabolites (Wilhelm and Selmar, 2011). Research on the yield of essential oil under different shade conditions indicates that species have different responses to light intensity, like *Lippia sidoides* Cham. (Souza et al., 2007) and *Ocimum basilicum* (Figueiredo et al., 2008) showed the increase of essential oil yields when grown at high light intensities. Kong et al. (2016) revealed that plastoglobules in the chloroplasts were significantly increased at higher light intensity for *M. bodinieri*. This study showed that not only the numbers of grana lamellae, grana and chloroplasts decreased with the increase of light irradiance intensity, but also the plastoglobules were obviously affected by the light intensity for *M. breviracema*. Notably, we revealed a positive correlation between the number of plastoglobules with the yield of essential oil at different growth stages in *M. breviracema*. The smaller amounts of plastoglobules were accumulated under I_{10}, which was accordance with a lower yield of essential oil. Our research showed significant chloroplast structural damage and increased plastoglobules with increasing light intensity. In particular, the grana totally ruptured and disappeared, but the chloroplasts were filled with abundant plastoglobules. The yield of essential oil rose sharply to 4.53 g kg^{-1} under I_{100}. These results are in accordance with that of Souza et al. (2007) and Figueiredo et al. (2008) in that a higher light intensity enhanced the accumulation and synthesis of essential oils. Accumulation and synthesis essential oils in *M. breviracem* mainly occur in chloroplasts. Furthermore, these results further confirmed that the increase of plastoglobules were closely related to the reduced chloroplast function and senescence under high light intensity (Biswal, 1995).

In general, the composition of essential oils is very sensitive and can suffer numerous reactions under stress factors (Selmar and Kleinwächter, 2012; Kleinwächter and Selmar, 2015). In the current study, the major and most representative component in all analyzed samples is the saturated fatty acid hexadecanoic acid which is exhibited in high percentage in the four samples (10.54-72.19%). This result is similar to that obtained in *M. bodinieri* (Liu et al., 2010a). Thereinto, the intermediate irradiance (I_{50}) was beneficial for increasing hexadecanoic acid. Moreover, in all these samples hexadecanoic acids were found in various forms such as ethyl ester, methyl ester. Hexadecanoic acid isolated from a marine red alga may be a lead compound of anticancer drugs (Harada et al., 2002) and presents important anti-inflammatory and analgesic activities properties (Aparna et al., 2012; Hamdi et al., 2018). Indeed, hexadecanoic acid derivatives showed and anti-nociceptive anti-inflammatory activities (Zitterl-Eglseer et al., 1997; Deciga-Campos et al., 2007). Such as, hexadecanoic acid, methyl ester is responsible for various pharmacological actions like antimicrobial and antioxidants activities (Tapiero et al., 2002). The presence of phytochemicals could be considered as sources of quality raw materials for food and pharmaceutical industries. Interesting, we found that α-ionone, another important composition in the oil of *M. breviracema*, showed highest content in full light conditions. Ionone has been reported as a product of oxidative rupture of β-carotene (Sánchez-Contreras et al., 2000). It is possible to increase the leaf temperature in the high light conditions, which is beneficial to the accumulation of carotenoids, while the high temperature stimulates the degradation of carotenoids and the accumulation of aromatic substances (ionone) (Kawakami and Kobayashi, 2002; Zepka and Mercadante, 2009; Zhan et al., 2012; Ramel et al., 2012). Carotenoids are sources of essential precursors for the biosynthesis of bioactive compounds in plants when oxidative cleavages occur to form carotenoids derivatives. These compounds serve as signal molecules (Ramel et al., 2012) and they have been implicated in the interactions of plants with their environment (light and temperature) (Walter and Strack, 2011; Nisar

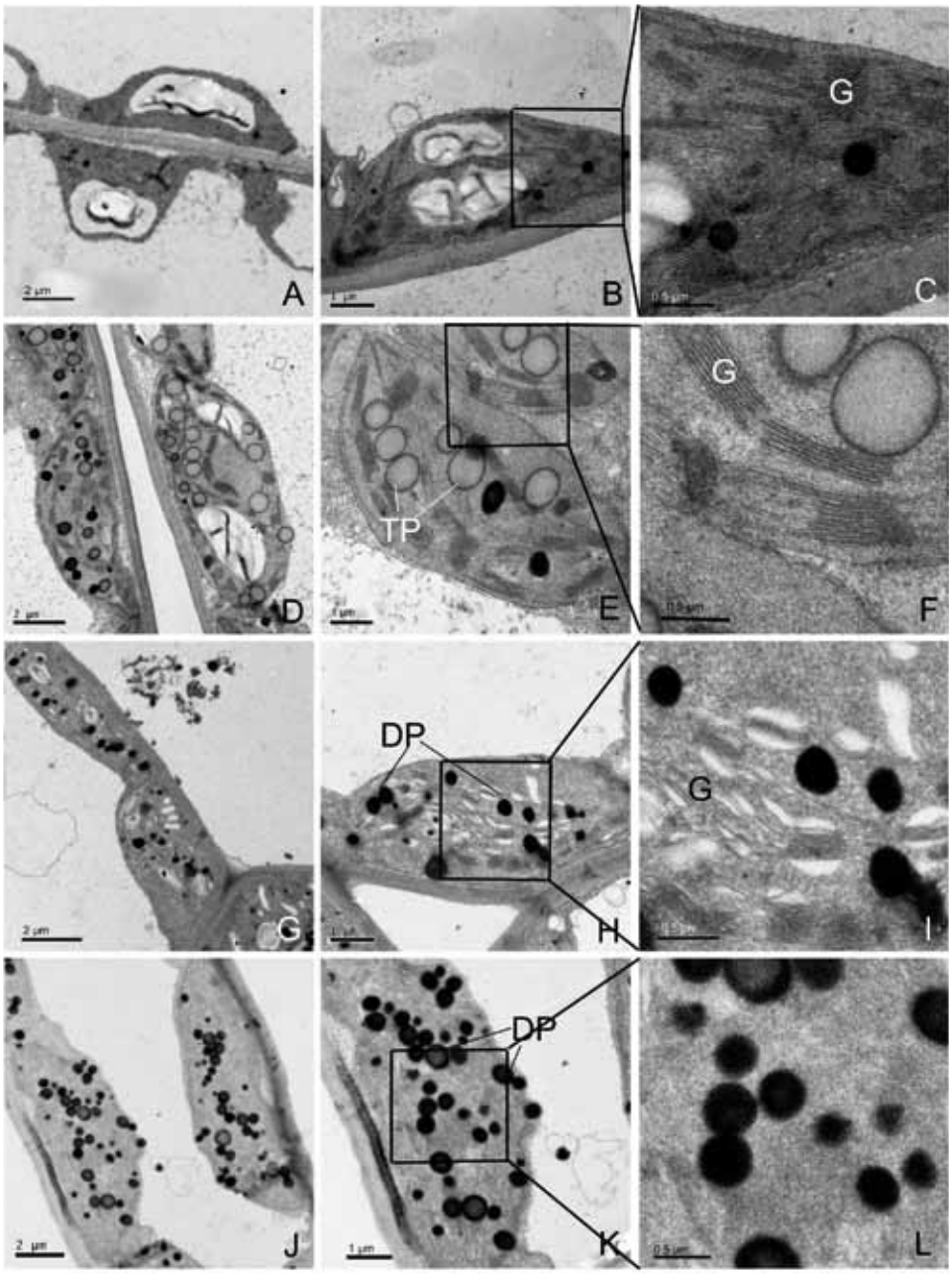

Fig. 4: Ultrastructure of chloroplast in the leaves of *M. breviracema* at different light intensities. I_{10} (A–C), I_{30} (D–F), I_{50} (G–I), I_{100} (J–L). DP: Electron-dense plastoglobules, G: Granum, TP: Electron-transparent plastoglobules.

et al., 2015; BRIARDO et al., 2016). Thereby, carotenoids play an important role on sensing and signalling oxidative stress, as chemical oxidation of *b*-carotene by $^1O_2$, forming a wide variety of products, such as *b*-Cyc *b*-ionone and *a*-ionone (RAMEL et al., 2012).

Hexadecanoic acid and α-ionone are the major components of *M. breviracema* leaf oils, suggesting a chemotype different from that described by LIU et al. (2010b), where the main component is 4-terpineol (43.73%) for *Mahonia duclouxiana* Gagnep. Hexadecanoic acid is used to treat blood lipids and cardiovascular diseases (CONSULTATION, 2003). α-ionone is an important material in the flavors and fragrances industry (SELL, 2006) Therefore, *M. breviracema* has important development potential and high medicinal value. Moreover, *M. breviracema* is a good ornamental plant.

Furthermore, the leaf oils also contained high levels of nerolidol and octadecanoic acid. It was reported that sesquiterpene indole (the main volatile of nerolidol) provided indirect plant defense against various herbivores (PACHECO et al., 2016). Octadecanoic acid is an inducible plant defense molecule against insects (MARKO-VARGA et al., 2008). Thereby, the increase of these compounds in leaves under high light intensity can indicate that there is an effective defense system for the adverse environmental conditions.

Conclusion

This research explores the significant differences in the content of alkaloid and chemical characteristics of leaf oil of *M. breviracema* grown under various light intensities. The contents of jatrorrhizine, palmatine and berberine were notably higher in root and stem samples compared to the leaf samples. By analyzing the variations of alkaloid contents and biomass, I_{30} and I_{50} are determined as the best

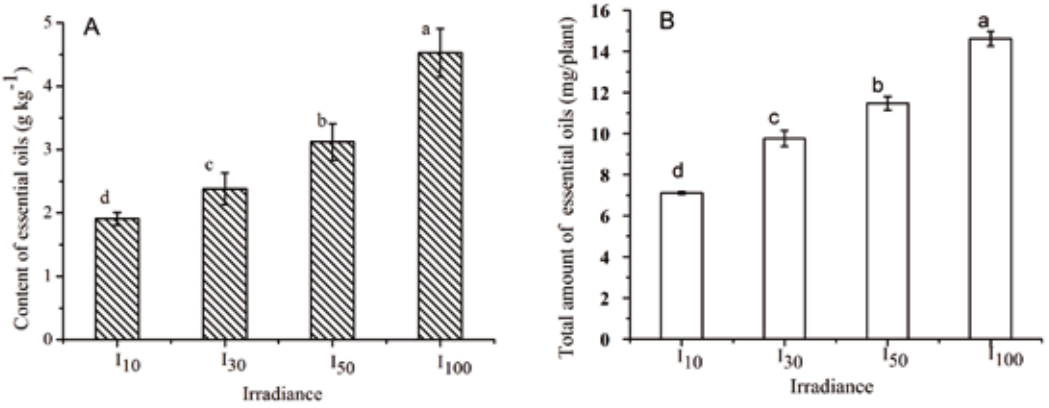

Fig. 5: The content of essential oil (A) and the total amount of essential oil (B) in leaves of *M. breviracema* under different light intensities. The columns with different uppercase letters show obvious differences (P<0.05).

Tab. 3: Chemical constituents (%) of essential oil in leaves of *M. breviracema* under different light intensities.

No	Compound	RI[a]	Relative content (%)[b]				Identification
			I_{10}	I_{30}	I_{50}	I_{100}	
1	2,2,4,6,6-Pentamthyl heptanes	1030	0.23±0.05[b]	0.43±0.05[b]	0.17±0.02[b]	10.37±1.17[a]	GC–MS, RI
2	2,6,8-Trimethyldecane	1121	0.39±0.05[b]	0.06±0.02[c]	0.06±0.06[c]	1.52±1.52[a]	GC–MS, RI
3	α-ionone	1366	1.92±0.18[b]	2.32±0.21[b]	1.25±0.16[b]	42.39±1.65[a]	GC–MS, RI, Co
4	2,6,10-Trimethyl dodecane	1429	0.18±0.03[b]	0.26±0.01[a]	0.05±0.01[c]	-	GC–MS, RI
5	Geranyl acetone	1434	0.22±0.02[a]	0.11±0.01[b]	0.04±0.01[c]	Tr	GC–MS, RI
6	2,6,10-Trimethyltetradecane	1555	0.90±0.37[a]	0.1±0.04[c]	0.16±0.03[bc]	0.51±0.02[ab]	GC–MS, RI
7	Nerolidol	1567	0.75±0.06[c]	0.54±0.03[c]	1.14±0.09[b]	2.75±0.20[a]	GC–MS, RI
8	Hexadecane	1600	2.71±0.29[a]	0.52±0.11[d]	0.91±0.11[c]	1.47±0.12[b]	GC–MS, RI
9	1,2,3-Popanetricarboxylic acid, 2-hydroxy-, triethyl ester	1655	1.19±0.05[a]	0.26±0.04[d]	0.42±0.10[c]	0.63±0.07[b]	GC–MS, RI
10	Phytane	1809	0.91±0.02[a]	0.53±0.11[b]	0.66±0.08[b]	0.93±0.10[a]	GC–MS, RI
11	6,10,14-Trimethyl-2-Pentadecanone	1843	-	1.43±0.11[a]	Tr	0.07±0.03[b]	GC–MS, RI
12	Hexadecanoic acid, methyl ester	1908	1.48±0.10[a]	0.46±0.08[d]	0.72±0.08[c]	1.20±0.20[b]	GC–MS, RI
13	α-Farnesylacetone	1914	2.46±0.08[a]	0.56±0.08[d]	0.96±0.10[c]	1.60±0.30[b]	GC–MS, RI
14	Methyl hexadecanoate	1924	2.50±0.04[a]	0.38±0.05[d]	0.64±0.04[c]	0.79±0.11[b]	GC–MS, RI
15	Hexadecanoic acid, ethyl ester	1968	1.83±0.16[a]	0.60±0.30[b]	0.79±0.09[b]	1.77±0.21[a]	GC–MS, RI
16	Hexadecanoic acid	1978	52.23±2.27[b]	60.05±7.02[b]	72.19±5.89[a]	10.54±2.74[c]	GC–MS, RI, Co
17	Octadecanoic acid	2002	6.54±0.88[a]	1.07±0.09[c]	1.44±0.21[c]	3.73±0.65[b]	GC–MS, RI
18	Isochiapin B	2005	1.01±0.03[a]	0.45±0.12[b]	0.50±0.10[b]	0.54±0.10[b]	GC–MS, RI
19	Eicosane	2008	0.41±0.09[a]	0.12±0.06[c]	0.12±0.08[c]	0.27±0.05[b]	GC–MS, RI
20	Phytol	2042	0.28±0.07[a]	0.27±0.05[a]	-	0.11±0.04[b]	GC–MS, RI
21	9,12,15-Octadecatrienoic acid, Methyl ester	2051	-	1.35±0.19[a]	1.01±0.19[b]	0.96±0.12[b]	GC–MS, RI
22	Methyl linoleate	2062	0.21±0.05[b]	0.44±0.56[a]	0.11±0.03[b]	0.10±0.02[b]	GC–MS, RI
23	9-Octadecenoic acid (Z)-, Methyl ester	2078	0.81±0.17[a]	0.18±0.06[b]	Tr	0.56±0.18[a]	GC–MS, RI
24	Octadecanoic acid, ethyl ester	2083	1.05±0.07[b]	12.94±3.01[a]	1.39±0.19[b]	0.76±0.22[b]	GC–MS, RI
25	Methyl linolenate	2102	-	0.33±0.11[a]	0.12±0.07[b]	Tr	GC–MS, RI
26	Ethyl linoleate	2165	1.81±0.38[a]	1.24±0.16[b]	0.71±0.29[c]	1.15±0.15[bc]	GC–MS, RI
27	Linolenic acid ethyl ester	2173	0.32±0.06[a]	Tr	Tr	0.22±0.01[b]	GC–MS, RI
28	3,7,11,15-tetramethyl-, [R-(R*,R*-(E))]-2-Hexadedcen-1-ol	2201	6.74±1.14[b]	9.01±0.63[a]	6.46±0.14[b]	3.07±0.18[c]	GC–MS, RI
29	Docosane	2208	0.55±0.10[b]	0.42±0.14[b]	0.36±0.04[b]	0.88±0.10[a]	GC–MS, RI
30	Carvacrol	2214	0.55±0.13[a]	0.25±0.10[b]	0.33±0.03[b]	0.56±0.02[a]	GC–MS, RI
31	Tricosane	2300	2.04±0.24[a]	0.66±0.23[b]	2.43±1.01[a]	1.93±0.17[a]	GC–MS, RI
	Monoterpenes		2.69	2.68	1.62	42.95	
	Sesquiterpenoids		6.72	1.33	1.49	3.37	
	Oxygenated sesquiterpenes		52.98	60.6	73.33	13.29	
	Hydrocarbons		6.62	2.25	4.48	5.48	
	Others		22.98	30.48	14.22	26.88	
	Total		91.99	97.34	95.14	91.97	

[a] RI=Retention indices on the basis of a homologous series of normal alkanes. [b] Data are represented as the average ± SD. Values sharing the same small letter within a line are not obviously different at P<0.05; GC–MS, gas chromatography – mass spectrometry; Co, co-injection with authentic compounds; –, not detected; Tr (Trace), relative content <0.1%.

growth environment to get the highest total alkaloids yields and enhance the value of medicinal plants. This study initially provides some information about the composition of *M. breviracema* leaf essential oils. Higher irradiance is conductive to increasing the yield of essential oil. The essential oil extracted from leaves was rich in hexadecanoic acid and α-ionone. Moderate light conditions (I_{30} and I_{50}) were suitable for accumulation and synthesis of hexadecanoic acid, and high light intensity (I_{100}) was beneficial for the accumulation and synthesis of α-ionone. By analyzing the changes in oil yields and compositions, I_{50} and I_{100} are determined the optimal growth environment to get the highest of leaf oils yield or high quality hexadecanoic acid and α-ionone. These results can be used as a reference for industrial exploiters of *M. breviracema* essential oils.

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References

ADAMS, RP., 2001: Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured Publishing Corporation, Carol Stream.

ALERIC, K.M., KIRKMAN, K.L., 2005: Growth and photosynthetic responses of the federally endangered shrub, *Linder amelissifolia* (Lauraceae), to varied light environments. Am. J. Bot. 92, 682-689. DOI: 10.3732/ajb.92.4.682

APARNA, V., DILEEP, K.V., MANDAL, P.K., KARTHE, P., SADASIVAN, C., HARIDAS, M., 2012: Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem. Biol. Drug. Des. 80, 434-439.

BISWAL, B., 1995: Carotenoid catabolism during leaf senescence and its control by light. J. Photoch. Photobiolo. B. 30, 3-13. DOI: 10.1111/j.1747-0285.2012.01418.x

BRIARDO, L., LUCIO, D., MANUEL, R.C., 2016: Evolutionary recycling of light signaling components in fleshy fruits: new insights on the role of pigments to monitor ripening. Front. Plant Sci. 7, 263. DOI: 10.3389/fpls.2016.00263

CHANG, X., ALDERSON, P.G., WRIGHT, C.J., 2008: Solar irradiance level alters the growth of *basil* (*Ocimum basilicum* L.) and its content of volatile oils. Environ. Exp. Bot. 63, 216-223. DOI: 10.1016/j.envexpbot.2007.10.017

CHINA PHARMACOPEIA COMMISSION, 2010: Pharmacopoeia of the People's Republic of China 2010, Vol. 1. Chinese Medical Science and Technology Press, Beijing, China.

CONSULTATION, J.W.E., 2003: Diet, nutrition and the prevention of chronic diseases. Eur. J. Clin. Nutr. 45, 619-623.

DE FALCO, E., MANCINI, E., ROSCIGNO, G., MIGNOLA, E., TAGLIALATELA-SCAFATI, O., SENATORE, F., 2013: Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. Molecules 18, 14948-14960. DOI: 10.3390/molecules181214948

DECIGA-CAMPOS, M., MONTIEL-RUIZ, R.M., NAVARRETE-VAZQUEZ, G., LOPEZ-MUNOZ, F.J., 2007: Palmitic acid analogues exhibiting antinociceptive activity in mice. Proc. West. Pharmacol. Soc. 50, 75-77.

DONG, L., MOU, F.H., YANG, X.H., CHEN, D., LIU, Y.Y., 2008: Study on chemical components of essential oil in leaf of *Mahonia bealei* (Fort.) Carr. by GC-MS. Spec. Wild Econ. Anim. Plant. Res. 1, 50-52.

FIGUEIREDO, A.C., BARROSO, J.G., PEDRO, L.G., SCHEFFER, J.J.C., 2008: Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour. Frag. J. 23, 213-226. DOI: 10.1002/ffj.1875

GHASEMZADEH, A., JAAFAR, H.Z.E., RAHMAT, A., WAHAB, P.E.M., HALIM, M.R.A., 2010: Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in Young Ginger varieties (*Zingiber officinale* Roscoe). Int. J. Mol. Sci. 11, 3885-3897. DOI: 10.3390/ijms11103885

HAMDI, A., MAIOULI, K., ABDELHAMID, A., MARZOUK, B., BELGHITH, H., CHRAIEF, I., BOURAOUI, A., MARZOUK, Z., VANDER HEYDEN, Y., 2018: Pharmacological activities of the organic extracts and fatty acid composition of the petroleum ether extract from *Haplophyllum tuberculatum* leaves. J. Ethnopharmacol. 216, 97-103. DOI: 10.1016/j.jep.2018.01.012

HARADA, H., YAMASHITA, U., KURIHARA, H., FUKUSHI, E., KAWABATA, J., KAMEI, Y., 2002: Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res. 22, 2587-2590.

HE, J.M., MU, Q., 2015: The medicinal uses of the genus *Mahonia* in traditional Chinese medicine: An ethnopharmacological, phytochemical and pharmacological review. J. Ethnopharmacol. 175, 668-683. DOI: 10.1016/j.jep.2015.09.013

KAWAKAMI, M., KOBAYASHI, A., 2002: Carotenoid-derived aroma compounds in tea. In: Winterhalter, P., Rouseff, R. (eds.), Carotenoid-derived aroma compounds, 145-161. Washington, DC: ACS Symposium Series 802.

KLEINWÄCHTER, M., SELMAR, D., 2015: New insights explain that drought stress enhances the quality of spice and medicinal plants: potential applications. Agron. Sustain. Dev. 35, 121-131. DOI: 10.1007/s13593-014-0260-3

KONG, D.X., HUANG, S.S., WANG, M.L., ZOU, R., TANG, H., JIANG, H.Y., 2011: Comparison study on FTIR characterization of chemical constituents and berbamine hydrochloride contents in *Mahonia breviracema* and *Mahonia healei*. Genomics. Appl. Biol. 30, 224-228.

KONG, D.X., LI, Y.Q., WANG, M.L., BAI, M., ZOU, R., TANG, H., WU, H., 2016: Effects of light intensity on leaf photosynthetic characteristics, chloroplast structure, and alkaloid content of *Mahonia bodinieri* (Gagnep.) Laferr. Acta. Physiol. Plant 38, 120. DOI: 10.1007/s11738-016-2147-1

LI, X., WANG, Y., YAN, X.F., 2009: Effect of light intensity on the contents of three main alkaloids in *amur corktree* seedlings. Acta. Ecol. Sin. 4, 1656-1659. DOI: 10.1007/s11738-016-2147-1

LI, Y.Q., KONG, D.X., WU, H., 2013: Analysis and evaluation of essential oil components of cinnamon barks using GC-MS and FTIR spectroscopy. Ind. Crop. Prod. 41, 269-278. DOI: 10.1016/j.indcrop.2012.04.056

LIAO, J.X., GE, Y., HUANG, C.C., ZHANG, J., LIU, Q.X., CHANG, J., 2005: Effects of irradiance on photosynthetic characteristics and growth of *Mosla chinensis* and *M. scabra*. Photosynthetica, 43, 1-4. DOI: 10.1007/s11099-005-1115-6

LIMA, M.C., AMARANTE, L., MARIOT, M.P., SERPA, R., 2010: Crescimento e producao de pigmentos fotossinteticos em *Achillea millefolium* L. cultivada sob diferentes niveis de sombreamento e doses de nitrogenio. Cienca. Rural. 3, 54-57. DOI: 10.1590/S0103-84782011000100008

LIU, P.W., LIANG, S.J., YAO, N., WU, H., 2012: Programmed cell death of secretory cavity cells in fruits of *Citrus grandis* cv. Tomentosa is associated with activation of caspase 3-like protease. Trees, 26, 1821-1835. DOI: 10.1007/s00468-012-0752-1

LIU, S.X., LIU, B.M., DONG, X.M., ZHANG, K.Y., LIN, X., CHEN, L., HE, K.J., 2010a: Analysis of essential oil form *Mahonia bodinieri* Gagnep, J. Guangxi Acad. Sci. 26, 216-217.

LIU, S.X., LIU, B.M., HE, K.J., LIN, X., LU, W.J., 2010b: Analysis of essential oil from *Mahonia duclouxiana*. J. Chin. Med. Mater. 33, 1099-1102.

MA, Z.Q., LI, S.S., ZHANG, M.J., 2010: Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of *Anoectochilus* plants. HortScience 45, 863-867.

MARKO-VARGA, G., NILSSON, J., LAURELL, T., 2008: Octadecanoic Acid (stearic acid). Encyclopedia of Genetics Genomics Proteomics & Informatics, 1387-1387.

NISAR, N., LI, L., LU, S., KHIN, N.C., POGSON, B.J., 2015: Carotenoid me-

tabolism in plants. Mol. Plant 8, 68-82. DOI: 10.1016/j.molp.2014.12.007

PACHECO, F.V., AVELAR, R.D.P., ALVARENGA, I.C.A., BERTOLUCCI, S.K.V., ALVARENGA, A.A.D., PINTO, J.E.B.P., 2016: Essential oil of monkey-pepper (*Piper aduncum* L.) cultivated under different light environments. Ind. Crop. Prod. 85, 251-257. DOI: 10.1016/j.indcrop.2016.03.016

RAMEL, F., BIRTIC, S., GINIES, C., SOUBIGOUITACONNAT, L., TRIANTAPHYLIDES, C., HAVAUX, M., 2012: Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc. Natl Acad. Sci. U.S.A. 109, 5535-5540. DOI: 10.1073/pnas.1115982109

SÁNCHEZ-CONTRERAS, A., JIMENEZ, M., SANCHEZ, S., 2000: Bioconversion of lutein to products with aroma. Appl. Microbiol. Biot. 54, 528-534. DOI: 10.1007/s002530000421

SELL, C., 2006: The chemistry of fragrances from perfumer to consumer, 2nd edn. The Royal Society of Chemistry, London.

SELMAR, D., KLEINWÄCHTER, M., 2013. Stress enhances the synthesis of secondary plant products: The impact of stress-related over-reduction on the accumulation of natural products. Plant Cell Physiol. 54, 817-826. DOI: 10.1093/pcp/pct054

SOUZA, G.S., CASTRO, E.M., PINTO, J.E.B.P., ALVES, E., BIAGIOTTI, G., DEUNER, S., 2007: Estrutura foliar e de cloroplastídeos de *Mikania laevigata* Schultz Bip. ex Bakerem diferentes condições de qualidade de luz. Rev. Bras. Biocien. 5, 78-80.

TANG, H., YUAN, H.Y., YU, W.W., SONG, L.L., WU, J.S., 2015: Growth, photosynthetic and physiological responses of *Torreya grandis* seedlings to varied light environments. Trees. 29, 1011-1022. DOI: 10.1007/s00468-015-1180-9

TAPIERO, H., TEW, K.D., NGUYEN, B.G., MATHÉ, G., 2002: Polyphenols, do they play a role in the prevention of human pathologies? Biomed. Pharmacother. 56, 200-207. DOI: 10.1016/S0753-3322(02)00178-6

WALTER, H.M., STRACK, D., 2011: Carotenoids and their cleavage products: biosynthesis and functions. Nat. Prod. Rep. 28, 663-692.

WILHELM, C., SELMAR, D., 2011: Energy dissipation is an essential mechanism to sustain the viability of plants: The physiological limits of improved photosynthesis. J. Plant Physiol. 168, 79-87. DOI: 10.1016/j.jplph.2010.07.012

YE, S.J., 2009: Herb verification and authentication of Chinese holly leaf and gonglao leaf. J. Zhejiang Coll. Tradit. Chin. Med. 33, 431-432.

ZENG, X.Y., DONG, Y.L., SHENG, G.Y., DONG, X.C., SUN, X.H., FU, J.M., 2006: Isolation and structure determination of anti-influenza component from *Mahonia bealei*. J. Ethnopharmacol. 8, 317-319. DOI: 10.1016/j.jep.2006.05.014

ZEPKA, L.Q., MERCADANTE, A.Z., 2009: Degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. Food Chem. 117, 28-34. DOI: 10.1016/j.foodchem.2009.03.071

ZHAN, J., ZHOU, F.F., HE, F., LI, W., WANG, T., GONG, C.R., 2012: Study on carotenoids degradation and color changes of flue-cured tobacco at leaf-drying stage during bulk curing. J. Fujian Agr. For. Universit. 41, 122-127.

ZITTERL-EGLSEER, K., SOSA, S., JURENITSCH, J., SCHUBERT-ZSILAVECZ, M., DELLA LOGGIA, R., TUBARO, A., BERTOLDI, M., FRANZ, C., 1997: Anti-oedematous activities of the main triterpendiol esters of marigold (*Calendula officinalis* L.). J. Ethnopharmacol. 57, 139-144. DOI: 10.1016/S0378-8741(97)00061-5

Address of the corresponding author:
H. Wu, E-mail: wh@scau.edu.cn