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Full Length Research Paper

Effect of potassium supplementation on monoterpene production of *Houttuynia cordata*

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Houttuynia cordata **Thunb. is an aromatic herb rich in monoterpenes. However, little information is available about the effect of potassium (K) on the monoterpene production. In this study, plants were cultured with different K levels and the monoterpenes in *H. cordata* were determined. Results showed that total monoterpene content only had a minor share (9.85%) of essential oil in the control treatment (0 mM), while it showed a remarkable increase in the treatments supplemented with potassium and 10.26 mM K resulted in the maximum total monoterpenes (43.25%). The total monoterpenes was strongly associated with the stimulation of monoterpene hydrocarbons. According to correlation analysis, the increase of monoterpenes was mainly attributed to potassium supplementation. This implies that potassium is very important for the monoterpene production of *H. cordata*.**

Key words: Essential oil, *Houttuynia cordata*, monoterpenes, potassium.

INTRODUCTION

Houttuynia cordata Thunb. is an aromatic herb native to Eastern Asia (Chen et al., 2008). It could be found in ravines, streamsides, forests, wetlands, slopes, thicket and field margins, trailsides, roadsides or ditch banks in these regions. In China, it is known as 'Yuxingcao', which means 'producing unique fishy smell', and is popularly used as wild vegetable. In addition, its plants are commonly used as a traditional Chinese medicine (Han et al., 2009). It therefore has been identified as one of the most potential medicinal and edible wild plant resources (Wu et al., 2005).

H. cordata processes essential oils rich in monoterpenes (Chen et al., 2008), which are the aroma agents and medicinal components. Some monoterpenes, such as α-terpineol and carveol are used as an important fragrance, and added to foods, drinks, perfumes, tobacco and other products (Verlet, 1993). These compounds exhibit a variety of pharmacological activities against cancer, oxidation, bacteria and viruses (Crowell, 1999; Martin and Bohlmann, 2004). Actually, *H. cordata* is effective in treating pneumonia, severe acute respiratory syndrome (SARS), human immunodeficiency virus (HIV) and influenza virus and refractory hemoptysis (Lau et al., 2008; Lu et al., 2006).

Due to its broad applications and commercial value, farmers are trying to increase its production by artificial cultivation in recent years. However, being different from field crops little information is available about the effects of fertilization on its quality. It is well known that potassium is quality element for plants (Besford and Maw, 1975). In previous study, optimum potassium was found to be important for its growth and development (Xu et al., 2011b). However, our preliminary study indicated that the optimum potassium could not result in desirable monoterpene production. Therefore, in the present study, we further investigate whether potassium supplemention levels influence the monoterpene composition and content in the essential oil of *H. cordata*.

MATERIALS AND METHODS

Plant materials and growth conditions

H. cordata W01-100 with desirable traits like disease resistance,

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Table 1. Monoterpenes composition in the leaves of *Houttuynia cordata* supplemented with different potassium levels.

S/N	Formula	Monoterpenes	RI	Potassium concentration (mM)				
				Control (0)	1.28	2.56	5.13	10.26
1	C10H16	α-Thujene	560	-	-	-	0.23 ± 0.03 b	0.35 ± 0.01 a
2	C10H16	α-Pinene	573	0.76 ± 0.19 e	1.24 ± 0.02 d	2.14 ± 0.34 c	2.96 ± 0.19 b	3.92 ± 0.07 a
3	C10H16	Camphene	602	0.30 ± 0.01 d	0.44 ± 0.09 d	0.82 ± 0.01 c	0.95 ± 0.06 b	1.36 ± 0.07 a
4	C10H16	β-Phellandrene	651	0.46 ± 0.01 d	-	1.07 ± 0.10 c	1.35 ± 0.13 b	2.58 ± 0.10 a
5	C10H16	β-Pinene	657	0.69 ± 0.23 c	1.09 ± 0.02 c	1.97 ± 0.33 b	2.39 ± 0.00 b	3.59 ± 0.13 a
6	C10H16	β-Myrcene	685	0.81 ± 0.44 e	1.91 ± 0.68 d	3.33 ± 0.40 c	6.83 ± 0.17 b	8.17 ± 0.06 a
7	C10H16	α-Phellandrene	712	-	-	-	0.46 ± 0.02 a	0.36 ± 0.12 a
8	C10H16	α-Terpinene	736	-	0.56 ± 0.01 b	0.81 ± 0.12 b	1.65 ± 0.10 a	1.74 ± 0.16 a
9	C10H14	p-Cymene	752	0.27 ± 0.01 a	0.56 ± 0.20 a	0.56 ± 0.21 a	0.58 ± 0.09 a	0.61 ± 0.14 a
10	C12H20O2	β-Terpinyl acetate	761	0.37 ± 0.04 d	0.88 ± 0.14 c	1.30 ± 0.04 b	1.36 ± 0.11 b	2.05 ± 0.06 a
11	C10H16	β-Ocimene	777	-	-	-	0.25 ± 0.00 a	0.23 ± 0.12 a
12	C10H16	γ-Terpinene	820	0.88 ± 0.12 d	1.95 ± 0.19 c	2.64 ± 0.15 b	3.97 ± 0.35 a	4.42 ± 0.17 a
13	C10H16	Terpinolene	879	0.33 ± 0.01 c	0.36 ± 0.01 c	0.64 ± 0.00 b	0.89 ± 0.16 a	0.88 ± 0.13 a
14	C10H18O	4-Terpineol	1049	2.01 ± 0.18 c	3.89 ± 0.54 b	4.34 ± 0.11 b	3.82 ± 0.11 b	5.72 ± 0.41 a
15	C10H18O	α-Terpineol	1075	-	0.53 ± 0.03 b	0.37 ± 0.15 b	0.33 ± 0.03 b	1.94 ± 0.17 a
16	C10H16O	Carveol	1205	0.89 ± 0.01 a	1.27 ± 0.28 a	1.36 ± 0.36 a	0.69 ± 0.06 a	1.35 ± 0.35 a
17	C12H20O2	Bornyl acetate	1244	2.07 ± 0.22 c	3.96 ± 0.16 b	5.25 ± 0.21 a	3.95 ± 0.18 b	5.04 ± 0.47 a

Values are means ± standard deviation of at least three replicates. Value represents percentage amounts in the essential oil. "-" means undetection. The values with different letters are significantly different by using LSD test at $P<0.05$.

high-quality and yield, was used in the present experiment. Its sterile plantlets were uniformly selected and cultured on MS (Murashige and Skoog, 1962) medium, supplemented with different potassium chloride levels (1.28, 2.56, 5.31 and 10.26 mM K). Potassium chloride was absent in the control treatment. Each treatment consisted of at least three replicates and maintained at 24 ± 2℃, under 12 h photoperiod with light intensity of 30 μmol m^{-2} s^{-1} provided by cool-white fluorescent lamps (Philips, China). After one month, the second fully expanded leaves were used for determination of monoterpenes in the essential oil of the plants.

Essential oil extraction

The essential oil was prepared by headspace solid-phase microextraction (HP-SPME) (Xu et al., 2011a). Briefly, the manual SPME holder was used with a 100 μm polydimethylsiloxane fibre assembly (Supelco, Bellefonte, USA) which was conditioned as recommended by the manufacturer. The sample (0.2 g) was hermetically sealed in a 4 ml vial. The fibre was equilibrated for 10 min in a thermostatic bath at 80℃.

Monoterpene determination

The compositional analysis of the essential oil was carried out by a GC (Agilent Technologies 6890N) interfaced with a mass selective detector (Agilent 5973B) equipped with an Agilent HP-5MS silica capillary column (30 m × 0.25 mm and film thickness 0.25 μm). The carrier gas was helium with a constant flow rate of 1 ml min^{-1}. Injector temperatures were 250℃. The oven temperature was set at 60℃ for 2 min, programmed to 110℃ at a rate of 10℃ min^{-1}, and held at 110℃ for 4 min, then heated to 220℃ at a r ate of 10℃ min^{-1}, isothermal at this temperature for 4 min. The mass spectra operating parameters were as follows: ionisation potential, 70 eV; interface temperature, 280℃; acquisition mass range, 50-450. T he identification of the components was based on comparison of the retention times with those of authentic samples, relative retention indices, and comparison of mass spectra from the published data and mass spectra library (NIST 2.0). The percentage of constituents in the essential oils was calculated in peak areas using the normalization method.

Statistical analysis

Values are presented as means of at least four replicates. Difference significance was separated statistically by analysis of variance (ANOVA) and least significant difference (LSD) test. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The *H. cordata* line W01-100 used belongs to chemotype myrcene, whose essential oil is dominated by β-myrcene and also contains numerous kinds of monoterpenes (Chen et al., 2008). On the basis of HP-SPME and GC-MS analysis in this study, 17 monoterpene constituents were identified in the essential oils of the leaves from *H. cordata* plantlets (Table 1). Of them, 12 Monoterpene hydrocarbons including α-thujene, α-pinene, camphene, β-phellandrene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, p-cymene, β-ocimene, γ-terpinene and terpinolene were quantified as the principal class of components and beyond them were placed the monoterpene alcohols including 4-terpineol, α-terpineol and carveol, and the monoterpene esters including β-terpinyl acetate and bornyl acetate. In general, the content of monoterpene hydrocarbons had the major

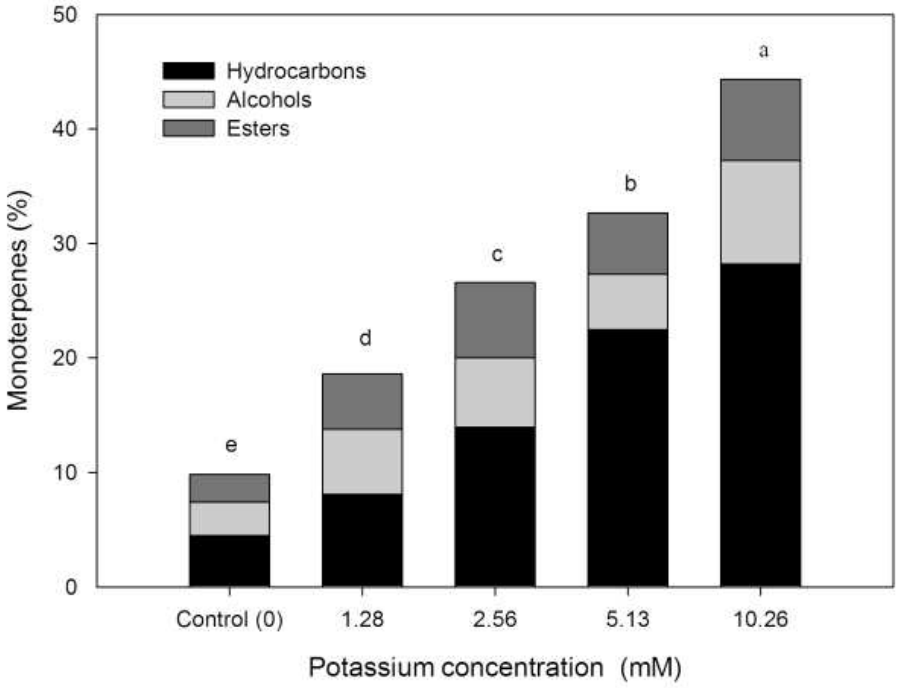

Figure 1. Changes of monoterpene hydrocarbons, alcohols and esters, as well as total monoterpenes in different potassium supplemention levels. The bars with different letters are significantly different by using LSD test at *P<0.05*.

share in the percentage of essential oil of *H. cordata in vitro* (Figure 1). The essential oil composition is well in agreement with the profile of essential oil from *H. cordata* W01-100 cultivated in the field (Chen et al., 2008).

The production of total monoterpenes was significantly affected by potassium supplementation levels (Figure 1). Total monoterpene content only had a minor share (9.85%) of essential oil in the control treatment (0 mM), while it showed a remarkable increase in the treatments supplemented with potassium and 10.26 mM K resulted in the maximum of total monoterpenes content (43.25%). Correlation analysis indicated that, increase of total monoterpenes may be mainly attributed to potassium supplementation (Table 2). This implies that potassium is very important for the monoterpene production of *H. cordata*.

Although, the changes of monoterpene hydrocarbon, alcohol and ester contents were almost in accordance with total monoterpenes in the treatments with potassium, the increase of total monoterpenes may be strongly associated with the stimulation of monoterpene hydrocarbons (Figure 1). Pearson correlation coefficients between total monoterpenes and hydrocarbons, alcohols, or esters were 0.98**, 0.86*, and 0.87* (**P<0.05*, ***P<0.01*), respectively. Hydrocarbon compounds had more share in the essential oil than alcohols as well as esters, particularly for high potassium supplementation (5.13 and 10.26 mM). Thus, total monoterpene content was mainly contributed by monoterpene hydrocarbons. This suggests that the biosynthesis of monoterpene hydrocarbons may be more sensitive to potassium levels than the other two. In detail, the increased potassium could improve the production of all monoterpene individuals (Table 1).

However, these monoterpenes were differently sensitive to potassium supplementation. According to correlation analysis, p-cymene, carveol, and bornyl acetate showed poor correlation with potassium, while the increase of α-pinene, camphene, β-phellandrene, β-pinene, β-myrcene, γ-terpinene, and β-terpinyl acetate is significantly related to potassium supplementation (Table 2).

Moreover, although α-thujene, α-phellandrene, β-ocimene, α-terpineol, and β-phellandrene could not be detected in the treatments of potassium starvation or/and low potassium levels, high potassium could stimulate their production. Thus, it is concluded that potassium could prove to be very useful for the biosynthesis of most monoterpenes.

Monoterpenes were synthesized from the common precursor geranyl diphosphate (GPP), catalyzed by monoterpene synthases, which was the key rate-limiting enzymes for monoterpene biosynthesis (Bick and Lange, 2003; Degenhardt et al., 2009). Multiple signaling molecules such as H_2O_2 prove to be involved in the regulation of the gene expression of monoterpene synthases (Zhao et al., 2005). Previous study indicated

Table 2. Pearson correlation coefficients between monoterpenes and potassium, respectively.

Coefficient	Monoterpenes						
Potassium	Total monoterpenes 0.96**	Hydrocarbons 0.96**	Alcohols 0.85*	Esters 0.75	α-Thujene -	α-Pinene 0.97**	Camphene 0.96**
Potassium	β-Phellandrene 0.99**	β-Pinene 0.98**	β-Myrcene 0.95**	α-Phellandrene -	α-Terpinene 0.88*	p-Cymene 0.65	β-Terpinyl acetate 0.94**
Potassium	β-Ocimene -	γ-Terpinene 0.92**	Terpinolene 0.86*	4-Terpineol 0.85*	α-Terpineol 0.87*	Carveol 0.22	Bornyl acetate 0.61

nt, not tested. *$P<0.05$, **$P<0.01$.

that, high potassium could result in the accumulation of reactive oxygen species such as H_2O_2, particularly at the level of 10.26 mM K (Xu et al., 2011b). Thus, the induced oxidative stress may partially contribute to the monoterpene production of *H. cordata* in the treatments with high potassium.

Similar to high potassium, potassium starvation also could lead to oxidative stress in plants (Xu et al., 2011b). However, the present study showed that potassium starvation resulted in the minimum total monoterpenes. It is well known that potassium is the major nutrient for plant growth and development, and plays an important role in metabolisms, like enzyme activities and protein synthesis (Pettigrew, 2008; Véry and Sentenac, 2003). Thus, the synthesis of monoterpene synthases may be suppressed in the treatments without potassium supplementation. In addition, potassium deficiency could markedly reduce chlorophyll content and net photosynthetic rate, and carbon assimilation would be very restrained (Gerardeaux et al., 2010; Xu et al., 2011b). The precursor GPP of monoterpenes is derived from isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate. IPP is synthetized by two basic metabolisms, mevalonate pathway and 2-C-methyl-D-erythritol 4-phosphate pathway (Laule et al., 2003). Thus, carbon fluxes suppressed by potassium deficiency would reduce the generation of GPP for isoprenoid biosynthesis, which belongs to secondary metabolism. This further supports that potassium is very important for monoterpene production of *H. cordata*.

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