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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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ABSTRACT

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.

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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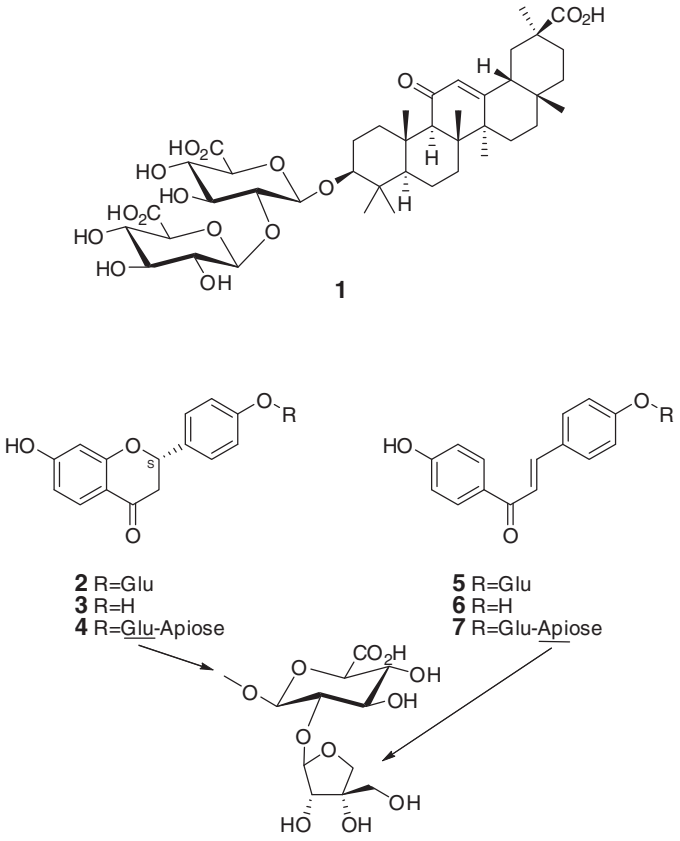

Currently, various chemical and physical factors affect the medicinal plant growth and secondary metabolite production had been researched widely. Moisture (Li et al., 2011), light (Hou et al., 2010; Afreen et al., 2005), salt (Wan et al., 2011), mineral elements (Yin et al., 2014; Wang et al., 2010; 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