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Original Article

Effect of exogenous phytohormones treatment on glycyrrhizic acid accumulation and preliminary exploration of the chemical control network based on glycyrrhizic acid in root of *Glycyrrhiza uralensis*

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A B S T R A C T

One-year-old *Glycyrrhiza uralensis* Fisch. ex DC, Fabaceae, was treated with three exogenous phytohormones in June and July, namely gibberellin, auxin (indole-3-acetic acid), methyl jasmonate at different concentrations. Control plants were treated with water. Roots of controls and hormones-treated *G. uralensis* plants were harvested at different times, and the contents of seven main chemical components were determined. Root glycyrrhizic acid content of plants treated in June increased significantly compared with controls, and the difference was significant. As for plants treated in July, root glycyrrhizic acid content increased in which sprayed with appropriate concentrations of hormones, but the effects of hormones were more evident in plants treated in June coincided with the vigorous growth period than those treated in July. Gibberellin at 40 mg/l and auxin at 40 mg/l applied in the two treatment periods significantly promoted the accumulation of glycyrrhizic acid in *G. uralensis* root. Treatment with methyl jasmonate at 100 and 25 mg/l in June and July, respectively, also increased glycyrrhizic acid content significantly. The determination of major active compositions indicated that liquiritin, isoliquiritin, isoliquiritin apioside and liquiritin apioside contents were positively related to glycyrrhizic acid content. The study preliminarily found phytohormones and the main chemical components associated with glycyrrhizic acid content, and these discoveries could provide a basis for establishing a chemical control network with glycyrrhizic acid as the core, confirming the secondary product metabolic pathways in the network and completely uncovering synthesis mechanism underlying glycyrrhizic acid-combined functional gene polymorphism.

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Introduction

Glycyrrhiza uralensis Fisch. ex DC, Fabaceae, is known to be the 'king' of traditional Chinese medicine. This plant is the most commonly used medicinal material and is an important additive in cosmetic, health product and tobacco industry. A high demand for *G. uralensis* is reported every year. Cultivation of *G. uralensis* has become the mainstream because of the lacking wild resources. However, a widespread problem has been reported regarding *G. uralensis* quality in terms of the substandard content of glycyrrhizic acid. Therefore, improving the quality of cultivated *G. uralensis* has become a focus of research in the field of Chinese medicine resources.

Glycyrrhizic acid (**1**), a triterpenoid saponins components, is the main bioactive component with anti-viral, anti-inflammatory, anti-tumor and other major pharmacological activities in *G. uralensis* root (Zhang and Ye, 2009). As another major effective components in *G. uralensis* root, flavonoids have significant anti-tumour (Zhang and Ye, 2009; Li et al., 2012), anti-oxidant activities (Zhang and Ye, 2009; Cai et al., 2004), and the most represented flavonoids are liquiritin (**2**), isoliquiritin (**3**), liquiritigenin (**4**), isoliquiritigenin (**5**), liquiritin apioside (**6**), and isoliquiritin apioside (**7**) (Zhang et al., 2013).

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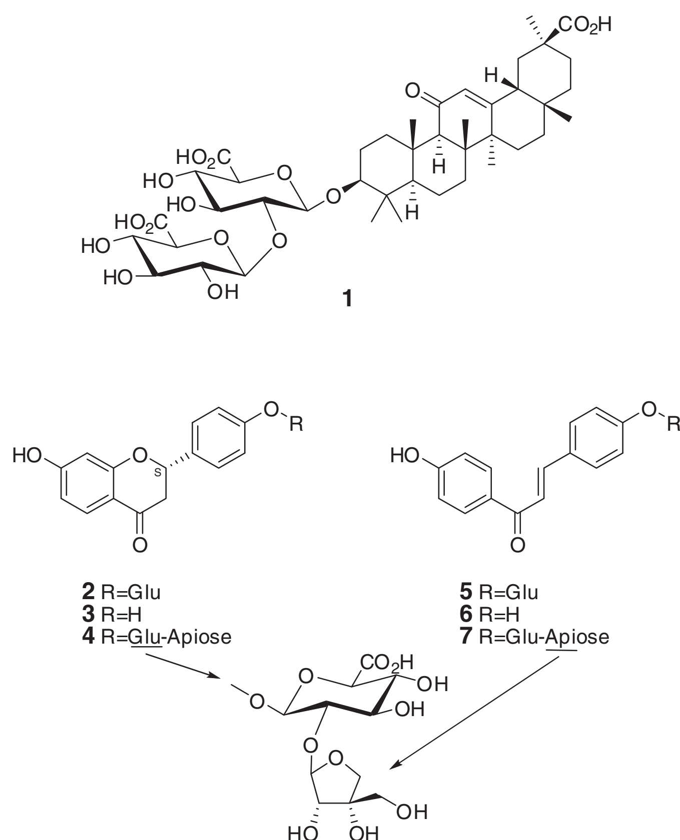

Currently, various chemical and physical factors affect the medicinal plant growth and secondary metabolite production had been researched widely. Moisture (Li et al., 2011), light (Hou et al., 2010; Afreen et al., 2005), salt (Wan et al., 2011), mineral elements (Yin et al., 2014; Wang et al., 2010; Liu and Wang, 2009) and other induced factors have been studied in relation to *G. uralensis* growth and accumulation of glycyrrhizic acid through field cultivation, *in vitro* culture and hairy root culture. However, studies on the regulatory effects of phytohormones on *G. uralensis* only focus in the aspect of plant growth, and in-depth research in the aspect of the secondary metabolism is lacking.

A large number of studies have suggested that phytohormones serve a crucial function in altering plant growth and secondary metabolism. Gibberellin (GA) is a widespread and widely studied phytohormone that could effectively regulate plant growth and formation of secondary metabolites. Zhang et al. (2005) reported that GA_3 can induce the transformation of artemisinic acid to artemisinin and stimulate artemisinin biosynthesis.

Auxin (indole-3-acetic acid, IAA) is a phytohormone that has close relationship and similar effects to GA. Studies have shown that IAA treatment can stimulate growth in hairy root culture, and produce different effects on secondary metabolites in different plants (Rhodes et al., 1994; Arroo et al., 1995). However, relatively few studies have focused on IAA's regulation on the metabolism of medicinal plants.

As growth regulator that widely exists in plants, methyl jasmonate (MeJa) can induce chemical defences that simulate biological stress, which is an exogenous inducer on induction of secondary metabolism in the plants, plant cells and calli (Qian et al., 2004; Yu et al., 2002; Zhao et al., 2001; Bulgakov et al., 2002). Exogenous MeJa increased the content of ginsenosides in *Panax ginseng* cell (Lu et al., 2001) and adventitious roots cultivation (Yu et al., 2002), and enhanced phenolic acid content in *Salvia miltiorrhiza* hairy root (Xiao et al., 2009).

Production and metabolism of each product in the plant are not isolated, and these processes should form an interrelated interaction network in which multiple metabolic pathways interconnect by nodes. Researchers have found an interplay between the end-product of different metabolic pathways in many plants. A theoretical metabolic network diagram that correlates with the content of glycyrrhizic acid (**1**) in *G. uralensis* root has been depicted based on a combination of research and literature (Fig. 1). The original view, which focuses on terpenoid metabolic pathway, has been amplified to cover all kinds of secondary metabolite biosynthesis pathways.

The current article aims to study root glycyrrhizic acid content of *G. uralensis* after treatments with three kinds of exogenous hormones, the correlation between major endogenous chemical components and glycyrrhizic acid content, then preliminarily find phytohormones and main chemical components associated with glycyrrhizic acid content, which could lay a solid foundation for defining constitution of chemical components and metabolic pathways in the control network based on glycyrrhizic acid, thereby completely explaining the underlying synthesis mechanism of glycyrrhizic acid combining functional gene polymorphism.

Materials and methods

Plant materials

One-year-old liquorice plant collected from Jingtai, Gansu Province, China were cultured in plastic pots in May 2014 filled with sandy loam soil (with identical composition and weight in each pot) in Beijing University of Chinese Medicine medical plant garden. Every pot contained eight *G. uralensis* plants, which were subsequently treated with exogenous hormones. These plants were managed in parallel according to the conventional cultivation method. The voucher specimen (No. GU-0010) of the sample, which was identified as *Glycyrrhiza uralensis* Fisch. ex DC, Fabaceae, by professor Chun-sheng Liu in the Beijing University of Chinese Medicine, was preserved in Beijing University of Chinese medicine specimen room.

Exogenous hormone treatment to the sample collection

GA_3 (BioDee), IAA (Bioway) and MeJa (Sigma) at l5, 25, 40 and 100 mg/l solutions, respectively, were used as exogenous hormone treatments. *G. uralensis* plants were separated into two batches. Leaves were sprayed with prepared hormone solutions in mid-to-late June and July. Control plants were sprayed with water. Exogenous hormones were sprayed every other day (three times in total) and marked when all were leaves moist and liquid was hanging on the leaf tips. Each concentration of three hormones was used on 16 pots of *G. uralensis* in randomised block arrangement. The pots were managed in parallel according to the conventional cultivation approach.

The first batch of *G. uralensis* (treated in June) was harvested five times on 10 July, 20 July, 20 August, 20 September and 20 October. The second batch of *G. uralensis* plants (treated in July) was harvested thrice on 15 August, 15 September and 15 October. At each sampling period, two pots (about sixteen plants) of *G. uralensis* plants belonging to different treatment groups (different concentration of hormone treatments and control plants) were harvested as one sample. Taproots (10 cm below the rhizome) of them were cut for content analysis.

Determination of the seven main components content of G. ularensis *root*

Chemicals and materials

Analyses were performed on Agilent-1200 high performance liquid chromatograph (HPLC) system equipped with quaternary

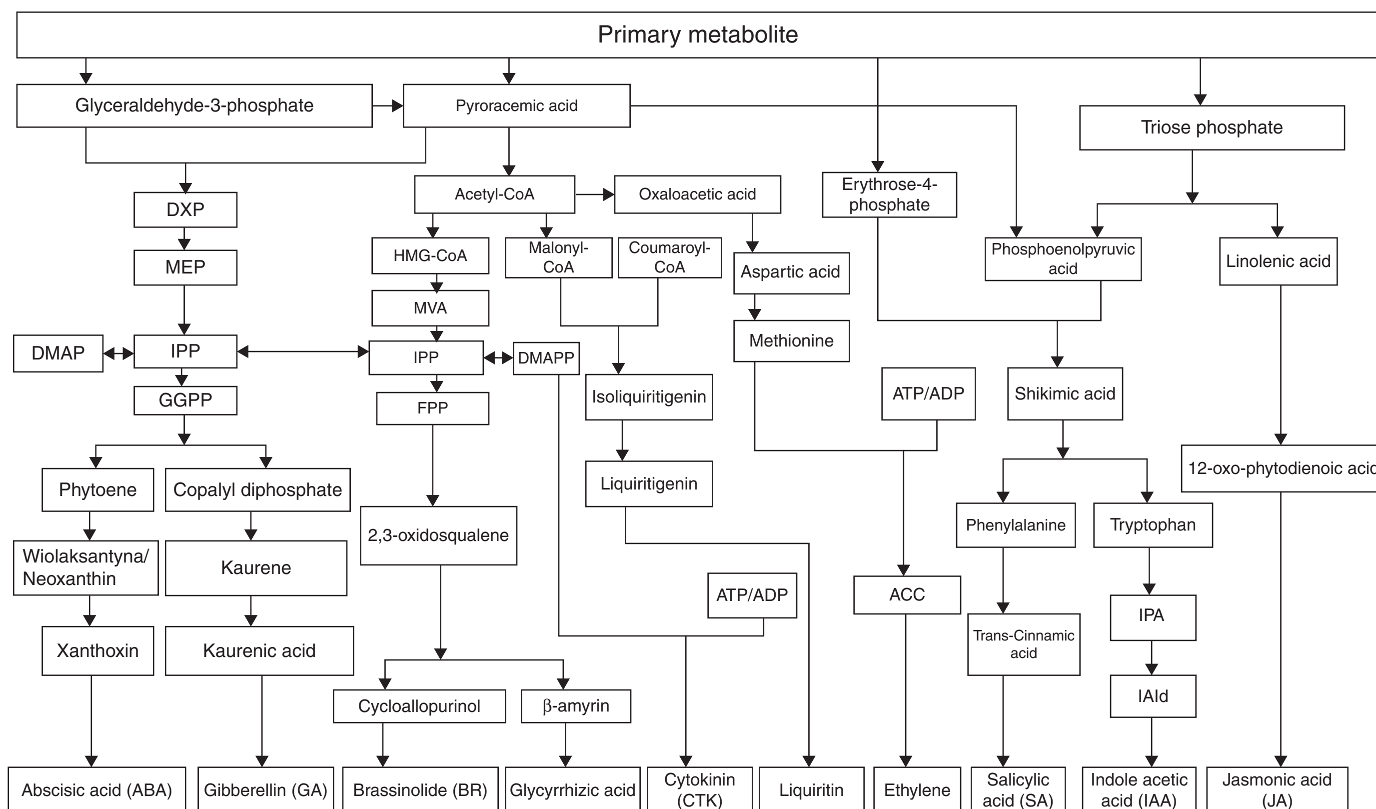

Fig. 1. Theoretically chemical control network based on glycyrrhizic acid in *Glycyrrhiza uralensis* root.

gradient pump, online degasser, and DAD detector. Glycyrrhizic acid (**1**) reference substance was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Batch No. 110731-201317). Reference substances, namely liquiritin (**2**), isoliquiritin (**3**), liquiritigenin (**4**), isoliquiritigenin (**5**) (DingGuoChangSheng), liquiritin apioside (**6**), isoliquiritin apioside (**7**) (Yuanmu), HPLC-grade acetonitrile (Fisher), analytically pure phosphoric acid, and ultrapure water.

Apparatus and conditions

Gradient elution was achieved using a Agilent TC-C18 column (250 mm × 4.6 mm, 5 μm), as follows: 0 min, acetonitrile–0.05% phosphoric acid (20:80); 8 min, acetonitrile–0.05% phosphoric acid (20:80); 30 min, acetonitrile–0.05% phosphoric acid (38:62); 42 min, acetonitrile–0.05% phosphoric acid (50:50). The detected wavelengths were as follows: 237 nm (0–15 min, liquiritin apioside, liquiritin), 365 nm (15–23 min, isoliquiritin apioside, isoliquiritin), 237 nm (23–30 min, liquiritigenin), 370 nm (30–37 min, isoliquiritigenin), 237 nm (37–42 min, glycyrrhizic acid). The column temperature and flow rate were set at 30 °C and 1 ml/min, respectively. In the above mentioned chromatography conditions, theoretical plate number was more than 5000 for glycyrrhizic acid and the degree of separation between adjacent chromatographic peak was greater than 1.5.

The appropriate seven reference substances were measured accurately as standard stock solution, the method consists of adding methanol to 0.2440 mg/ml liquiritin apioside, 0.7500 mg/ml liquiritin, 0.2280 mg/ml isoliquiritin apioside, 0.3300 mg/ml isoliquiritin, 0.2820 mg/ml liquiritigenin, 0.3240 mg/ml isoliquiritigenin and 1.0280 mg/ml glycyrrhizic acid. The seven above mentioned standard stock solutions were measured accurately as 1.00, 1.00, 0.40, 0.60, 0.20, 0.08, 0.80 ml, respectively, and were subsequently transferred in 10 ml volumetric flask. Methanol was added to scale to produce a mixed reference solution per millilitre containing 24.40 μg liquiritin apioside, 75.00 μg liquiritin, 9.12 μg isoliquiritin apioside, 19.80 μg isoliquiritin, 5.64 μg liquiritigenin, 2.59 μg isoliquiritigenin and 246.72 μg glycyrrhizic acid.

Approximately 0.2 g of *G. uralensis* root sample powder (passing 60 mesh) was measured accurately and placed inside a covered conical flask. About 50 ml 70% ethanol was added. The plug was subsequently closed, and the samples were weighed. After 30 min of ultrasonic treatment and cooling, the samples were weighed again. Complement weight loss, filtering and collecting subsequent filtrate passing through a 0.45 μm millipore filter, namely test samples in the present study.

Each hormone-treated *G. uralensis* root sample was used as test sample based on the abovementioned method. Moreover, 10 μl samples were injected at the set HPLC conditions. Thus, the contents of the seven main components were calculated by using the external standard method.

Method inspection of content determination

Different concentrations of the mixed reference solution were accurately measured at 10 μl respectively and injected into HPLC. Injection volume was the abscissa, and the peak area was the ordinate, plotting standard curve and calculating regression equation (Table 1).

G. uralensis root sample powders at 0.2 g were used to make the test solution based on the above method of test sample preparation. Moreover, the test sample was continuously injected six times at the set HPLC conditions. The peak area RSD ($n = 6$) of liquiritin apioside, liquiritin, isoliquiritin apioside, isoliquiritin, liquiritigenin, isoliquiritigenin and glycyrrhizic acid were 1.2%, 1.8%, 1.6%, 1.1%, 1.0%, 1.1%, 0.8%, respectively. These values indicate a good precision of the apparatus.

Six parallel copies of accurately measured sample powders of about 0.2 g in weight were used in the test solution based on the above method of test sample preparation and were injected

Table 1
Regression equation, correlation coefficient and linearity range of the seven main components in *Glycyrrhiza uralensis* root.

Component	Regression equation	*r*	Linearity range (μg/ml)
Liquiritin apioside (**6**)	$Y = 13.905X + 0.8565$	0.9997	2.44–24.4
Liquiritin (**2**)	$Y = 16.152X + 1.9249$	0.9998	7.5–75
Isoliquiritin apioside (**7**)	$Y = 30.667X - 0.3979$	0.9998	0.912–9.12
Isoliquiritin (**3**)	$Y = 35.502X - 0.8873$	0.9997	1.98–19.8
Liquiritigenin (**4**)	$Y = 34.028X - 0.3297$	0.9997	0.564–5.64
Isoliquiritigenin (**5**)	$Y = 69.708X - 0.2655$	0.9997	0.2592–2.592
Glycyrrhizic acid (**1**)	$Y = 5.9346X - 4.7191$	0.9999	24.672–246.72

at the set HPLC conditions. The peak area RSD of liquiritin apioside, liquiritin, isoliquiritin apioside, isoliquiritin, liquiritigenin, isoliquiritigenin and glycyrrhizic acid were 1.9%, 1.5%, 1.8%, 1.1%, 0.6%, 0.9%, 1.2%, respectively, which also indicates repeatability of the method.

The same test solution was injected after the sample was placed at room temperature for 0, 2, 4, 8, 12, 24 h. The peak area RSD of liquiritin apioside, liquiritin, isoliquiritin apioside, isoliquiritin, liquiritigenin, isoliquiritigenin and glycyrrhizic acid were 1.4%, 1.5%, 1.2%, 0.8%, 0.4%, 0.4% and 0.4%. These values indicate that the seven main components in the test solution were stable for 24 h.

Six copies of content-known sample powders were individually and accurately measured to be about 0.2 g. Subsequently, standard stock solutions of liquiritin apioside, liquiritin, isoliquiritin apioside, isoliquiritin, liquiritigenin, isoliquiritigenin and glycyrrhizic acid were precisely added at the following respective volumes: 0.51, 0.78, 0.25, 0.30, 0.14, 0.07 and 1.44 ml. The test solution was made based on the above method of test sample preparation and was injected under set HPLC conditions. Afterwards, the recovery rate was calculated. The average recovery rate ($n = 6$) of the seven above mentioned components were 103.3%, 98.2%, 101.5%, 98.1%, 99.6%, 103.2%, 98.8% and the RSD were 1.9%, 1.0%, 2.9%, 1.5%, 0.6%, 0.5%, and 0.6%.

The chromatogram of reference substance and sample (Fig. 2) indicated that the peak shape and separation were good.

Statistical analyses

Statistical analyses were performed using the SPSS17.0 statistical package. The difference between mean values of each treatment was determined by Duncan's multiple range test and was considered significant at $p < 0.05$. Correlation among components was analysed using Pearson bivariate correlation. Figures were constructed using Excel 2007.

Results

Influence on glycyrrhizic acid accumulation in G. uralensis *root of exogenous hormone treatment.*

Effect of exogenous GA_3 treatment on glycyrrhizic acid content

Root glycyrrhizic acid (**1**) content of *G. uralensis* plants treated with different concentrations of GA_3 in June showed obvious differences (Fig. 3). Glycyrrhizic acid contents of most *G. uralensis* treated with GA_3 were higher compared with control plants in each sampling period. Glycyrrhizic acid contents of *G. uralensis* treated in July also had differences, but were not as obvious as those treated in June. Two batches of *G. uralensis* that were subjected to different GA_3 treatments were compared for glycyrrhizic acid contents. GA_3 at 40 mg/l greatly improved glycyrrhizic acid content in all eight sampling periods, and the difference between treated plants and controls was significant. The contents increased by 22.73%, 12.42%, 34.73%, 69.02%, 22.34%, 14.66%, 36.43% and 12.09%, respectively. Therefore, 40 mg/l exogenous GA_3 could significantly promote glycyrrhizic acid accumulation in one-year-old *G. uralensis* root. In addition, GA_3 treatment in June which was a vigorous growth period of plant was appropriate.

Effect of exogenous IAA treatment on glycyrrhizic acid content

Similar to GA_3, root glycyrrhizic acid contents of *G. uralensis* root treated with different concentrations of IAA in June were obviously different (Fig. 4). Glycyrrhizic acid contents of most IAA-treated plants were higher than control plants in each sampling period. Root glycyrrhizic acid contents of *G. uralensis* plants treated in July also showed differences compared with the control, but the differences were not as obvious as those of plants treated in June. Root glycyrrhizic acid contents of plants treated with IAA at 40 mg/l improved and showed a significant difference compared with controls in the eight sampling periods of two batches. The increase in content for the eight sampling periods were 18.40%, 6.20%, 18.93%, 33.86%, 24.20%, 13.03%, 30.03% and 4.23%, but the increase was less than that observed in GA_3-treated plants. Hence, 40 mg/l exogenous IAA could obviously promote glycyrrhizic acid accumulation in one-year-old *G. uralensis* root, and glycyrrhizic acid accumulation was higher when exogenous hormone treatments were conducted in June than in July.

Effect of exogenous MeJa treatment on glycyrrhizic acid content

In general, root glycyrrhizic acid content of *G. uralensis* treated with different concentrations of MeJa in June obviously increased compared to controls in each period, but treated in July was not that obvious (Fig. 5). MeJa at 100 mg/l applied in June obviously increased glycyrrhizic acid content in the five sampling periods, and the differences were significant. The percentages of increase were 21.36%, 32.79%, 11.65%, 46.40% and 37.84%. In plants treated in July, MeJa at 25 mg/l obviously influenced glycyrrhizic acid content, and the percentages of increase in content were 23.11%, 63.00% and 1.46% in the three sampling periods. Therefore, exogenous MeJa at 100 mg/l and 25 mg/l treatments in mid-to-late of June and July, respectively could significantly promote glycyrrhizic acid accumulation in one-year-old *G. uralensis* root. In addition, similar to GA_3 and IAA treatments, MeJa application in June was more appropriate.

Correlation between the main components and glycyrrhizic acid content in G. uralensis *root*

Correlation analyses (Table 2) indicated that liquiritin (**2**) content had significant positive correlation with glycyrrhizic acid (**1**) content at 0.01 level ($r = 0.799$), which was statistically significant because of $p = 0.000 < 0.01$. Isoliquiritin (**3**), $r = 0.725$ and $p = 0.000 < 0.01$, was significantly and positively correlated with glycyrrhizic acid at 0.01 level. Isoliquiritin apioside (**7**) had a positive and statistically significant correlation with glycyrrhizic acid content at 0.01 level, $r = 0.418$, $p = 0.000 < 0.01$. In addition, liquiritin apioside (**6**) and glycyrrhizic acid contents were also positively and statistically significantly correlated at 0.05 level, $r = 0.222$ and $p = 0.023 < 0.05$. Therefore, the main components that were quantified in *G. uralensis* root were positively correlated with glycyrrhizic acid content including liquiritin, isoliquiritin, isoliquiritin apioside and liquiritin apioside.

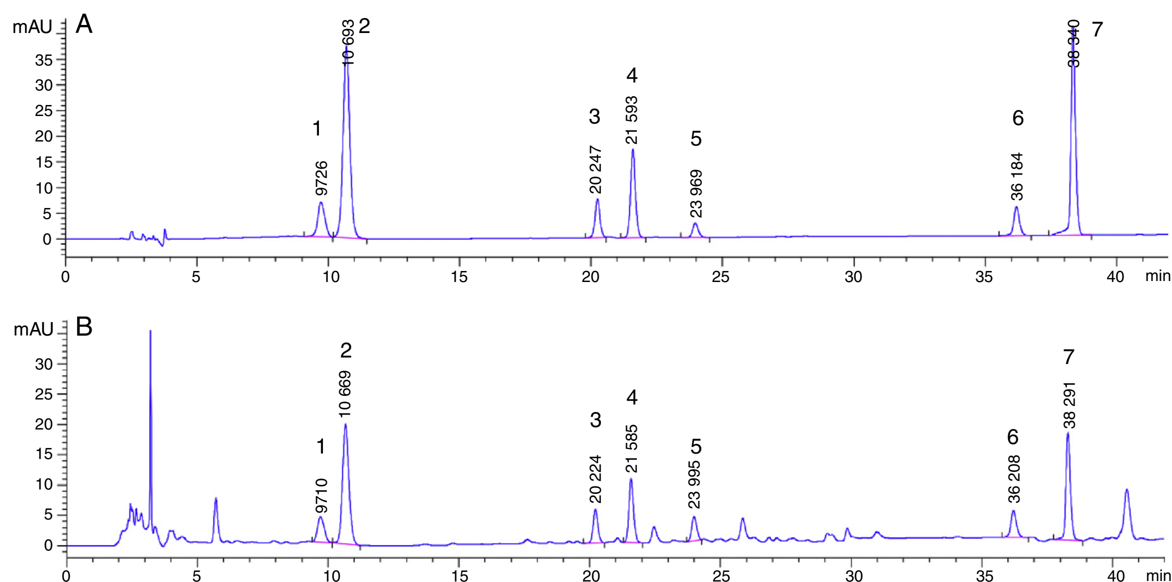

Fig. 2. HPLC chromatogram of the mixed reference substance (A) and sample (B). (**1**) Liquiritin apioside, (**2**) liquiritin, (**3**) isoliquiritin apioside, (**4**) isoliquiritin, (**5**) liquiritigenin, (**6**) isoliquiritigenin and (**7**) glycyrrhizic acid.

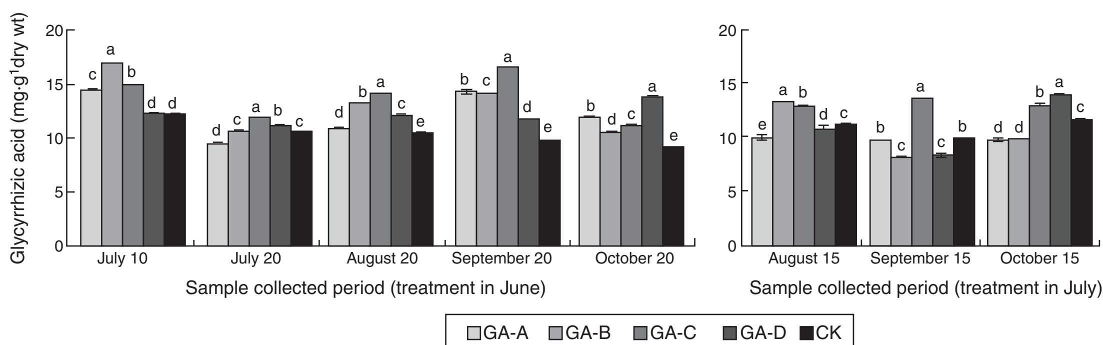

Fig. 3. The effect of GA_3 treatment on glycyrrhizic acid content in *G. uralensis* root (GA-A, GA-B, GA-C and GA-D stand for 15, 25, 40 and 100 mg/l GA_3 treatment, respectively. Different letters followed by mean ± standard error of three replicates indicated significant differences at $p < 0.05$).

Discussion

Currently, wide-ranging studies on MeJa's influence on *G. uralensis* have been conducted. Shabani et al. (2009) found that MeJa treatment increased glycyrrhizic acid content in 65-day-old *in vitro* plantlet roots of *G. uralensis*, but restrained root growth. These results were consistent with our study results. Besides the influence of MeJa on glycyrrhizic acid (**1**), Yang et al. (2008) indicated MeJa treatment improved total flavonoids production in suspension culture cells of *Glycyrrhiza inflata* but restrained cells growth. Hayashi et al. (2003) reported that MeJa promoted soya saponin accumulation because it is connected with a key enzyme activity that could improve biosynthesis or gene expression quantity. In the endogenous chemical components network of *G. uralensis*, many studies confirmed that liquiritin, a major flavonoid component, was significantly positively correlated

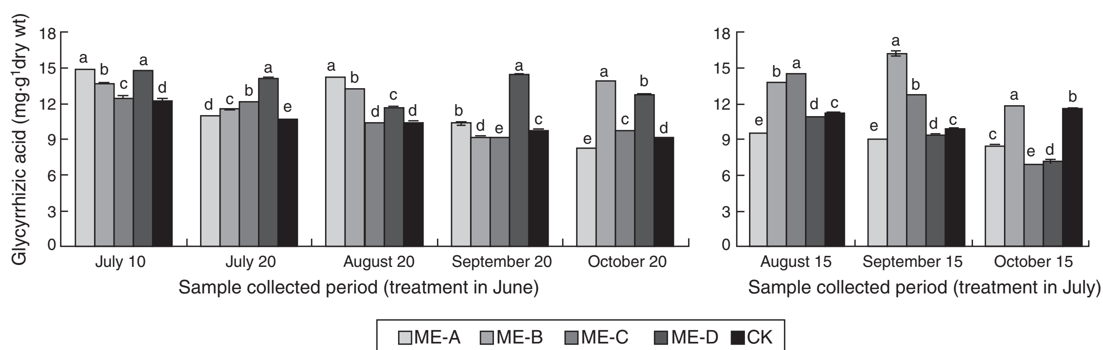

Fig. 4. The effect of IAA treatment on glycyrrhizic acid content in *G. uralensis* root (IAA-A, IAA-B, IAA-C and IAA-D stand for 15, 25, 40 and 100 mg/l IAA treatments, respectively. Different letters followed by mean ± standard error of three replicates indicated significant differences at $p < 0.05$).

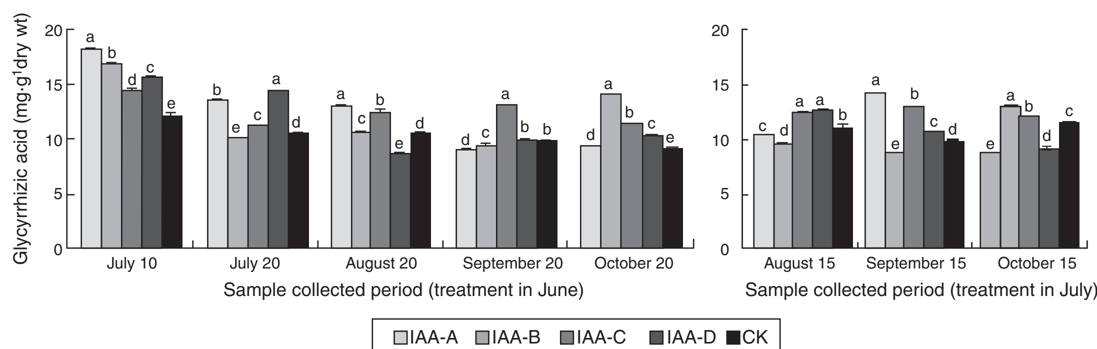

Fig. 5. The effect of MeJa treatment on glycyrrhizic acid content in *G. uralensis* root (MeJa-A, MeJa-B, MeJa-C and MeJa-D stand for 15, 25, 40 and 100 mg/l MeJa treatments, respectively. Different letters followed by mean ± standard error of three replicates indicated significant differences at $p < 0.05$).

with glycyrrhizic acid (Guo et al., 2014), which was consistent with the results obtained in the present study. Other than secondary metabolites like phytohormones and medicinal components, primary metabolites of medicinal plants were also essential 'sources' in the chemical network. Many researchers have studied physicochemical properties and functional characteristics of polysaccharide in *G. uralensis* (Wan and Cheng, 2009), and reported that starch content had a positive correlation with glycyrrhizic acid (Liu et al., 2009). All these data laid a solid foundation for establishing a chemical regulation network based on glycyrrhizic acid.

Chemical regulation, one of the main control technologies of crops and medicinal plants in the recent years, could adjust plant growth and metabolism by changing the endogenous hormone system. This study investigated the effects of different hormone treatments (GA_3, IAA and MeJa), times of application, concentration and sample collection period on root glycyrrhizic acid content. Phytohormones, appropriate concentration and treatment time, which could significantly affect glycyrrhizic acid content were primarily defined, providing a significant guide for producing and cultivating medicinal plants. Changes of glycyrrhizic acid content showed some regularities under different hormone treatments and sampling times. Parallel conventional cultivation and hormone treatments as well as content determination of group sampling were maximum reduced error, thereby ensuring the accuracy and reliability of experimental results. Results from different time nodes showed the whole trend was that most glycyrrhizic acid content in *G. uralensis* root at first sampling time after hormone treatment obviously increased then evidently declined, after which content increased again and tended to steady. Finally, a slight drop in content was observed. The possible reason for this phenomenon was that the hormone treatment stimulated the plant, thereby inducing stress response and metabolising more secondary products. Afterwards, a large amount of hormones was absorbed, thereby accelerating metabolism or the mobilisation of reserves in plants and leading to the decrease in hormone content. Then, a relatively balanced state is reached through autogenous regulation, and a great increase of glycyrrhizic acid content at this period confirmed that phytohormones could promote accumulation of glycyrrhizic acid. As growth period progressed, during which metabolism shifted to dormancy period, synthesis rate of secondary metabolites in plant also gradually decreased. Results from different concentrations of exogenous hormones indicated too-low concentrations weakly affected glycyrrhizic acid content and showed shorter maintenance time than the optimum concentration. Too-high concentrations of hormones likely inhibited growth. The optimum concentration and treatment time varied according to plant species. The chemical control network based on glycyrrhizic acid in root of *G. uralensis* should include GA_3, IAA, MeJa and liquiritin, isoliquiritin, isoliquiritin apioside and liquiritin apioside. Besides the three kinds of hormones studied, ABA, cytokinin (CTK), brassinolide (BR) have also been investigated in preliminary experiments, and results indicated appropriate concentration of ABA and CTK could significantly improved glycyrrhizic acid content. However, the effects of different hormone treatments applied at different times on *G. uralensis*, endogenous hormones levels after exogenous hormones absorbed by leaves and transited to root and on the glycyrrhizic acid anabolism mechanism need to be studied further from the perspectives of plant physiology, phytohormones synthetic pathway and action mechanism and biosynthetic pathways.

Plant is an organic unity, and metabolic pathways are not isolated. Because of the close relationship among various metabolic pathways, the change in activity of one metabolic pathway could obviously affect the other metabolic pathways, thereby resulting in the final content. Researchers have reported that improvement in the functional gene expression quantity of one metabolic pathway could enhance the others', thereby increasing the content of the end product. More interestingly, improved functional gene expression quantity of one metabolic pathway could also reduce that of other pathways, thereby leading to the decrease in the content of the end product. Regulatory mechanisms of glycyrrhizic acid theoretical core network could be categorised into two kinds, as follows: each branch pathway in the network enjoys a common substrate, and change of different branch metabolic fluxes could control glycyrrhizic acid content. Endogenous chemical component content transformation in *G. uralensis*, especially when the concentration of a phytohormone is maintained at a high level for a long time because of gene polymorphism, thus influencing biosynthesis

Table 2
Correlation among the main components and glycyrrhizic acid content in *Glycyrrhiza uralensis* root.

Glycyrrhizic acid (**1**)	Liquiritin (**2**)	Isoliquiritin (**3**)	Isoliquiritin apioside (**7**)	Liquiritin apioside (**6**)	Liquiritigenin (**4**)	Isoliquiritigenin (**5**)
Pearson correlation significance (bilateral)	0.799[a]	0.725[a]	0.418[a]	0.222[b]	0.095	−0.045
	0.000	0.000	0.000	0.023	0.338	0.654
n	104	104	104	104	104	104

[a] Significant correlation at 0.01 level (bilateral).
[b] Significant correlation at 0.05 level (bilateral).

enzyme activity of glycyrrhizic acid and regulating glycyrrhizic acid content. Studying functional gene polymorphism in the core control network of active components content could entirely uncover genetic mechanisms underlying the changes in the active component concentration in medicinal plants.

Authors' contribution

YL and CY, as joint first authors, designed the study and wrote the manuscript under the guidance of CL. YL, JQ and YZ performed the experiments. LW and XZ was in charge of culturing *G. uralensis* plants. YX and GR were responsible for statistical analysis. All authors contributed extensively to the work presented and approved this manuscript.

Conflicts of interest

The author declares no conflicts of interest.

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