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Distribution patterns for metabolites in medicinal parts of wild and cultivated licorice

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ABSTRACT

Licorice is a famous Chinese medicinal material widely applied worldwide in food and drugs. It possesses a substantial share in the international and domestic markets. While the demand is continuously increasing, wild-type of licorice is gradually disappearing. Its cultivated-type is significantly different compared to its wild-type, especially the bioactive compounds in dried roots and rhizomes (the medicinal parts of wild and cultivated licorice) are responsible for their quality difference. In this study, a total of thirty-two constituents, including seventeen secondary compounds and fifteen primary metabolites, were simultaneously analyzed by UFLC coupled with triple quadrupole-linear ion trap mass spectrometry (UFLC-QTRAP-MS/MS) in different medicinal parts of licorice. Our findings indicated that the content of major bioactive compounds in wild licorice were significantly higher than those in its cultivated-type. The metabolites in rhizomes showed totally different outcomes between wild and cultivated licorice. Remarkably high level of some important amino acids related to abiotic stress (drought and salt) were found in wild licorice. Notably, the high contents of phenylalanine and compounds in upstream of flavonoid synthesis in cultivated licorice suggested that the synthesis may differ at the initial stage. The distribution pattern of metabolites in different medicinal parts of wild and cultivated licorice will not only provide a novel clue in agricultural breeding but also facilitate the further study on their quality formation.
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1. Introduction

The application of licorice could date back to 4000 years ago in China. It is originally recorded in Shennong Materia Medica [1]. More than 60% of the prescriptions of traditional Chinese medicine contain licorice because of its importance in clinic [2,3]. In addition to the prescriptions requiring large amounts of licorice, glycyrrhizin, the most important compound with highest content in licorice, has great commercial value and is often used as sweetener and flavoring agent in baked goods, frozen dairy products, beverages, and light industrial products all over the world [4]. Currently, there are eight Glycyrrhiza species in China, and among them only three species (*Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat., and *Glycyrrhiza glabra* L.) are recorded in Chinese Pharmacopoeia (2015 edition) as the origins of medicinal licorice [5]. In the light of the initial description on plant morphology and the legendary depiction, the majority of licorice described in ancient books is *Glycyrrhiza uralensis* Fisch., which is also the mainstream species and holds a large proportion of more than 90% of licorice production [6]. At present as a result of excessive and unsustainable harvesting, the wild resources of licorice are rapidly decreasing. The cultivated licorice is widely distributed in the northwest of China [7]. In the therapy, the dried roots and rhizomes are the primarily medicinal parts of licorice [8]. There is almost no differentiated treatment to the medicinal parts even though the morphological properties of the rhizomes of the cultivated licorice have changed a lot and the quality of cultivated-type has already declined. Under extreme living environment, wild licorice has been enduring mainly drought and salt stress for the whole lifespan. On the contrary, cultivated licorice may suffer less with relatively rich fertilizer which leads to

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remarkably different morphological properties and quantity of primary and secondary metabolites. Therefore, the investigation of the quality formation of licorice and the breeding of the high-quality cultivated strain should be put on agenda.

To investigate the difference of the quality formation between wild and cultivated licorice, a total of thirty-two constituents consisting of the major active compounds (triterpene saponins and flavonoids) and the key amino acids against abiotic stress were simultaneously determined by UFLC-QTRAP-MS/MS. The results showed that in general both primary and secondary metabolites accumulated more in wild-type than in cultivated-type. Contents of metabolites in two ecotypes of licorice showed the opposite results and the rhizomes of the cultivated licorice seemed morphologically quite different from those of the wild licorice. Contents of metabolites in different parts of roots tended to the same levels except for the middle part, which could be explained by its supporting and sending nutrients to the whole plant body. Our investigation will help to understand the formation law of licorice quality under abiotic stress and provide a scientific basis for the breeding of the high-quality cultivated licorice.

2. Materials and methods

2.1. Materials and chemicals

Five batches of more than four-year-old *Glycyrrhiza uralensis* Fisch (both cultivated and wild plants) were collected in September 2017 in Yanchi County, Ningxia Province, China (E107.41°, N37.78°). Each of them was carefully washed and divided into five segments: three different parts of taproots (upper, middle, and lower), lateral roots and rhizomes (Fig. 1). The botanical origins of the materials were identified by Professor Xunhong Liu. (Department for Authentication of Chinese Medicines, School of Pharmacy, Nanjing University of Chinese Medicine, PR China), Voucher specimens were deposited at Herbarium in School of Pharmacy, Nanjing University of Chinese Medicine.

Thirty-two chemical standards of glycyrrhizic acid (1), neoliquiritin (6), ononin (9), isoliquiritigenin (12), formononetin (14), licochalcone B (15), licoflavone A (10), and 4′,7-dihydroxyflavone (16) were purchased from Chengdu Chroma-Biotechnology Co., Ltd. (Chengdu, PR China); liquiritin (2), liquiritigenin (8), licochalcone A (13), glycyrrhetinic acid (17), and echinatin (11) were obtained from Liangwei Chemical Reagent Co., Ltd. (Nanjing, PR China); isoliquiritin (3) and neoisoliquiritin (7) were offered by Chengdu Purechem-Standard Co., Ltd. (Chengdu, PR China); liquiritin apioside (4) and isoliquiritin apioside (5) were brought from Nanjing Jingzhu Bio-technology Co., Ltd. (Nanjing, PR China). All amino acids were provided by Shanghai Yuanye-Biotechnology Co., Ltd. (Shanghai, PR China). The purities of all chemical standards were greater than 98% by HPLC analysis. HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Milli-Q purifying system was used to obtain ultrapure water (Millipore, Bedford, MA, USA). All other chemicals used in the experiments were of analytical reagent grade (Shanghai Yuanye-Biotechnology Co., Ltd., PR China).

2.2. Preparation of standard and sample solutions

Thirty-two reference compounds were prepared by completely dissolving in 70% methanol and their concentrations were as follows: (1) 10.05 mg mL^{-1}; (2) 5.190 mg mL^{-1}; (3) 5.088 mg mL^{-1}; (4) 1.060 mg mL^{-1}; (5) 1.060 mg mL^{-1}; (6) 1.175 mg mL^{-1}; (7) 0.925 mg mL^{-1}; (8) 1.093 mg mL^{-1}; (9) 1.22 mg mL^{-1}; (10) 1.005 mg mL^{-1}; (11) 1.12 mg mL^{-1}; (12) 0.965 mg mL^{-1}; (13) 1.225 mg mL^{-1}; (14) 1.330 mg mL^{-1}; (15) 1.16 mg mL^{-1}; (16) 1.71 mg mL^{-1}; (17) 1.42 mg mL^{-1}; (18) 5.087 mg mL^{-1}; (19) 4.096 mg mL^{-1}; (20) 1.087 mg mL^{-1}; (21) 0.992 mg mL^{-1}; (22) 1.086 mg mL^{-1}; (23) 1.022 mg mL^{-1}; (24) 1.061 mg mL^{-1}; (25) 0.8320 mg mL^{-1}; (26) 1.004 mg mL^{-1}; (27) 1.017 mg mL^{-1}; (28) 0.7570 mg mL^{-1}; (29) 0.5680 mg mL^{-1}; (30) 1.103 mg mL^{-1}; (31) 1.084 mg mL^{-1}; (32) 0.8770 mg mL^{-1}. The mixed standard stock solution containing thirty-two reference compounds was further diluted with 70% methanol for the establishment of the calibration curves. All solutions were stored at 4° before analysis.

All samples were pulverized and screened through the 60-mesh sieve. Approximately 0.5 g of each sample was weighed and extracted by ultra-sonication in 25 mL 70% methanol for 1 h. The mixture was centrifuged at 12,000 rpm for 10 min. The supernatant of mixture was collected, diluted tenfold and filtered through 0.22 μm membrane prior to UFLC-QTRAP-MS/MS analysis.

2.3. UFLC-QTRAP-MS/MS conditions

UFLC-QTRAP-MS/MS analysis was performed on a triple quadrupole-linear ion trap mass spectrometer (QTRAP-4500) (AB Sciex, Framingham, MA, USA). The MS was equipped with an electrospray ionization (ESI) source operating in multiple-reaction monitoring (MRM) and under both positive and negative ion modes. The MS parameters were set as follows: gas temperature, 550°; pressures of nebulizer of MS, 5500 V (positive) and −4500 V (negative); GSI flow, 65 L min^{-1}; CUR flow, 30 L min^{-1}. All MS data were acquired using the Analyst 1.6.3 software to

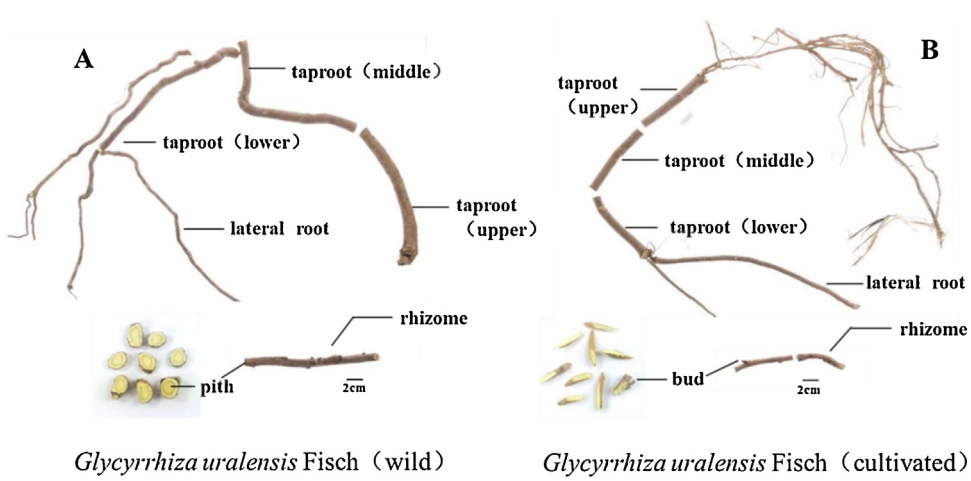

Fig. 1. Five segments (upper of taproot, middle of taproot, lower of taproot, lateral root, rhizome) of *Glycyrrhiza uralensis* Fish. (A) wild (B) cultivated.

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