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Original Article

Effects of 5-aminolevulinic Acid on the Photosynthesis, Antioxidant System, and α-Bisabolol Content of *Matricaria recutita*

Xiaomeng LIU[1], Li ZHU[1], Qiling SONG[1], Jie CHANG[2,3], Jiabao YE[1], Weiwei ZHANG[1], Yongling LIAO[1], Feng XU[1]*

[1]*Yangtze University, College of Horticulture and Gardening, Jingzhou 434025, Hubei, China; LiuXM925@163.com; zhuli322@163.com; song70@126.com; yejiabao13@163.com; wwzhangchn@163.com; liaoyongling@yeah.net; xufeng198@126.com (*corresponding author)*
[2]*Hubei Collaborative Innovation Center of Targeted Antitumor Drug, Jingmen 448000, Hubei, China; 18986662379@163.com*
[3]*Jingchu University of Technology, College of Chemical Engineering and Pharmacy, Jingmen, 448000, Hubei, China*

Abstract

Matricaria recutita is a widely used medicinal plant with broad pharmacological effects, and α-bisabolol is the main active ingredient of this plant. To improve its α-bisabolol content, *M. recutita* was sprayed with different concentrations (1.0, 2.0, and 4.0 mmol·L^{-1}) of 5-aminolevulinic acid (ALA) or with water as a control to study the effects of ALA treatment on the photosynthesis, antioxidant system, and α-bisabolol content of *M. recutita*. Results showed that the photosynthetic rate, transpiration rate, stomatal conductance, intercellular CO_2 concentration, soluble protein, total amino acids, soluble sugar, and α-bisabolol of *M. recutita* were significantly increased. Moreover, the activities of superoxide dismutase, peroxidase, and catalase of *M. recutita* were also enhanced by ALA treatment. Optimal results were obtained when the concentration of ALA was 2.0 mmol·L^{-1}. Results showed that ALA treatment could improve the α-bisabolol content of *M. recutita*, and the underlying physiological mechanism was analyzed. ALA treatment was an effective measure for improving the medicinal value of *M. recutita*.

Keywords: antioxidant system; α-bisabolol; 5-aminolevulinic acid; *Matricaria recutita*; photosynthesis

Introduction

Chamomile (*Matricaria recutita* L.) is an annual or perennial herb of Chrysanthemum, which is native to Europe (Farhoudi *et al*., 2013).The whole plant of *M. recutita* is aromatic and rich in essential oil, and this plant exhibits various effects such as anti-inflammatory, antioxidant, antibacterial, anti-inflammatory, spasm relief, and analgesic (Srivastava *et al*., 2010). Thus, this medicinal plant is remarkably developed and has high utilization value. *M. recutita* is widely used as additive to spices, cosmetics, medicines, and other products (Ma *et al*., 2007). The main active ingredients of the essential oil of *M. recutita* are terpene compounds, of which α-bisabolol is the most important terpenoid (Raal *et al*., 2012). Therefore, improving α-bisabolol content in *M. recutita* has become an important concern in the cultivation of this plant.

5-Aminolevulinic acid (ALA) is a non-protein amino acid widely found in the living cells of bacteria, fungi, animals, and plants (Xu *et al*., 2015). ALA is a key precursor of many tetrapyrrols, such as including porphyrins for chlorophyll and haeme biosynthesis (Naeem *et al*., 2011). Moreover, ALA has great potential in agriculture as a new type of plant growth regulator (Watanabe *et al*., 2000). High dosage of ALA has been used as a biodegradable photodynamic herbicide in agricultural production (Duke *et al*., 1994). Meanwhile, low concentration of exogenous ALA has many physiological effects on plant growth regulation, such as the enhancement of yields of some plants (Hotta *et al*., 1997a). Hotta *et al*. (1997b) showed that low concentrations of ALA can improve plant photosynthesis and increase plant growth. Moreover, numerous studies have shown that low ALA concentration can promote salt resistance in cotton seedlings (Liu *et al*., 2011), cold tolerance in rice (Hotta *et al*., 1998), and herbicide tolerance in oilseed (Zhang *et al*., 2008). Under low light and cold environment, ALA can also improve chlorophyll content, gas exchange ability, and photosynthesis rate (*P*n) of melon seedling leaf (Wang *et al*., 2004). Low concentration of ALA could accelerate the production of reactive oxygen species, such as H_2O_2, and increase the

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activity of key antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and dehydroascorbate reductase (DHAR) (Xu *et al*., 2009). In addition, our previous work also showed that exogenous ALA could increase the biosynthesis of anthocyanin in peach skin (Ye *et al*., 2017). Therefore, there is reason to believe that ALA can promote plant secondary metabolites, such as the synthesis of flavonoids and terpenoid compounds. However, the application of ALA in *M. recutita* has not been reported yet. In this study, the effects of ALA on the growth, photosynthesis, antioxidant system, and α-bisabolol content of *M. recutita* were investigated by spraying *M. recutita* with different concentrations of exogenous ALA solutions. Theoretical and experimental bases for improving the medicinal value of *M. recutita* were provided.

Materials and Methods

Plant materials and treatments

The experiment was conducted in the glass greenhouse of Yangtze University from November 2014 to May 2015. The test material is the seed of *M. recutita* sown in a nutrition bowl (20 × 20 cm) after immersion and germination on November 6, 2014. The culture medium was vermiculite, coconut shell powder and pearl, uniformly mixed with the ratio of 1: 1: 1 and placed in the greenhouse for seedling cultivation. The day/night light cycle was 16 h/8 h, temperature was 24 ℃/18 ℃, and relative humidity was 70%. Two weeks after seed germination, *M. recutita* was planted in a nutrition bowl, and three strains of *M. recutita* were planted in each pot, for a total of 50 pots.

Then, 15 days after transplantation, 36 pots of morphologically uniform *M. recutita* were selected and foliary sprayed with aqueous solution of ALA at 1.0, 2.0, and 4.0 mmol·L^{-1}. In the control treatment, distilled water was used instead of ALA. Each treatment was repeated thrice, and each replicate involved three pots. ALA is easily decomposed under irradiation. Thus, foliar spraying with a handheld atomizer was performed at night when the light was weak. The plants were treated once every two weeks, for a total of 8 sprays. During this period, the cultivation and management measures were undertaken to maintain consistency until *M. recutita* blossomed. Then, the whole plants were marked, frozen (roots were cleaned, and surface water was drained with filter paper), and stored in liquid nitrogen for subsequent determination of fresh weight and physiological characteristics. Three technical replicates were used.

Determination of growth and physiology characteristics

Fresh weight and dry weight (ground and underground), which were expressed in milligrams, were measured separately after *M. recutita* blossom. After sample collection, the roots were cleaned, and moisture on the surface was drained with a filter paper. The samples were immediately weighed and then placed in a hot-air oven at 80 °C until weighed at constant weight.

Photosynthetic parameters, namely, intercellular CO_2 concentration (*Ci*), stomatal conductance (*Gs*), photosynthetic rate (*Pn*), and transpiration rate (*Tr*), were measured using the Li6400 portable photosynthesis system on the fifth leaf of each *M. recutita* from top to bottom. Given that the leaf area of *M. recutita* was less than 6 cm^2, a leaf area meter should be used to measure the leaf area of the collected fresh leaves.

Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were determined according to the method of Wellburn (1994). Chlorophyll content [mg/g FW (fresh weight)] was calculated at 665 and 649 nm by spectrophotometer (UV-2800, Unico, GER).

Soluble sugar content was determined using anthrone colorimetric method (Moustakas *et al*., 2011). Fresh tissue samples (5 g) were collected and ground into a homogeneous slurry. Then, the ground samples were extracted with 6 ml of sulfosalicylic acid for 2h, filtered by a funnel, and centrifuged at 4,000×*g* for 5 min. The supernatant after centrifugation was used to calculate the soluble sugar content [mg/g FW (fresh weight)] at a wavelength of 630 nm by a spectrophotometer.

Soluble protein contents [mg/g FW (fresh weight)] were determined according to the specification of a protein quantification kit.

The activities of antioxidant enzymes, namely SOD, CAT, POD, APX, were determined according to the instructions of corresponding kits.

α-Bisabolol in of *M. recutita* was determined according to the method of Irmisch *et al*. (2012). Essential oil from the sample of the test tissue was extracted and analyzed by gas chromatography-mass spectrometry (GC-MS, Agilent 6890N gas chromatography system and Agilent 5975B mass spectrometer) to determine the α-bisabolol content.

Statistical analysis

Data are presented as mean value of each treatment. The results represent the means ± standard error with each experiment performed in triplicate. Data were analyzed with one-way ANOVA using SPSS 10.0 for Windows (SPSS Inc., IL, USA), and differences in the treatment means were compared with Duncan's multiple range test at the $P \leq 0.05$ level.

Results

Effects of ALA treatments on growth

Compared with the control group, ALA treatments at 1.0, 2.0, and 4.0 mmol·L^{-1} resulted in corresponding 23.0%, 27.0% and 23.3% improvement in the dry weight of aboveground, underground, and the total dry weight of *M. recutita* (Fig. 1). Thus, 2.0 mmol·L^{-1} ALA provided the best results.

Effects of ALA treatments on photosynthesis and chlorophyll content

1.0, 2.0 and 4.0 mmol·L^{-1} concentrations of ALA could increase the photosynthetic parameters (*Pn*, *Tr*, *Ci*, and *Gs*) of *M. recutita* leaves to different extents (Table 1). Treatment using 2.0 mmol·L^{-1} ALA resulted in the best values for *Pn*, *Tr*, and *Gs* at 38%, 27%, and 38% higher than the control group, respectively. *Ci* values at 2.0 mmol·L^{-1} and

4.0 mmol·L^{-1} ALA treatment were significantly higher than those of the control by 30% and 34%, respectively. These results indicated that photosynthesis was significantly improved by spraying ALA on the leaf of *M. recutita*.

The chlorophyll *a*, chlorophyll *b*, and chlorophyll (*a* + *b*) were significantly higher in the leaves of *M. recutita* sprayed with exogenous AIA than in the control (Fig. 2). ALA at 2.0 mmol·L^{-1} showed the best effect, resulting in

Table 1. Gas exchange parameters of *M. recutita* leaves sprayed with different concentrations of ALA

ALA/ (mmol·L^{-1})	P_n (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	T_r (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	C_i (μmol $CO_2 \cdot mol^{-1}$)	G_s (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)
0	9.04+0.25 b	8.73+0.18 c	233.74+10.18 c	0.429+0.014 b
1	12.25+0.43 a	8.36+0.26 c	271.14+13.24 b	0.532+0.015 a
2	12.46+0.23 a	11.09+0.35 a	303.86+18.27 a	0.591 +0.021a
4	12.32+0.57 a	10.01+0.48 b	313.09+11.93 a	0.537+0.014 a

Each value represents the mean of three replicates of each treatment, and the different normal letters in the same columns mean significant differences at $P < 0.05$.

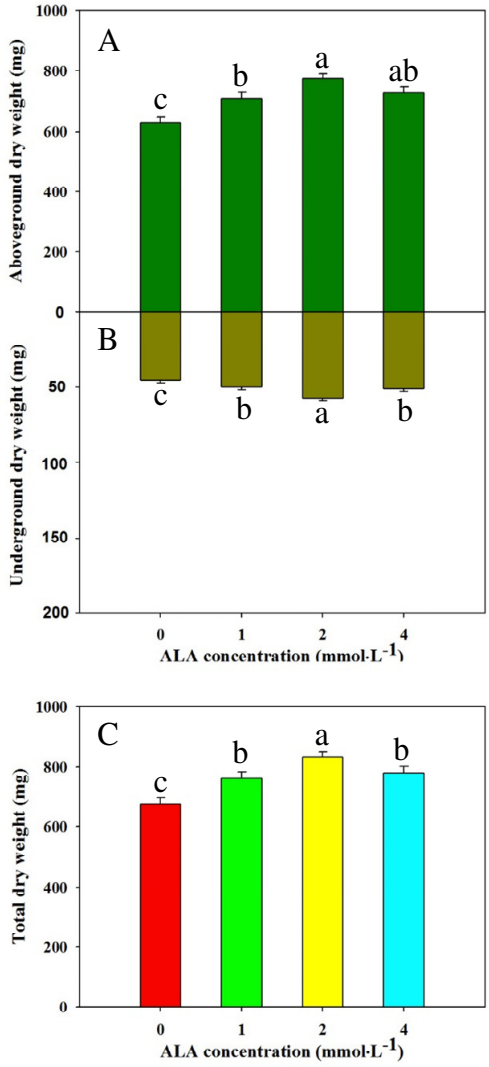

Fig. 1. Dry weight of *M. recutita* treated with different concentrations of 5-aminolevulinic acid (ALA). A, B, and C represent the aboveground dry weight, underground dry weight and total dry weight of *M. recutita*, respectively. Each value represents the mean of three replicates of each treatment, and different normal letters in the same columns indicate significant differences at $P < 0.05$

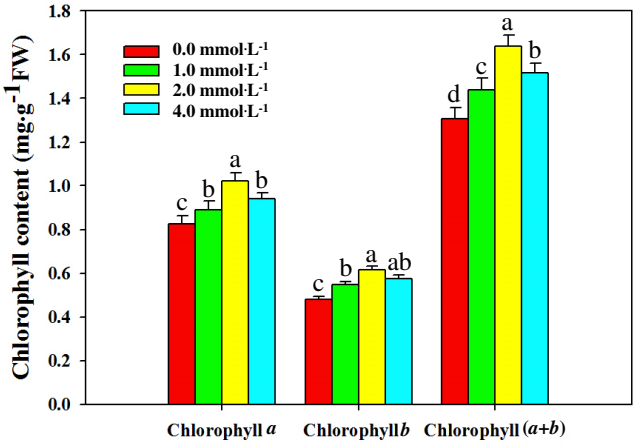

Fig. 2. Chlorophyll content of leaves of *M. recutita* sprayed with different concentrations of ALA. Each value represents the mean of three replicates of each treatment, and different normal letters in the same columns indicate significant differences at $P < 0.05$

24%, 28%, and 32.1% higher chlorophyll *a*, chlorophyll *b* and chlorophyll (*a* + *b*) contents, respectively, compared with those of the control group.

Effects of ALA treatments on photosynthetic products

The contents of photosynthetic products (soluble protein, total amino acids, and soluble sugar) of chamomile under ALA treatments were significantly higher than those of control (Fig. 3). Treatment with 2.0 mmol·L^{-1} ALA resulted in the highest levels for soluble protein, total amino acids, and soluble sugar, which were 19%, 39%, and 33% higher than that of the control group, respectively. The results indicated that ALA had a significant effect on carbohydrate accumulation.

Effects of ALA treatments on antioxidant enzymatic activities

The activities of SOD, POD, and CAT in *M. recutita* treated with 1.0, 2.0, and 4.0 mmol·L^{-1} ALA were significantly higher than those in control group (Fig. 4). The

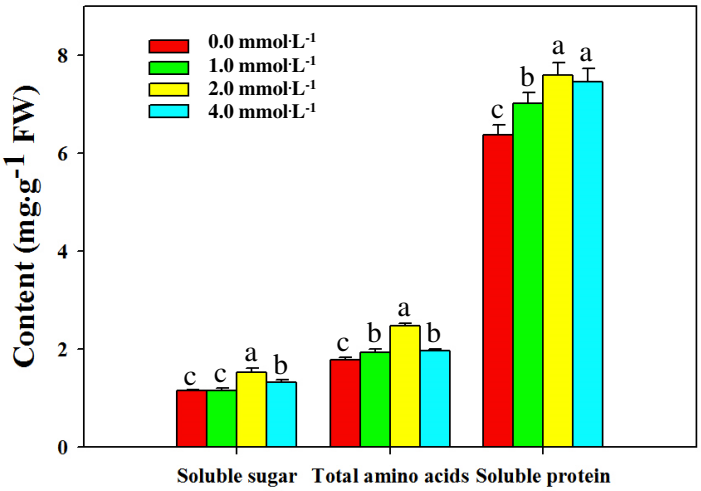

Fig. 3. Effects of ALA treatment on soluble protein, total amino acid, and soluble sugar content of *M. recutita*. Each value represents the mean of three replicates of each treatment, and different normal letters in the same columns indicate significant differences at $P< 0.05$

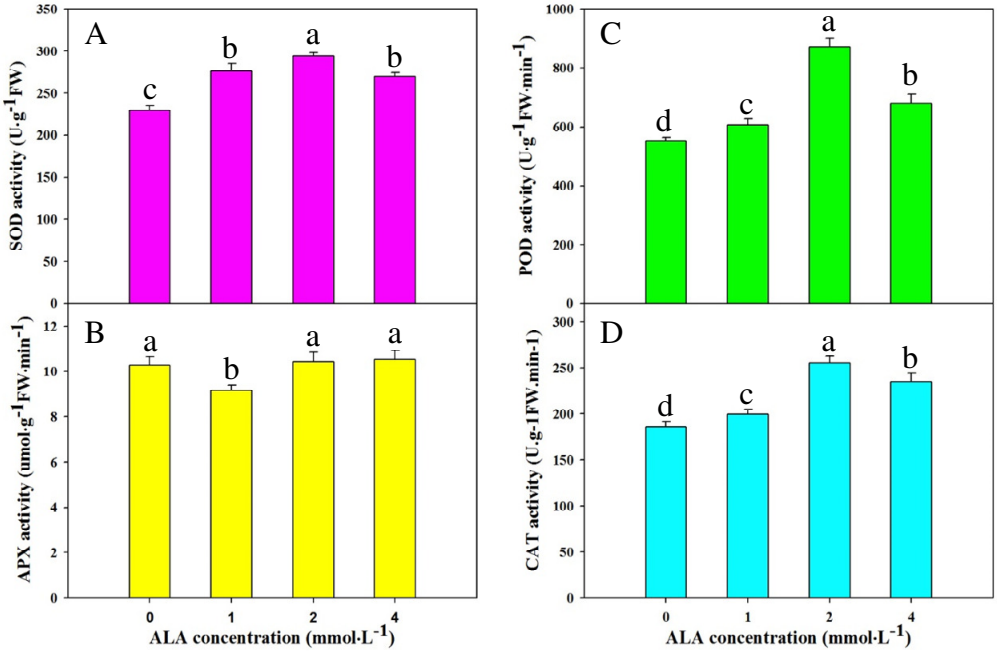

Fig. 4. Activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) of *M. recutita* with ALA treatment. A, B, C and D represent the activity of CAT, SOD, APX and POD enzyme of *M. recutita* under the treatments with different ALA concentrations, respectively. Each value represents the mean of three replicates of each treatment, and the different normal letters in the same columns indicate significant differences at $P< 0.05$

highest activity was obtained at 2.0 mmol·L^{-1} ALA treatment with corresponding values 58%, 28%, and 37% higher than in the control. ALA treatment had no effect on APX activity, but APX was significantly lower than that of the control at 2.0 mmol·L^{-1} ALA.

Effects of ALA Treatment on α-Bisabolol Content

The content of α-bisabolol in *M. recutita* treated with different concentrations of ALA was significantly higher than that of control (Fig. 5). Treatment with 2.0 mmol·L^{-1} ALA yielded the highest value, which was 21% higher than the control. This result indicates that ALA can promote the biosynthesis of α-bisabolol.

Discussion

This experiment is the first to verify that exogenous ALA treatment can significantly improve the growth, photosynthesis, antioxidant system, and the α-bisabolol of *M. recutita* (Fig. 6). These findings are consistent with those obtained by Akram *et al.* (2012) in sunflower and Ali *et al.* (2013) in *Brassica napus*. Photosynthetic pigments are the

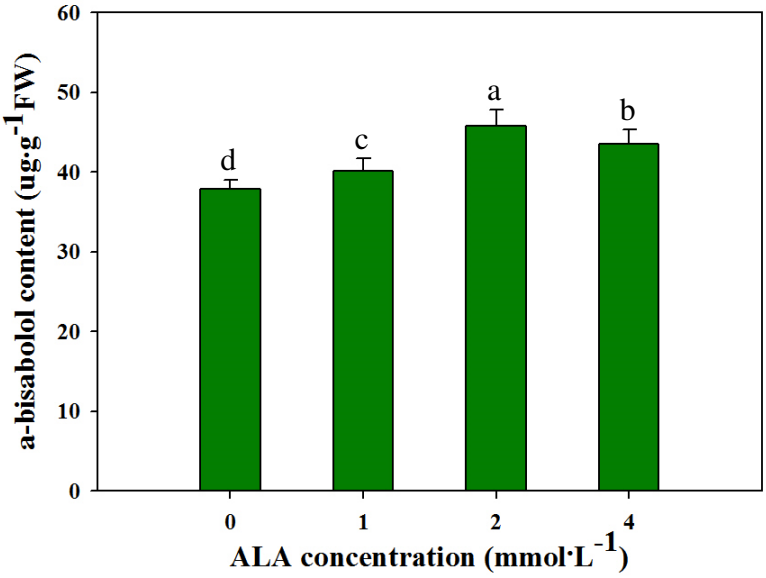

Fig. 5. Effects of ALA treatment on α-bisabolol content of *M. recutita*. Each value represents the mean of three replicates of each treatment, and different normal letters in the same columns indicate significant differences at $P < 0.05$

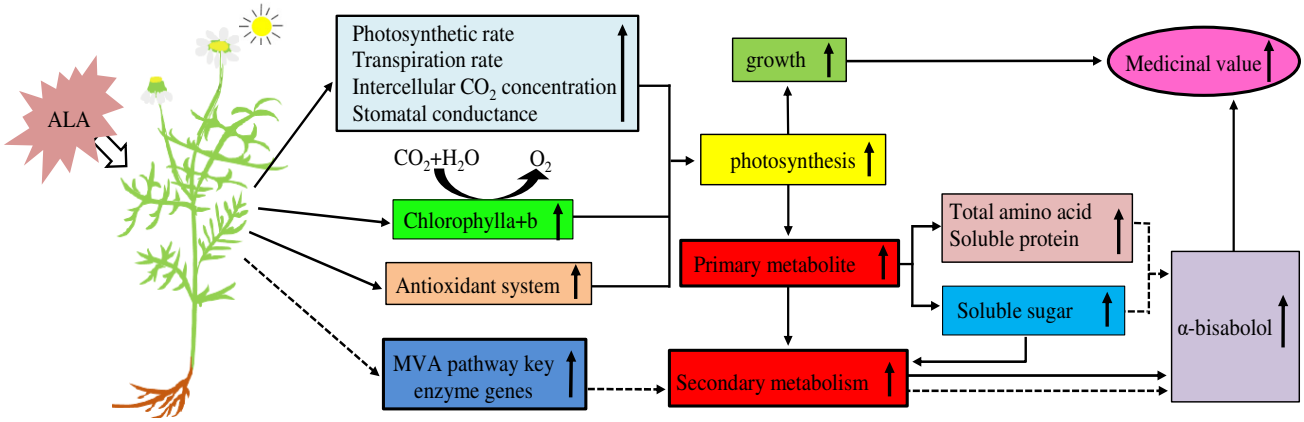

Fig. 6. Simulation diagram of the effects of ALA spraying on *M. recutita*. The dotted line indicates that the result is to be verified in further study. The arrow in the box indicates the increase of content, enzyme activity or metabolic flux

primary factors of plant photosynthesis and are important pigments involved in photosynthesis and in the transfer and transformation of light energy (Castelfranco *et al*., 1983). Therefore, the photosynthetic pigment in leaves is important, because it reflects the photosynthetic capacity of a plant. The change in environmental factors can alter the photosynthetic pigment content, which in turn varies the photosynthetic performance (Ding *et al*., 2006). Second, soluble sugar could maintain cell osmotic potential and cell water balance through its osmotic regulation.

Thus, soluble sugar plays a significant role in the physiological effects of photosynthesis. Therefore, the improvement of photosynthesis of *M. recutita* by spraying ALA in this experiment may be due to the increase in photosynthetic pigments and soluble sugar content (Fig. 6). ALA is a precursor material of chlorophyll synthesis and forms protochlorophyllide in the proplastid or chloroplast after a series of enzymatic activities. The protochlorophyllide forms chlorophyll *a* through the reduction of light, and then chlorophyll *a* is oxidized to form chlorophyll *b*, preventing the decomposition of chlorophyllase (Wang *et al*., 2009). The results study show that exogenous ALA treatment could improve the chlorophyll content of *M. recutita* (Fig. 2), and this result is consistent with the results of Hotta *et al*. (1997b). Thus, the increase in chlorophyll content is one of the reasons for the improved photosynthesis after ALA treatment.

Exogenous ALA treatment significantly increased the content of soluble protein, total amino acids, and soluble sugar (Fig. 3). We inferred that ALA could be a precursor of the chlorophyll biosynthesis, directly involved in photosynthesis of plants, and promote accumulation and transformation of organic matter. Plant photosynthesis is an important source of plant carbon, biomass accumulation, and growth and development. This process can convert inorganic substances into organic matter. Photosynthesis provides the material and energy for plant growth and development (Shao *et al*., 2009). In addition, ALA

promotes the accumulation of photosynthetic products. This process may also be related to ALA promotion of plant uptake and utilization of nutrients, because ALA can be combined with trace metal gold and iron and can effectively promote plant growth (Zhang and Zhou, 2000).

This study shows that ALA treatment can improve the activity of antioxidant enzymes in *M. recutita*. This result is consistent with the results of Nishihara *et al*. (2003) in *Spinacia oleracea* and Xu *et al*. (2009) in *Ginkgo biloba*. SOD is an enzyme that removes superoxide anion from cells, while POD, APX, and CAT are cleared of H_2O_2 produced by decomposition of SOD (Leonardis *et al*., 2000). These enzymes can effectively remove the oxygen free radicals produced during photosynthesis, inhibit membrane lipid peroxidation, and maintain the balance of metabolism of oxygen free radicals, thereby enhancing the resistance of plants (Liangju *et al*., 1999). Liu *et al*. (2005) found that promotion of the Pn of winter strawberry leaves by ALA treatment was related to the increase in the leaf SOD and POD activities. Similarly, in this study, ALA treatment was also found to improve the activities of POD, CAT, and SOD in *M. recutita*. POD is an enzyme, with ferroheme as the prothetic group, while ALA is a precursor of the ferroheme biosynthetic. Therefore, the mechanism by which the antioxidant enzyme activity of *M. recutita* cells was increased may be that ALA was converted to ferroheme, and its activity was increased by increasing the prothetic group of the antioxidant enzyme (Huystee *et al*., 1997). This process thereby reduced the damage of membrane lipid peroxidation, which maintained the integrity of the membrane system and the normal operation of the photosynthetic system and improved photosynthesis (Fig. 6).

Plant primary metabolism through photosynthesis and tricarboxylic acid (TCA) cycle provides energy and a few small molecular compound materials for secondary metabolism. Secondary metabolism is the continuation and development of primary metabolism under specific conditions to avoid the excessive accumulation of certain intermediates or products in the primary metabolic process of toxic effects on the body (Wahid *et al*., 2007). The secondary metabolites of plants resulted from the interaction between plants during long-term evolution. Many secondary metabolites are important components of traditional Chinese herbal medicine. Among these compounds, alkaloids, flavonoids, saponins, terpenoids, and other compounds, which are indirectly associated with photosynthesis, are the material bases for the efficacy of many traditional Chinese medicines (Su *et al*., 2005). Plants combine CO_2 and water into sugars by photosynthesis, and in different manners produce adenosine triphosphate (ATP), coenzyme (NADH), pyruvate (PA), phosphor-enolpyruvic acid (PEP), 4-phosphate-erythrose (E4P), and ribose, which are the indispensable substances in maintaining body life activity. PEP and E4P can further synthesize shikimic acid (CAS). Meanwhile, PA is hydrogenated and decarboxylated to form acetyl-CoA (AcSCoA), enters the TCA cycle, generates a series of organic acids and malonyl-CoA, and obtains a series of amino acids through nitrogen fixation reaction. These processes are the primary metabolic processes. Under certain conditions, several important primary metabolites, such as AcSCoA, malonyl-CoA, CAS, and some amino acids, act as raw materials or substrates, and further different secondary metabolic processes, result in isoprene compounds (e.g. terpenoids), phenolic compounds (e.g. flavonoids) and nitrogen-containing compounds (e.g. alkaloids), and so on (Shao *et al*., 2008). α-Bisabolol is a secondary metabolite of *M. recutita,* a terpenoid compound with multiple therapeutic effects, and synthesized by mevalonate (MVA) pathway. The results of this study show that exogenous ALA spraying on *M. recutita* can increase the α-bisabolol content (Fig. 5). Combined with the relationship between secondary metabolism and primary metabolism, we believe that the increase of α-bisabolol content of *M. recutita* sprayed ALA may be at the substrate level. PA is a photosynthetic carbon metabolite and also a substrate for the MVA synthesis pathway. Thus, ALA can improve the secondary metabolites of α-bisabolol content (Fig. 6). Numerous studies have analyzed the phenotypic and evolutionary patterns of carbon compounds in the distribution of plant secondary metabolites (Heyworth *et al*., 1998; Mosaleeyanon *et al*., 2005). The theory of metabolic overflow indicates that when the carbon element compounds exceed the amount required for plant growth, the excess will be diverted to the synthesis of the plant secondary metabolites (Matsuki *et al*., 1996). Therefore, the increase in the α-bisabolol content in *M. recutita* by ALA treatment may also be attributed to the increase in carbohydrate content, such as soluble sugar, amino acids, and soluble proteins, in the leaves (Fig. 6).

The biosynthetic pathway of chamomile α-sweet myrrh has been relatively elucidated. α-Bisabolol is synthesized by MVA pathway. This process is mainly based on pyruvic acid derived from TCA as a substrate and forms AcSCoA. Then, AcSCoA and a series of hydroxylation, acylation, and other enzymatic reaction result in the final synthesis of α-bisabolol (Vranová *et al*., 2012). In recent years, domestic scholars have cloned the several key enzyme gengs in the synthesis of α-bisabolol from *M. recutita*. α-Bisabolol content was found to be closely related to the expression level of its key enzyme gene, such as 3-hydroxy-3-methylglutaryl-CoA synthase (*HMGS*) (Tao *et al*., 2016a), acetyl-CoA C-acetyl-transferase (*AACT*) (Tao *et al*., 2016b), and (−)-α-bisabolol synthase (*BAS*) (Son *et a*l., 2014). Exogenous ALA in addition to the physiological level can increase the α-bisabolol content, whether it can also regulate the α-bisabolol synthesis pathway key enzyme genes expression level to improve the activity of key enzymes, thereby increasing the α-bisabolol, still need further study.

Conclusions

In this experiment, ALA improved the growth, photosynthesis, antioxidant system, and α-bisabolol content of *M. recutita.* Treatment with 2.0 mmol·L^{-1} ALA provided the best results. α-Bisabolol content was one of the important indices of *M. recutita* medicinal value. Spraying ALA on the leaf can be used as an effective cultivation and control measure to improve the medicinal value of *M. recutita*. ALA is a photosensitive material, that is, it easily decomposes when irradiated. Thus, using ALA is safe to animals, plants, and the environment. The results of this

study provide new and effective cultivation measures to improve the medicinal value and economic value of *M. recutita*.

Acknowledgements

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