A strategy for promoting lipid production in green microalgae Monoraphidium sp. QLY-1 by combined melatonin and photoinduction

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HIGHLIGHTS

• Lipid content of QLY-1 increased by 1.32-fold under photo-melatonin induction.
• Influence of melatonin on microalgal physiology was investigated.
• ROS and lipid biosynthesis-related enzyme were related to lipid accumulation.
• The strategy could be developed for efficient microalgal lipid production.

GRAPHICAL ABSTRACT

ABSTRACT

Microalgae lipids are potential candidates for the production of renewable biodiesel. The combination of plant hormones and two-step cultivation regulates lipid production in microalgae. A strategy for promoting lipid accumulation in Monoraphidium sp. QLY-1 by combining exogenous melatonin (MT) and photoinduction was developed. The effects of melatonin on the lipid content, reactive oxygen species (ROS), and activities of three key fatty acid biosynthetic enzyme in Monoraphidium sp. QLY-1 were investigated. The lipid content increased by 1.32-fold under 1 μM melatonin treatment. The maximum lipid content achieved was 49.6%. However, the protein and carbohydrate contents decreased rapidly from 57.21% to 47.96% and from 53.4% to 37.71%, respectively. Biochemical and physiological analyses suggested that the ROS and lipid biosynthesis-related enzyme activities correlated with increased lipid accumulation under photo-melatonin induction conditions.

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ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form 15 March 2017
Accepted 20 March 2017
Available online 22 March 2017

Keywords:
Lipid
Monoraphidium sp. QLY-1
Melatonin
Photoinduction

1. Introduction

Microalgae has attracted significant interest from researchers as a biodiesel feedstock due to their capacity to accumulate substantial amounts of biomass and lipids, fast growth rate, and economic and environmental benefits (Yu et al., 2012). Moreover, enhancement of microalgae lipid content has further strengthened the case for microalgae as a sustainable feedstock for biodiesel production, and could improve the economics of biodiesel production (Singh et al., 2016a). Microalgae, although cultivated through traditional methods, have also been cultivated under stress conditions, e.g., nutrient starvation, high salinity, and flashing light, to accumulate...
lipids (Singh et al., 2016b; Lawton et al., 2015; Abu-Ghosh et al., 2016). Recently, several strategies, including the combination of stress factors and addition of phytohormones and chemicals, were explored to overcome the challenges of conventional approaches, such as low yield, high cost of biomass production, single nutrient limitation and induction of stress by controlled cultivation, and then achieve maximum possible outcomes in terms of lipid content, (Yang et al., 2015; Jusoh et al., 2015; Wang et al., 2016; Singh et al., 2016a).

Lipid production can still be improved by customizing the conditions conducive for lipid biosynthesis. *Monoraphidium* sp. has been considered for lipid accumulation and is a promising feedstock for biodiesel (Yu et al., 2012; Holbrook et al., 2014; Yang et al., 2014). The growth and lipid profiles of *Monoraphidium* sp. vary greatly depending on the growth conditions and nutrients (Yu et al., 2012; Yang et al., 2014; Huang et al., 2014). Nevertheless, Che et al. (2016) and Zhao et al. (2016) investigated "combination of fulvic acid and two-step cultivation" and "heterotrophic cultivation and photo-chemical modulator induction" for higher biomass and lipid production in *Monoraphidium* sp., respectively. The heterotrophic microalgal cells transferred to the light environment, and the added phytohormones or chemical inducers were efficient for the lipid induction in microalgae. Lu and Xu (2015) summarized the implication of phytohormone manipulation for developing microalgae feedstock for biofuels. Therefore, exploring new plant hormones for lipid accumulation in microalgae under autotrophic condition is important.

Melatonin (N-acetyl-5-methoxytryptamine) (MT) is an important natural signal molecule during plant developmental processes and multiple biotic and abiotic stress responses (Shi et al., 2015a). This indoleamine has different functions in many aspects of plant growth, development of plants, and affects the physiology of microalgae (Zhang et al., 2015; Tal et al., 2015). It acts as a plant growth regulator and plays a role similar to that of indole-3-acetic acid (IAA) and salicylic acid (SA), i.e., promoting rooting, vegetative growth, and the differentiation of cells, tissues, and organs (Sarroú et al., 2015). MT is an antioxidant similar to butylated hydroxyanisole and glycine betaine (GB); it may accelerate lipid accumulation in microalgae under high light stress (Franz et al., 2013; Zhao et al., 2016). Nevertheless, no information regarding the application of MT to lipid production and biosynthesis-related mechanism in microalgae under photoinduction has been reported.

In this study, a novel lipid induction strategy combining melatonin and photoinduction was developed for lipid accumulation. The effect of melatonin on lipid production, reactive oxygen species (ROS), fatty acids composition, and several lipid biosynthesis-related enzyme activities in QLY-1 was investigated under photoinduction conditions. High lipid content was achieved, and correlation analysis was performed to determine the relationship between enzyme activities and lipid accumulation in QLY-1 under MT treatment. Our work provided valuable insights into the physiology and biotechnology of QLY-1; our results can be used in further manipulation of this microalgae for improved lipid production.

2. Materials and methods

2.1. Microalgae strain, culture conditions, and melatonin treatment

The *Monoraphidium* sp. QLY-1 used in this paper was obtained from Biorefinery Laboratory of Faculty of Life Science and Technology, Kunming University of Science and Technology (Kunming, China) (Zhao et al., 2016). The culture medium BG11 was reported by Yu et al. (2012). The seed cells were cultured in flasks containing the above medium under 30 μmol m⁻² s⁻¹ light intensity at 25 ± 1 °C and 150 r min⁻¹ for 15 days. For heterotrophic growth, 500 mL of seed culture was inoculated into a 10-L fermentor with 5 L BG11 medium and 10 g L⁻¹ glucose at 25 ± 1 °C. MT (purity, ≥98.0%) was purchased from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China).

The heterothrophic algal cells were collected through centrifugation (5804R, Eppendorf) and re-suspended at a density of ca. 1 g L⁻¹ in a 500 mL Erlenmeyer flask containing 300 mL of fresh medium under 100 μmol m⁻² s⁻¹ light intensity. Besides, different concentrations of MT (0, 1, 10, and 100 μM) were applied for lipid induction and each treatment was conducted in triplicate. The samples were harvested within a 2 day interval.

2.2. Measurement of cell growth and lipid content

Fresh algal cells were harvested through centrifugation. The dry weight of the algal biomass was estimated after freeze drying for 12 h. The algal powders were stored at −20 °C for total lipid analysis.

Weighed dried samples were subjected to total lipid extraction by using the procedures described by Bligh and Dyer (1959). Specific operational steps were recommended by Zhao et al. (2016). The sample was ground into fine powder. Briefly, 300 mg to 500 mg of the powder (w₁) was blended with 3 mL of chloroform/methanol (2:1, v:v). The mixture was agitated for 20 min in an orbital shaker (TS-2102GZ, TENSEUC) at 150 rpm and room temperature. The solvent phase was recovered through centrifugation at 2000 rpm for 10 min. The pellet was re-extracted in 3 mL of chloroform/methanol solution twice. The total lipid was transferred into a pre-weighed 50 mL centrifuge tube (w₂), dried to constant weight at 40 °C, and then weighed (w₃). Lipid content (LC, %) was calculated as follows:

\[
\text{Lipid content (\%)} = \left(\frac{w_3 - w_2}{w_1}\right) \times 100
\]

Lipids were fractioned into neutral lipids, glycolipids, and phospholipids on a column (25 mm × 550 mm) of silicic acid (30 g) by sequential elution with chloroform, acetone, and methanol, respectively (Zhao et al., 2016).

2.3. Biomolecular analysis

To analyze the cellular constituents, i.e., chlorophyll a (Chl a), proteins, and carbohydrates, 5 mL of sample cells was harvested by centrifugation during the induction phase. Samples were then pulverized using a mortar and pestle. The amount of Chl a was measured spectrophotometrically (SmartSpec Plus, Bio-Rad), the absorbance of extracts was read at 663 and 645 nm, as described by Wellburn (1994). Protein was analyzed following the Bradford assay method, using bovine serum albumin as standard (Berges et al., 1993). Carbohydrates were quantified by using the method of Jia et al. (2015) with glucose as standard.

2.4. MT concentration measurement

MT concentration was determined using a high-performance liquid chromatography (HPLC) apparatus with a photodiode array detector (Waters 996, USA) and a reverse-phase C18 column (15 cm × 4 mm, 5 μm particle size, Waters) by comparing with authentic standards (Sigma) at known concentrations. Samples of 20 μL microdialysis perfusate were injected into the HPLC system. MT was eluted with a mobile phase at a flow rate of 1 mL/min. The mobile phase contained methanol and 0.2% acetic acid (1:1, v:v). The temperature of the column heater was kept at 30 °C. MT was detected at 277 nm against standards to calculate the concentration.
2.5. Measurement of ROS

The intracellular ROS levels were monitored as previously described (Che et al., 2017). 2,7'-Dichlorodihydrofluorescein diacetate (DCFH-DA; Beyotime, China) was used as a probe for ROS measurement. DCFH-DA crosses cell membranes and is hydrolyzed enzymatically by intracellular esterase to nonfluorescent DCFH. To detect intracellular ROS after induction by MT at high light intensity, 10 μM (final concentration) of DCFH-DA was added to the cultures and incubated in a shaker at 37 °C in the dark for 30 min. Then, the cells were washed thrice with the same buffer. The fluorescence of the samples was measured with a fluorospectrophotometer (Shimadzu RF-540, Japan) with an excitation wavelength of 488 nm and an emission band between 500 and 600 nm. The average fluorescence density of the intracellular areas was measured to indicate the ROS level.

2.6. Determination of enzymatic activities

Centrifugation (12,000 rpm, 5 min at 4 °C) was performed to harvest 5 mL of the fresh culture. The cell pellet was washed twice, frozen with liquid nitrogen, and ground with a mortar and pestle. Enzyme activity was determined subsequently. Malic enzyme (ME), acetyl-CoA carboxylase (ACCase), and phosphoenolpyruvate carboxylase activity of microalgae were analyzed with colorimetric quantitative detection kit (Suzhou Comin Biotechnology Co., Ltd., Suzhou, China) according to the manufacturer’s instructions (Xue et al., 2015; Ma et al., 2016). One unit (U) of enzyme activity is defined as the amount of enzyme catalyzing the formation/consumption of 1 nM of each enzymatic reaction product/substrate per minute or hour under the aforementioned conditions.

2.7. Transesterification and fatty acid composition analysis

The extracted lipids were added with 2 mL of 3% H₂SO₄ in a methanol solution to prepare fatty acid methyl ester. The mixture was heated at 70 °C for 4 h in a water bath. After methylation, 2 mL of n-hexane was added into a vial and was left to stand for 4 h. The top n-hexane layer was then removed and placed into another vial for gas chromatography/mass spectrometry analysis. The detailed method of determination of fatty acid composition was described by Zhao et al. (2016).

![Fig. 1. Effects of MT on the biomass (A) and lipid content (B) of Monoraphidium sp. QLY-1 during photoinduction days. The error bars indicate the standard deviations from three independent samples. d, days. *Indicates statistical significance (P < 0.05); **indicates statistical significance (P < 0.01) compared with the control.](image)
2.8. Statistical analysis

Mean ± standard deviation was derived from all data and statistically analyzed using one-way ANOVA (SPSS 19.0). A least-significant difference–multiple comparison test was performed to investigate the differences among groups of different trials. P values less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Cell growth and lipid content of Monoraphidium sp. QLY-1 under MT induction

Numerous studies have investigated the effects of MT on plant growth (Hernández et al., 2015; Li et al., 2016). However, few studies have focused on the influence of MT on photoinduction conditions to investigate the extent and mechanism of lipid accumulation by microalgae. As shown in Fig. 1A, the biomass concentration of MT-treated cells was slightly lower than that of the control during induction day 3. This phenomenon might be due to the direct transformation of heterotrophic microalgae cells into the photoinduction conditions, and the sole energy source was insufficient for cell growth (Fan et al., 2012; Zhao et al., 2016). However, no significant difference was noted between cells grown with MT addition and that of the control during the induction phase. The results are similar to those obtained in a previous study. The addition of MT did not affect Chlamydomonas reinhardtii cell growth (Tal et al., 2015). In addition, Zhao et al. (2016) explained that the biomass concentration of the glycine betaine-treated cells did not significantly decrease or increase.

The lipid content increased sharply with the increase of MT from 0 μM to 100 μM concentration during the first induction day, and a higher lipid content (46.13%) was obtained in treatment with 100 μM MT. Furthermore, the 1, 10, and 100 μM MT-treated samples exhibited a higher lipid content (i.e., 1.32-, 1.24-, and 1.16-fold, respectively) than the control on day 3. The highest lipid content (49.6%) was obtained in the 1 μM MT induction (Fig. 1B). This lipid content was higher than most values reported in the related literature (Bogen et al., 2013; Holbrook et al., 2014; Shrivastav et al., 2015; Jiang et al., 2016).

Lipid content is upregulated by exogenous MT treatment. However, the detailed molecular mechanism of MT induction in microalgae cells remains unclear. Plants treated with 1 μM MT yielded the highest essential oil content with 0.46% compared with the control plants (0.39%) (Sarrou et al., 2015). Wei et al. (2015) showed that melatonin application during germination significantly promotes soybean growth and seed production and enhances soybean tolerance to salt stress by modifying the oxidoreductase activity/process and secondary metabolic processes. MT may also affect gene expressions of HSP70B, FeSOD, and MnSOD via positive involvement in oxidative stress protection and carotenoids accumulation (Tal et al., 2015). Moreover, Shi...
et al. (2015a) and Qian et al. (2015) showed that MT as a messenger enhanced endogenous salicylic acid (SA) and NO level with great efficacy against bacteria in plant. Several studies have shown that SA was an inducer for the growth and metabolite production in several microalgae species (Czerpak et al., 2002; Raman and Ravi, 2011; Li et al., 2015).

Fig. 3. Effect of 1 μM MT on biochemical composition of Monoraphidium sp. QLY-1 during photoinduction days. Chl a (A), protein (B), carbohydrate (C). *Indicates statistical significance ($P < 0.05$); **indicates statistical significance ($P < 0.01$) compared with the control.
After the enhancement of total lipid content, lipid classes, especially neutral lipid content, should be considered. Lipid classes under 1 μM MT-treated cells showed that the NL content was higher than in the control (Fig. 2A). The contents of NL and PL increased by 28.67% and 9.08%, which was in line with the decreased GL (26.35%). The increase in NL may be mainly attributed to the transformation of GL in QLY-1 with MT treatment (Yang et al., 2014; Zhao et al., 2016).

In this present study, the highest lipid content was obtained after treatment with 1 μM MT. At a low dosage, elicitor interaction sites in cells were speculated to be insufficiently utilized to activate the secondary metabolite synthesis, whereas a high dosage may cause cytotoxic effects (Liang et al., 2010; Zhao et al., 2015). To confirm the uptake of MT by algal cells treated in 1 μM MT for 7 days, the consumption levels of MT were measured. Fig. 2B shows that the MT concentration decreased sharply during the first 3 days, which was accompanied by the initial fast lipid accumulation. We deduced that high levels of lipid accumulation may be associated with MT consumption under photoinduction. In addition, chlorophyll, protein, and carbohydrate are also the main microalgae components, and their contents may be changed and converted to lipids under photo-MT induction (Che et al., 2017). However, the regulatory and physiological roles of MT in lipid biosynthesis remain unclear.

3.2. Physiological response to MT

The physiological responses of Monoraphidium sp. QLY-1 under 1 μM MT are shown in Fig. 3. The chlorophyll content had a very similar trend compared with cell biomass (Fig. 3A). The Chl a content was not significantly unregulated in response to 1 μM MT treatment, and the highest Chl a content (20.22 μg ml⁻¹) occurred on the last induction day. This result is in agreement with that obtained in several studies, which reported that 1 mM MT did not induce a significant effect on chlorophyll content relative to the control cells (Tal et al., 2015). However, MT has been observed to preserve chlorophyll and improve the photosynthetic efficiency of chloroplasts in plants under stress (Tan et al., 2012). This phenomenon may be relevant to different species and different concentrations of MT.

QLY-1 cells synthesized protein in a steady state on the first 3 days under MT treatment. The highest protein content was 58.06% on day 3. The MT-treated algal cells increased to 11.06% compared with the control (Fig. 3B). Under photoinduction conditions, protein content dropped to 47% on day 7 in response to MT. Moreover, the protein content decreased from 52.28% to 42.41% in the control sample. These results were consistent with several studies. Fan et al. (2012) reported that heterotrophic Chlorocella cells were diluted and transferred to autotrophic condition; the protein content increased rapidly after several hours and reached approximately 55% after 48 h. Under stress conditions, high light and nitrogen starvation led to a sharp decrease in protein level of Nanochloropsis oceanaica (Ma et al., 2016). The protein content might be related to irradiance, nitrogen starvation, and carbon reallocation of carbohydrates, lipids, and proteins in response to MT induction (Jia et al., 2015; Ma et al., 2016; Che et al., 2017).

Fig. 3C showed that the carbohydrate content decreased sharply under photoinduction condition during the first 3 days. These data were similar to the study of Fan et al. (2012), which reported carbohydrate content was reduced by nearly 30% throughout the whole photoinduction process. The carbohydrate content in 1 μM MT-treated cells was higher than that of control, and MT was assumed to be tolerant. However, its content was also reduced rapidly from 53.4% to 39.56% (Fig. 3C), and the carbohydrates were probably converted to lipids during high light induction day 3 (Fig. 1B). Furthermore, Shi et al. (2015b) indicated exogenous melatonin involved major reorientation of photosynthetic and carbohydrate and nitrogen metabolism in Bermuda grass under abiotic stress. Thus, the levels of carbohydrate in QLY-1 might be related to a complex metabolic process by combined melatonin and high light induction.

3.3. ROS involvement in the lipid accumulation process

Fig. 4 illustrates the intracellular ROS during photoinduction with 1 μM MT treatment. The results showed that the level of ROS increased in cells exposed to light stress. The ROS content of the control and experimental group were enhanced by 1.17- and 1.15-fold on day 3, respectively. The highest level of ROS in MT-treated cells decreased by 19.26% compared with that of the control. However, the ROS content increased with the MT consumption (Figs. 2 and 4). These data indicated that the extraneous MT was an efficient antioxidant against the toxic ROS. A previous study demonstrated that MT had the greatest impact on C. reinhardtii under conditions promoting photo-oxidative stress (Tal et al., 2015). Szafranska et al. (2016) also explained that single melatonin application into the seeds during presowing priming improved oxidative stress tolerance of growing seedlings exposed to paraquat. Furthermore, most antioxidants are secondary metabolites and can considerably reduce intracellular ROS levels. Such antioxidants are present in Haematococcus pluvialis, which can produce lipid triacylglycerol and carotenoids under photo-oxidative stress conditions (Gwak et al., 2014). The results were consistent with the analysis of the lipid content in QLY-1 (Fig. 1B). However, the specific protective mechanism of MT as an antioxidant that protects against ROS and upregulates lipid accumulation in QLY-1 under photoinduction requires further research.

3.4. Biochemical activities analysis of lipid biosynthetic enzymes

Studying the relationship between the regulation of lipid biosynthesis enzymes and lipid accumulation can increase our understanding of the underlying lipid mechanism in QLY-1. In our study, the activity levels of three lipid biosynthetic enzymes were analyzed between the control and 1 μM MT samples over 7 days (Fig. 5).

ACCase is considered the key enzyme in the fatty acid biosynthesis pathway, which is in the upstream of triacylglycerol. This enzyme, a single polypeptide with multi-functional domains in
eukaryotes, converts acetyl-CoA into malonyl-CoA (Chang et al., 2016). Notably, ACCase increased 30.25% and 24.33% under MT treatment, more than that of controls on days 1 and 3, respectively (Fig. 5A). The lipid content of MT treatments increased significantly from day 1 in this study (Fig. 1B), and the results showed that ACCase preceded the initial fast lipid accumulation. Che et al.
(2017) observed a significant improvement in ACCase activity on the whole phase under fulvic acid (FA) induction. The highest lipid content occurred in high light and nitrogen deficiency, and the ACCase activity increased threefold compared with that of the control (Ma et al., 2016).

ME is involved in pyruvate metabolism and carbon fixation, which catalyzes the irreversible oxidative decarboxylation of malate to pyruvate, thereby yielding pyruvate, NADH, and CO₂ (Vongsangnak et al., 2012). The activity of ME significantly increased under photo-MT induction (Fig. 5B) and increased by 1.37- and 1.3-fold in MT-treated cells compared with the controls on days 1 and 3, respectively. Previous studies suggested that high levels of lipid accumulation in microorganisms and microalgae were caused by ME overexpression. Such overexpression in Rhodotorula glutinis significantly enhanced ME enzymatic activity and lipid content, and mitochondrial ME also had a significant impact on its enzymatic activity and lipid accumulation in Phaeodactylum tricornutum (Li et al., 2013; Xue et al., 2015). Therefore, the lipid accumulation of QLY-1 under photo-MT induction may be associated with ME activity.

Oxaloacetate, an intermediate of the TCA cycle, can flux out of the cycle using the decarboxylation enzyme phosphoenolpyruvate carboxylase (PEPC), thereby decreasing the carbon flow directed to fatty acid biosynthesis (Yang et al., 2016). Fig. 5C showed that PEPC carboxylase (PEPC), thereby decreasing the carbon flow directed to may be associated with ME activity.

ME overexpression in Phaeodactylum tricornutum levels of lipid accumulation in microorganisms and microalgae were caused by ME overexpression. Such overexpression in Rhodotorula glutinis significantly enhanced ME enzymatic activity and lipid accumulation in Phaeodactylum tricornutum (Li et al., 2013; Xue et al., 2015). Therefore, the lipid accumulation of QLY-1 under photo-MT induction may be associated with ME activity.

Oxaloacetate, an intermediate of the TCA cycle, can flux out of the cycle using the decarboxylation enzyme phosphoenolpyruvate carboxylase (PEPC), thereby decreasing the carbon flow directed to fatty acid biosynthesis (Yang et al., 2016). Fig. 5C showed that PEPC level during MT treatment decreased during the photoinduction phase. Although the enzyme activity increased on day 3, it was still significantly lower than in the control. Similar to a previous research, knockdown of PEPC in combination with nitrogen starvation significantly enhanced lipid accumulation to a significant extent (Yang et al., 2016). In addition, Che et al. (2017) also suggested that higher lipid accumulation with the PEPC activity decreased under FA treatment in Monoraphidium sp. FXY-10. The relative contribution of individual lipid biosynthetic enzymes at the activity level to the overall lipid production remains unknown, and the high level of lipid accumulation may also be correlated with carbon fixation and lipid biosynthetic genes under stress.

| Table 1 |
|-----------------|-----------------|
| Fatty acids     | Control         | 1 μM MT         |
| C16:0           | 0.16 ± 0.01     | 0.1 ± 0.06      |
| C17:0           | 0.86 ± 0.03     | 0.82 ± 0.04     |
| C18:0           | 0.12 ± 0.01     | 0.09 ± 0.01     |
| C16:1           | 38.4 ± 2.54     | 38.19 ± 1.24    |
| C17:0           | 1.18 ± 0.06     | 1.23 ± 0.02     |
| C18:0           | 0.22 ± 0.01     | 0.25 ± 0.01     |
| C18:1           | 2.81 ± 0.14     | 2.18 ± 0.72     |
| C18:0           | 17.89 ± 0.76    | 19.81 ± 0.92    |
| C18:2           | 14.31 ± 0.56    | 16.28 ± 0.67    |
| C18:3           | 17.95 ± 0.84    | 14.83 ± 1.2     |
| C18:4           | 1.62 ± 0.02     | 1.75 ± 0.03     |
| C20:0           | Nd              | 0.15 ± 0.01     |
| C20:1           | 0.07 ± 0.01     | 0.183 ± 0.07    |
| C20:3           | 0.06 ± 0.01     | Nd              |
| C20:5           | 0.17 ± 0.01     | 0.079 ± 0.01    |
| C22:0           | 0.36 ± 0.03     | 0.3955 ± 0.04   |
| C22:1           | 0.65 ± 0.06     | 0.7215 ± 0.03   |
| C24:0           | 2.82 ± 0.19     | 2.695 ± 0.35    |
| C24:1           | 0.35 ± 0.04     | 0.3955 ± 0.02   |
| SFA             | 45.75 ± 2.56    | 44.768 ± 1.06   |
| MUFA            | 20.13 ± 1.22    | 22.345 ± 0.92   |
| PUFA            | 34.11 ± 2.17    | 32.905 ± 1.96   |
| DU              | 88.36 ± 3.23    | 88.155 ± 2.99   |

Nd = below the limit of detection.

References


Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant Nos. 21266013 and 21666012), The Natural Science Foundation of Yunnan Province, China (Grant No. 2010CD028).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.03.114.
Terrestrial plants, endogenously, accumulate melatonin in response to environmental cues. Exogenous melatonin influences the development and physiology of plants, acting as a powerful regulator of plant growth and development.


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