Pak. J. Bot., 53(4): 1219-1225, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-4(1)

EFFECTS OF 6-BENZYLADENINE AND KINETIN ON GROWTH AND SECONDARY METABOLITES OF *HOUTTUYNIA CORDATA* THUNB. (SAURURACEAE)

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Abstract

The plant height, root length, fresh weight, morphological characters, phenolics, and volatiles of *Houttuynia cordata*, cultured in the presence of different concentrations of 6-benzyladenine (6-BA) or kinetin (KT) in the culture medium, were analyzed. Treatment with 6-BA and KT markedly inhibited the height and root length of *H. cordata*, and significantly promoted fresh weight. Treatment with either 6-BA or KT stimulated non-normal plantlet development. Treatment with either 6-BA or KT markedly inhibited the accumulation of the phenolics chlorogenic acid, rutin, quercitrin, and isoquerietrin by *H. cordata*, and significantly promoted the accumulation of *α*-pinene, *β*-pinene, *trans*-2-hexenal, *trans*-2-hexen-1-ol, decanal, linalool, decanol, and borneol. Treatment with 6-BA inhibited the accumulation of bornyl acetate, *β*-myrcene and *cis*-3-hexen-1-ol, whereas KT inhibited the accumulation of cineole. These findings provide theoretical support for improving the concentration of secondary metabolites in *H. cordata,* of potential value in the pharmacological utilization and exploitation of these compounds.

Key words: 6-Benzyladenine; Kinetin; Phenolics; Volatiles.

Introduction

Auxins play important role in promoting the formation of plant organs, apical dominance, tissue differentiation, and so on. Endogenous auxins are synthesized in germinating seeds, shoot tips, young leaves, root tips, etc. of plants, where cell division is active, maintaining a dynamic balance between synthesis and catabolism in plants, and are transported to all parts of the plant through polarity and free diffusion through cell membranes, achieving differential distribution in plants. Thus, the symmetrical nature of organs and leaf morphology on both sides of the plant distribution of auxins are regulated (Ren *et al*., 2012; Li & Chong, 2006; Lü & Yu, 2004; Shuai *et al*., 2016). Endogenous auxins are synthesized within the plants and also through adding some amino acid, intermediate products and/or specially enzymes (Liu *et al*., 2011; Li *et al*., 2012; Zhang, 2009). It has been reported that combining application of exogenous auxins with specific receptors (e.g. amino acids, sucrose, inositol, proteins, polypeptides etc.) result in specific biochemical reactions, which influence plant growth and the accumulation of secondary metabolites (Wang *et al.*, 2006; Li & Qu, 2006; Zou *et al.*, 2015). As a consequence, it is worthwhile to study the effects of exogenous auxins on secondary metabolite concentrations in plants.

The two processes (including primary and secondary metabolites) are involved in growth and development in plants. Primary metabolites are usually compounds essential for plant growth and development, but secondary metabolites are usually non-essential small molecules (Fang *et al*., 2014). Plant secondary metabolism includes biosynthetic pathways of phenylpropanoids, isoprenes, and alkaloids, among others, and biosynthesis is affected by environmental factors (e.g. light, soil properties, microorganisms, etc.) (Xing *et al*., 2015; Chen *et al*., 2016; Fan *et al*., 2018). Recent research has mainly focused on the effect of exogenous auxins on plant morphology, physiology and biochemistry. For example, indole-3-acetic acid, indole butyric acid and naphthalene acetic acid significantly influence the growth of corn seedlings, uptake of cadmium, and concentrations of malondialdehyde, proline and glutathione as well as antioxidant activities (Chen *et al*., 2016). The effects of endogenous hormones on plant growth may be one of the important factors causing changes in plant secondary metabolite accumulation because endogenous hormones were considered to mainly control cell division, cell elongation, or the synthesis of protein signals. However, the effects of exogenous cytokinins on plant secondary metabolites, especially in medicinal plants, have been little reported, so the mechanism by which exogenous cytokinins control plant secondary metabolism is an important problem which needs to be addressed urgently.

Houttuynia cordata Thunb., belongs to the family Saururaceae, is a dual-purpose plant, which can be used as both food and medicine, being particularly rich in secondary metabolites (such as volatiles, phenolics (including flavones), alkaloids, polysaccharides, etc.), which have not only medicinal value, such as antioxidant, antibacterial, and anti-inflammatory properties as well as being capable of inhibiting virus, improving immunity, etc., but it is also has a high nutritional value. *H. cordata* has been considered to be a suitable plant to develop in terms of its uses, having broad market prospects (Bernardi *et al*., 2019; Chen *et al*., 2016). The concentrations of secondary metabolites in *H. cordata* are affected by environmental factors, so our research is focused on analysing the effects of exogenous cytokinins on *H. cordata* growth and secondary metabolite profiles (namely phenolics and volatiles) in response to treatment with different exogenous cytokinins. This will provide theoretical support for improving the accumulation and potential value of secondary metabolites in *H. cordata,* while also providing new avenues to study the regulation of natural secondary metabolites by cytokinins.

Materials and Methods

Instruments and reagents: Instruments: A high-performance liquid chromatography (HPLC) system (LC-20AT, Shimadzu, Kyoto, Japan), including a diode array detector (DAD, SPD-M20A), system controller (SC, CBM-20A) and chromatography workstation (LC-solution, 2.50) was used. Agas chromatography–mass spectrometer (GCMS-QP2010, Shimadzu, Kyoto, Japan), including chromatography workstation (GCMS-Solution 2.10), was coupled to the HPLC. Other equipment and instruments used included an intelligent artificial climate incubator (BIC-300, Boxun, Shanghai, China), an electronic balance (LE204E, Mettler-Toledo, China), a vertical flow clean bench (ZHIJH-C106B, Zhicheng, Shanghai, China), and a steam sterilizer (YXQ-30SII, Boxun, Shanghai, China).

Reagents: 6-benzyladenine (6-BA) and kinetin (KT) were purchased from Sigma-Aldrich Co., Ltd. Chlorogenic acid, rutin, isoquercitrin, quercitrin, afzelin and quercetin were purchased from the Chinese Traditional Medicines Division of the Institute in Drug Quality Determination. *α*-Pinene, camphene, *β*-pinene, *β*-myrcene, limonene, cineole, *trans*-2-hexenal, *γ*-terpinene, *cis*-3-hexenyl-acetate, hexanol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, decanal, linalool, bornyl acetate, *β*-caryophyllene, 2-undecanone, borneol, decanol and 3-tetradecanone were purchased from J & K Scientific, and other reagents were obtained from chemical suppliers and were of analytical grade.

Medium: Murashige & Skoog (MS) medium was used, consisting of MS salts 4.42 g/L, sucrose 30 g/L, and agar 8 g/L, with the pH adjusted to 5.8.

Experimental design: Individuals from the same batch of *H. cordata* tissue-cultured plantlets were selected for use as experimental material. Stem segments with one leaf were taken and used as explants, which were incubated on MS medium containing different concentrations of 6-BA or KT. Each cytokinin treatment included four concentrations of either 6-BA or KT, namely 0 (as CK), 1, 2, and 3 mg/L, respectively. *H. cordata* explants, one per flask, were incubated in 10 replicate transparent flasks of MS medium containing the respective cytokinin concentration. The *H. cordata* explants were cultured in the growth incubator, under standardized ambient conditions (temperature 25±1ºC, light intensity 2000IX, and photoperiod 14 h/d).

Morphological analysis: The *H. cordata* explants cultured for 45 d were removed, then five plants from each cytokinin concentration were taken at random, and their leaf number, plant height, main root length, and fresh weight were determined. The leaf number was based on the replicate plant with the largest leaf number from each treatment. Plant height was recorded from the top of the main stem to the base of the plant, measured, using vernier calipers. Root length was recorded based on the distance from the tip of the main root to the base of the plant, measured using vernier calipers (Li *et al*., 2013). Fresh weights based on the weights of all leaves, stems and roots of the plant, accurate to 0.0001 g were recorded.

HPLC analysis of secondary metabolites

Analysis of phenolic contents: Determination of the contents of chlorogenic acid, rutin, isoquercitrin, quercitrin, afzelin and quercetin in *H. cordata* was carried out using a slightly modified version of the method reported by Yang *et al.*, (2014). The HPLC conditions involved a Shim-pack CLC-ODS chromatographic column (150 × 6.0 mm i.d., No: 61529098B), with detection at the wavelength 345 nm and a column temperature of 40°C. The linear solvent gradient used for sample elution is shown in Table 1, consisting of eluent A (acetonitrile: methanol=11:5 (v/v)) and eluent B (0.1% formic acid (v/v)).

Table 1. Gradient elution program for separation of *Houttunyia cordata* phenolics by high-performance liquid chromatography.

Times (min)	Flow rates (mL/min)	Ratios of mobile phase (%)	
		A*	B*
1	1.2	6	94
6	1.39	13.3	86.7
9	1.11	18.1	81.9
11	1	18.9	81.1
31	1.1	29.2	70.8
48	1.2	38	62
55	1.39	100	0

*****eluent A: Acetonitrile: Methanol = 11:5 (v/v); eluent B: 0.1% formic acid (v/v).

Gas chromatography–mass spectrometry (GC–MS) analysis of volatile contents: Determination of the contents of *α*-pinene, camphene, *β*-pinene, *β*-myrcene, limonene, cineole, *trans*-2-hexenal, *γ*-terpinene, *cis*-3-hexenyl-acetate, hexanol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, decanal, linalool, bornyl acetate, *β*-caryophyllene, 2-undecanone, borneol, decanol and 3-tetradecanone was performed using the method reported by Yang *et al*., (2016), with some modifications. The GC conditions involved a, VF-WAXms capillary column (30 m × 0.25 mm × 0.25 μm), at a column temperature program of 40°C (3 min), 40~100°C (5.6°C/min), 100°C (1 min), 100~125°C (3.1°C/min), 125~230°C (15°C/min), and 230°C (5.44 min), with an injector temperature of 250°C, a helium (He) (99.999%) carrier gas, a flow velocity of the carrier gas of 0.90 mL/min, a split ratio, splitless, and an injector volume of 1 μL. The mass spectrometry conditions involved an electron ionization (EI) ion source, an ionization energy of 70 eV, an ionization temperature of 200°C, an interface temperature of 260°C, a detector voltage of 1.2 KV, a solvent delay time of 3 min, and a single ion monitoring (SIM) scan mode.

Data handling and statistical analysis: Microsoft Office Excel 2010 was used to analyze the data from *H. cordata* secondary metabolites (namely phenolics and volatiles) and morphological parameters, with each parameter being measured on three independent replicate samples, with results being expressed on a fresh weight basis. The concentration of individual phenolics was calculated by the external standard method, based on the peak areas from a HPLC chromatographic file of phenolics. The concentrations

of volatiles were calculated using the internal standard method based on the peak areas from a GC-MS chromatographic file of volatiles. Each experimental treatment of *H. cordata* culture was carried out with 10 biological replicates. Analysis of variance (ANOVA), with pairwise multiple comparison carried out by Least Significant Difference (LSD), was conducted for all variables, using SPSS (v. 19.0; IBM, Armonk, NY, USA).

Results and discussion: The mean plant height, root length and fresh weight of *H. cordata* tissue-cultured plantlets produced in response to different concentrations of 6-BA or KT are shown in Table 2. The results obtained showed that the mean plant height of *H. cordata* plantlets treated at 1, 2 and 3 mg/L 6-BA or KT was significantly shorter than that produced under CK (control). For example, the mean plant height of *H. cordata* treated at CK was 5.15 cm, and the mean plant heights of *H. cordata* treated at 1, 2 and 3 mg/L cytokinin were 3.82, 3.81, 4.02 cm for 6-BA, respectively, and 3.91, 4.83, 3.12 cm for KT, respectively. No *H. cordata* roots were detectable on plantlets cultured at 1, 2 or 3 mg/L 6-BA. The mean root length of *H. cordata* plantlets treated with 1 mg/L 6-BA was 3.12 cm, which was significantly longer than that in the CK treatment (2.72 cm). At 2 mg/L KT, the mean root length of *H. cordata* (2.73 cm) was not significantly different from that in the CK treatment (2.72 cm), whereas no roots developed on *H. cordata* plantlets treated at 3 mg/L KT. The mean fresh weights of *H. cordata* treated with 1, 2 and 3 mg/L 6-BA and KT were significantly heavier than that of the CK plantlets. The mean fresh weight of CK *H. cordata* plantlets was 0.98 g/plant, compared with mean weights of *H. cordata* plantlets treated with 1, 2 and 3 mg/L cytokinin of 2.46, 1.98 and 1.68 g/plant for 6-BA, respectively, and 1.56, 2.55 and 2.37 g/plant for KT, respectively.

The morphological features of plantlets treated with 6-BA or KT are shown in Table 3 and Fig. 1. The morphological features of a typical CK *H. cordata* plantlet after 45 d culture were as follows: single plant, seven leaves, fibrous roots, two buds and two developing leaves at each bud. When *H. cordata* was treated with 1 mg/L 6-BA, the plantlets progressively deteriorated, with an increase in the number of cluster buds, callus induced from leaves, and no roots. When *H. cordata* was treated with 2 or 3 mg/L 6-BA, the plantlets deteriorated to only 2–3 leaves, a decline in the number of cluster buds, and no roots. When *H. cordata* plantlets were treated with 1 mg/L KT in tissue culture, the typical plantlet developed three shoots, which had four and twi leaves, respectively, and no roots. When *H. cordata* was treated with 2 mg/L KT in tissue culture, the plantlet developed two shoots, and progressively deteriorated to two leaves, which had five and five leaves, respectively, and fibrous roots. When *H. cordata* was exposed to 3 mg/L KT in tissue culture, the plantlet developed a single shoot, with two cluster buds, and progressively deteriorated to three leaves, and no roots.

Table 2. Effects of 6-benzyladenine and on plant height, root length and fresh weight of *H. cordata* (mean ± standard deviation).

Treatments	Cytokinin	Cytokinin concentration (mg/L)	Plant height (cm)	Root length (cm)	Fresh weight (g)
CK		0	5.18 ± 0.12a	2.72 ± 0.07b	0.98 ± 0.03e
B1	6-BA	1	3.82 ± 0.11c	-	2.46 ± 0.17ab
B2		2	3.81 ± 0.04c	-	1.98 ± 0.06c
B3		3	4.02 ± 0.09c	-	1.68 ± 0.12d
K1	KT	1	3.91 ± 0.0ca	3.21 ± 0.14a	1.56 ± 0.08d
K2		2	4.83 ± 0.09b	2.73 ± 0.09b	2.55 ± 0.11a
K3		3	3.12 ± 0.12d	-	2.37 ± 0.04b

Note: "-" means that the fibrous roots of *H. cordata* tissue-cultured plantlets did not emerge. Any two samples in the same column with common letters were not significantly different (p>0.05) level

Table 3. Effects of 6-BA and KT on plant morphology of *H. cordata*. The leaf number was based on the replicate plant with the largest leaf number from each treatment.

Treatments	Cytokinin	Treatment concentration (mg/L)	Plant morphology*
CK		0	Single plantlet, seven leaves, fibrous roots, two buds, with two leaves at each bud
B1	6-BA	1	More cluster buds, callus was induced from leaves, no roots were formed
B2		2	Three leaves, fewer cluster buds, and no roots
B3		3	Two leaves, fewer cluster buds, and no roots
K1	KT	1	Plantlet split into three shoots, with four, four, and two leaves, respectively, and no roots
K2		2	Plantlet divided two shoots, two cluster buds, with five and five leaves, respectively, and fibrous roots
K3		3	Plantlet formed a single shoot with two cluster buds, three leaves, and no roots

Fig. 1. The morphology of *H. cordata* cultured in the presence of 6-BA and KT (From left to right: CK, B1 and K2; see).

The effects of 6-BA and KT on the plant height, root length, fresh weight and morphology of *H. cordata* plantlets showed that the effect of exogenous treatment with different cytokinins on growth, development, and morphology of *H. cordata,* was different. The results obtained showed that, at the concentrations used, 6-BA caused inhibition of the root system of *H. cordata,* even inducing callus formation from *H. cordata* leaves at a low concentration. This suggested that 6-BA could be considered for callus culture of *H. cordata* at a low concentration. Lin *et al*., (2012) reported that callus was produced on leaves and stems of *Scindapsus aureus*, even at higher 6-BA concentrations. KT treatment also inhibited the formation or length of *H. cordata* roots. The results obtained showed that the different effects of cytokinins at different locations of *H. cordata* resulted in their morphological differences. The different concentrations of the cytokinins used promoted or inhibited the growth and development of *H. cordata* plantlets. High concentrations of cytokinins combined with specific receptors, such as amino acid, sucrose, inositol, protein, polypetide, *etc.,* in *H. cordata*. This produced the specific biochemical, physiological and morphological changes in the different *H. cordata* phenotypes as a result of altered growth and development.

The changes in concentration of chlorogenic acid, rutin, isoquercitrin, quercitrin, afzelin and quercetin in *H. cordata* treated with 6-BA or KT are shown in Fig. 2. At 1, 2 and 3 mg/L 6-BA or KT, the concentrations of chlorogenic acid, rutin, isoquercitrin, and quercitrin (undetected, 2.56, 24.60, and 5.27 µg/g, respectively) for 6-BA, and undetected, 1.58, 2.06 µg/g, and undetected, respectively, for KT) were significantly lower than the corresponding concentrations in control (CK) plantlets (3.63, 41.65, 6.84 and 2.26 µg/g, respectively). Quercitrin in *H. cordata* treated with 2 mg/L 6-BA was neither detected, nor was isoquercitrin and quercitrin of *H. cordata* treated with 3 mg/L 6-BA or KT. The concentrations of chlorogenic acid, rutin, isoquercitrin, and quercitrin of *H. cordata* plantlets treated with 1 mg/L KT (1.01, 29.39, 3.48 µg/g and undetected, respectively) were significantly lower than those of CK plantlets, whereas the concentrations following plantlet treatment with 2 mg/L KT (8.42, 81.41, 12.26 and 3.21 µg/g, respectively) were significantly higher and the concentrations at 3 mg/L KT (3.24, 14.32, 3.81 µg/g and undetected, respectively) were significantly lower than those in the CK plantlets. Neither afzelin nor quercetin were detected in *H. cordata* from the CK, 6-BA or KT treatments.

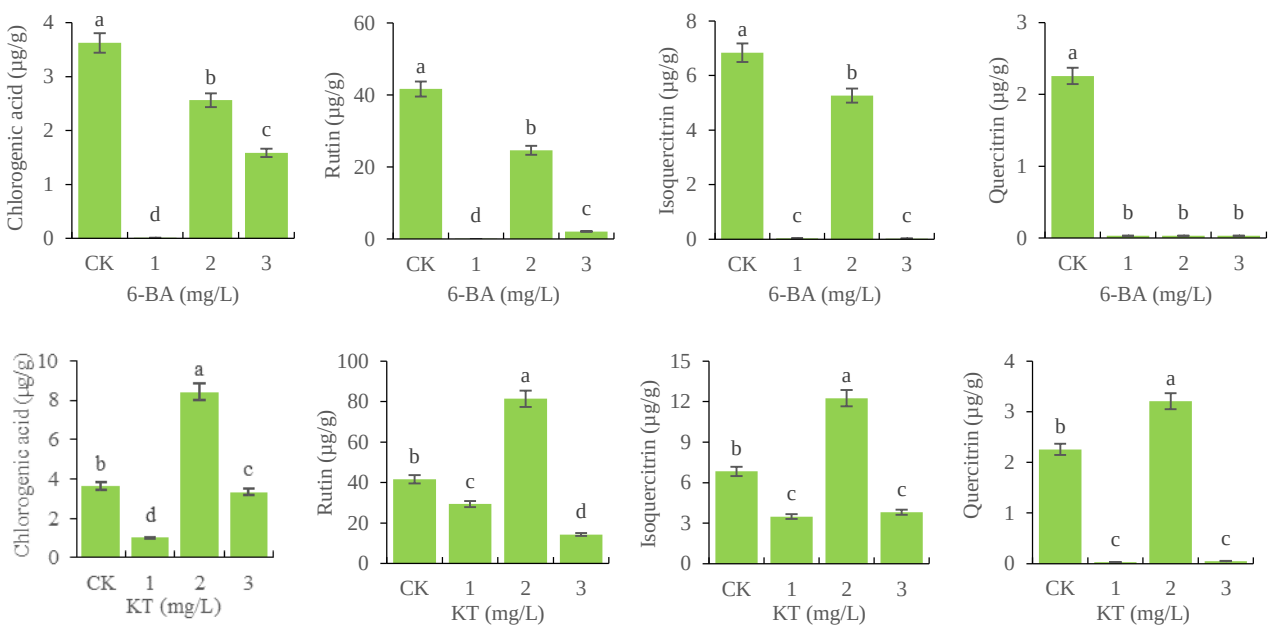

Fig. 2. Effects of 6-benzyladenine (6-BA) and kinetin (KT) on phenolic compounds in *H. cordata.* Afzelin and quercetin in *H. cordata* in the CK, 6-BA and KT treatments were not detected. CK: control; 6-BA: 5-benzyladenine; KT: kinetin. Any two samples in the same column with common letters were not significantly different (p>0.05) level.

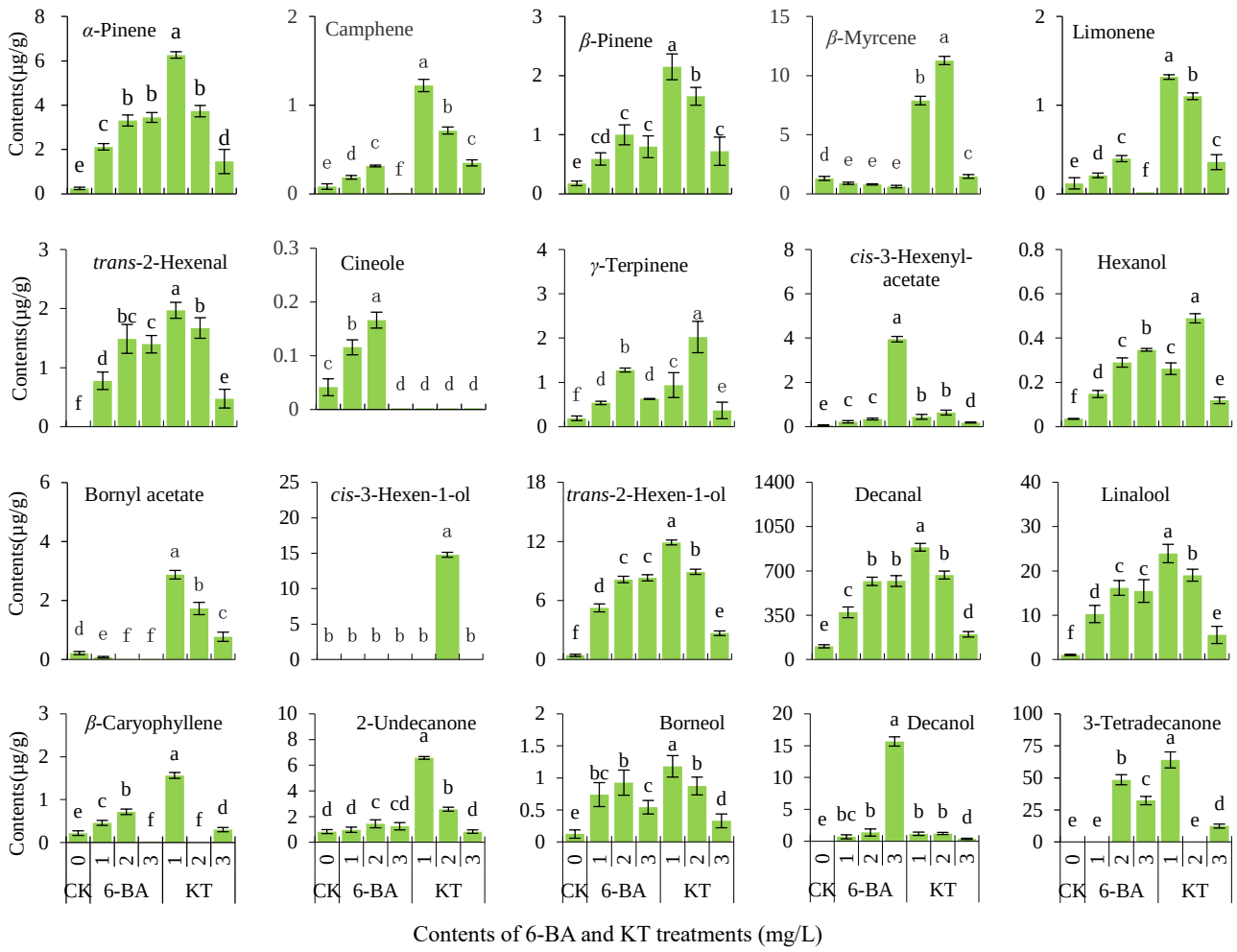

Fig. 3. Effects of 6-benzyladenine (6-BA) and kinetin (KT) on volatile profiles in *H. cordata*. CK: control; 6-BA: 5-benzyladenine; KT: kinetin. Any two samples in the same column with common letters were not significantly different (p>0.05) level.

The changes in concentrations of individual volatiles in *H. cordata* in response to treatment with 6-BA or KT are shown in Fig. 3. The concentrations of *α*-pinene (1.46~6.27 μg/g) in *H. cordata* treated at 1, 2 and 3 mg/L 6-BA or KT were significantly higher than that in CK plantlets (0.24 μg/g). Except for 3 mg/L 6-BA, the concentrations of camphene and *β*-pinene (0.19~1.22 μg/g and 0.59~2.15 μg/g, respectively) of *H. cordata* treated with 6-BA or KT were significantly higher than those in CK (0.08 and 0.18 μg/g, respectively). The concentrations of *β*-myrcene (0.61~0.89 μg/g) of *H. cordata* treated with 6-BA and KT were significantly lower than that in CK (1.30 μg/g), whereas the concentrations (1.46~11.27 μg/g) in *H. cordata* treated with KT were significantly higher than that in CK. With 3 mg/L 6-BA, the concentration of limonene (0.02 μg/g) of 6-BA-treated *H. cordata* was significantly lower than that of CK (0.12 μg/g), whereas the limonene concentrations (0.21~1.32 μg/g) in *H. cordata* treated with lower 6-BA concentrations were significantly higher than that of CK. The concentrations of cineole (0.12~0.16 μg/g) of *H. cordata* treated with 1 or 2 mg/L 6-BA were significantly higher than that of CK (0.04 μg/g), whereas cineole was not detected in plants treated with 3 mg/L 6-BA or with any KT concentrations. The concentrations of *trans*-2-hexenal (0.48~1.97 μg/g), *γ*-terpinene (0.37~1.28 μg/g), *cis*-3-hexenyl-acetate (0.18~3.95 μg/g), hexanol (0.12~0.49 μg/g), *trans*-2-hexen-1-ol (2.68~11.90 μg/g), decanal (198.84~886.27 μg/g), linalool (5.56~23.94 μg/g), borneol (0.33~1.18 μg/g), and decanol (0.37~15.64 μg/g) were significantly higher in plants treated with any concentration of either cytokinin than that in CK plants (0.01, 0.19, 0.05, 0.04, 0.42, 104.39, 1.03, 0.42, and 0.02 μg/g, respectively). The concentrations of *cis*-3-hexen-1-ol (14.78 μg/g) in *H. cordata* treated with 2 mg/L KT were significantly higher than CK (not detected), whereas this volatile was not detected in plants treated with other concentrations of either cytokinin. The concentrations of bornyl acetate (0.02~0.78 μg/g) in *H. cordata* treated with 1, 2, or 3 mg/L 6-BA were significantly lower than that of CK plants (0.22 μg/g), and the concentrations (0.77~2.88 μg/g) in plantlets treated with 1, 2, or 3 mg/L KT were significantly higher than that of CK plants. The concentrations of 2-undecanone (0.874 and 0.832 μg/g) in *H. cordata* plants treated with 1 mg/L 6-BA or 3 mg/L KT were not significantly different from that of CK plants (0.829 μg/g), whereas their concentrations (1.44~6.57 μg/g) in plants treated with other concentrations of the two cytokinins were significantly higher than that CK. The concentrations of *β*-caryophyllene (0.02 μg/g and not detected) in *H. cordata*

treated with 3 mg/L 6-BA and 2 mg/L KT, respectively, were significantly lower than that in CK plants (0.21 μg/g), whereas the concentrations (0.32~1.56 μg/g) in plants treated with other concentrations of the two cytokinins were significantly higher than that in CK plants. The concentrations of 3-tetradecanone (0.02 μg/g) in *H. cordata* treated with 1 mg/L 6-BA or 2 mg/L KT, respectively, were essentially in agreement with that in CK (0.02 μg/g), but their concentrations (12.47~64.05 μg/g) in plants treated wit other concentrations were significantly higher than that in CK plants.

The results obtained showed that 6-BA could inhibit the accumulation of chlorogenic acid, rutin, and quercitrin of *H. cordata*. This agreed with the results obtained by Lee *et al.,* (2011). The synthesis of rutin in *H. cordata* were induced by adding either 6-BA or KT into the culture medium. Jia *et al.*, (2015) reported that, over a certain range of concentrations, 6-BA could promote synthesis of a secondary metabolite (ponicidin) in petal callus of *Rabdosia rubescens.* Results from the current study showed that regulation of the metabolism of chlorogenic acid, rutin, isoquercitrin and quercitrin in *H. cordata,* using 6-BA, were different from the effects obtained using KT, while the effects of cytokinin concentration on these secondary metabolites also differed between 6-BA or KT. The 6-BA inhibited the accumulation of chlorogenic acid, rutin, and quercitrin of *H. cordata,* whereas KT inhibited or promoted the accumulation of chlorogenic acid, rutin, and quercitrin of *H. cordata* at different concentrations.

The experimental results obtained indicated that 6-BA could promote accumulation of *α*-pinene, *β*-pinene, *trans*-2-hexenal, *γ*-terpinene, *cis*-3-hexenyl-acetate, hexanol, *trans*-2-hexen-1-ol, decanal, linalool, borneol and decanol in *H. cordata,* the effects being positively associated with cytokinin concentration, whereas 6-BA inhibited the accumulation of *cis*-3-hexen-1-ol, *β*-myrcene and bornyl acetate, the effects being negatively associated with cytokinin concentration. KT could promote synthesis of *α*-pinene, camphene, *β*-myrcene, limonene, *trans*-2-hexenal, *γ*-terpinene, *cis*-3-hexenyl-acetate, hexanol, *trans*-2-hexen-1-ol, decanal, linalool, borneol, decanol, and bornyl acetate in *H. cordata,* the effect being positively correlated with cytokinin concentration, and inhibited cineole accumulation in *H. cordata,* the effects being negatively correlated with cytokinin concentration. These findings were consistent with the study of Zhang *et al.*, (2018). Abscisic acid (ABA) influenced synthesis of different terpenes of *Tripterygium wilfrodii* over a range of concentrations and the dosage-response curve was a *β* curve, which conformed to the hormesis effect (Zhang *et al.*, 2018). This might hint at a similar regulatory mechanism between ABA and the cytokinins. Accumulation of secondary metabolites in *H. cordata* was regulated by exogenous application of cytokinins, which may represent a new technical way for increasing the concentrations of active components of Traditional Chinese Medicines (TCMs), and be significant in improving quality control of TCMs.

Treatments with 6-BA or KT inhibited plant height, root length, and promoted fresh weight, though these results were classified as being preliminary. At the same time, 6-BA and KT could also regulate accumulation of the phenolics and volatiles of *H. cordata,* so that the concentrations were changed. This information will provide a theoretical basis for improvement of the accumulation of the secondary metabolites, and promote the exploitation and utilization of pharmacological and possibly other functions in *H. cordata*.

Acknowledgements

We thank Yuan-Shuai Wang and Hai-Tao Ye at Guizhou Normal University for assistance in determination of secondary metabolites of *H. cordata*. This work was supported by the National Natural Science Foundation of China (grant number 41761010 and 81260641), Science and Technology Foundation of Guizhou Province of China (QKHJC[2017]1121), and Project of Natural Science Research in Education Office of Guizhou Province (QJHKY[2021]002).

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(Received for publication 25 September 2019)