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Relationships between the bioactive compound content and environmental variables in *Glycyrrhiza uralensis* populations in different habitats of North China

Relaciones entre el contenido de compuestos bioactivos y las variables ambientales en *Glycyrrhiza uralensis* en diferentes hábitats del Norte de China

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Abstract. The content of active compounds in *Glycyrrhiza uralensis* may vary among populations in different regions, and be influenced by environmental variables. We determined the effects of soil and climate on contents of glycyrrhizic acid and liquiritin in various populations of *Glycyrrhiza uralensis*. Fifty individuals from 5 rangeland populations in arid and semi-arid regions of North China were collected and analyzed. The contents of glycyrrhizic acid and liquiritin were determined using the HPLC method. Contents of glycyrrhizic acid and liquiritin varied significantly among populations as follows: Chifeng > Hangjinqi > Minqin > Aletai > Kashi. These contents were significantly correlated with soil nutrients (e.g. P, K, N), organic matter, and annual precipitation. However, soil P and K, and precipitation were the key factors influencing the contents of active compounds. Environmental variables can be useful to predict the contents of glycyrrhizic acid and liquiritin in *Glycyrrhiza uralensis*.

Keywords: Licorice; Glycyrrhizic acid; Liquiritin; Soil nutrients; Precipitation; *Glycyrrhiza uralensis*; China.

Resumen. El contenido de compuestos activos en *Glycyrrhiza uralensis* puede variar entre poblaciones en diferentes regiones, y estar influenciado por las variables ambientales. Se determinaron los efectos del suelo y el clima en los contenidos de ácido glycyrrhizic y liquiritin en varias poblaciones de *Glycyrrhiza uralensis*. Se muestrearon y analizaron 50 individuos de 5 poblaciones naturales en las regiones árida y semiárida del Norte de China. Los contenidos de ácido glycyrrhizic y liquiritin se determinaron usando el método HPLC. Dichos contenidos variaron significativamente entre poblaciones de la siguiente manera: Chifeng > Hangjinqi > Minqin > Aletai > Kashi. Estos contenidos estuvieron correlacionados significativamente con los nutrientes del suelo (ej., P, K, N), la materia orgánica, y la precipitación anual. Sin embargo, el P y K del suelo y la precipitación fueron los principales factores que influenciaron el contenido de los compuestos activos. Las variables ambientales pueden ser útiles para predecir los contenidos de ácido glycyrrhizic y liquiritin en *Glycyrrhiza uralensis*.

Palabras clave: Regaliz; Ácido glycyrrhizic; Liquiritin; Nutrientes del suelo; Lluvias; *Glycyrrhiza uralensis*; China.

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INTRODUCTION

Licorice is the dry roots and rhizomes of *Glycyrrhiza* plants, which belong to the Leguminosae. Licorice is one of the most useful Chinese herbal medicines; licorice extracts are widely used as cosmetics, food additives, tobacco flavors, and confectionery foods. Furthermore, *Glycyrrhiza* plants are used for land reclamation in arid and semi-arid areas of China (Zhou, 2006; Zhang & Chen, 2007). Uses of licorice in medicine include several chemicals with biological activities: glycyrrhizin, liquiritin, flavonoids, coumarins, polysaccharides and alkaloids. It also has pharmacological effects against pain, cough, inflammatory processes, allergy, detoxification, HIV, etc. (Hu & Shen, 1995; Shen et al., 2003; Ji et al., 2004). Among these compounds, glycyrrhizic acid and liquiritin are commonly used to evaluate licorice quality (Zhou, 2006; Hayashi & Sudo, 2009).

Three species, *Glycyrrhiza uralensis, G. inflata* and *G. glabra* are used as medicinal resources in China, but Ural licorice (*G. uralensis*) is the most abundant (Zhao et al., 2006). The annual production of herbal medicine from this species is over 60000 ton in China. *Glycyrrhiza uralensis* is widely distributed from east to west in north China (Zhang et al., 2006). There were many studies on *G. uralensis* in the past decade, most of which concentrated on the classification and identification of licorice (Hu & Shen, 1995; Zhang et al., 2001; Pan & Zhang, 2002); cultivation techniques (Zhou, 2003, 2006); chemical composition, and separation and effective biological components of licorice extracts (Hurst et al., 1983; Sun et al., 2003; Sabbioni et al., 2005; Zhang & Ye, 2009). The effects of environmental variables on the content of active chemicals have not been sufficiently studied in licorice. The present study reports on the relationships between contents of glycyrrhizic acid and liquiritin in *G. uralensis versus* various climate and soil variables. The objective of this study was: to test the hypothesis that (1) populations in different environments have different contents of glycyrrhizic acid and liquiritin; and (2) contents of glycyrrhizic acid and liquiritin are correlated with some important factors. Additionally, models to predict contents of glycyrrhizic acid and liquiritin using environmental variables were established.

MATERIALS AND METHODS

Sampling. After a general distribution survey of *G. uralensis*, five study sites were established: (1) Chifeng (in Inner Mongolia Autonomous Region); (2) Hengjinqi (in Inner Mongolia Autonomous Region); (3) Minqin (in Ganshu Province); (4) Aletai (in Xinjiang Autonomous Region), and (5) Kashi (in Xinjiang Autonomous Region) (Fig. 1). These sites included various plant populations exposed to different climate and soil conditions (Tables 1 and 2). The area of each sampling site was about 40 ha. Ten quadrats of 1 m × 1 m were randomly established at each site (Zhang, 2004). Whole plants of *Glycyrrhiza uralensis* (including roots and rhizomes) were collected and stored in zip-lock, plastic bags. A total of 50 individuals were collected from the five study sites.

Fig. 1. Locations of sampling sites for the five populations of *Glycyrrhiza uralensis* in North China. Chifeng and Hangjinqi were in Inner Mongolia autonomous region, Minqin in Ganshu province and Aletai and Kashi in Xingjiang autonomous region.
Fig. 1. Ubicación de los sitios de muestreo para las cinco poblaciones de *Glycyrrhiza uralensis* en el Norte de China. Chifeng y Hangjinqi se encuentran en la región autónoma más interna de Mongolia, Minqin en la Provincia de Ganshu, y Aletai y Kashi en la región autónoma de Xingjiang.

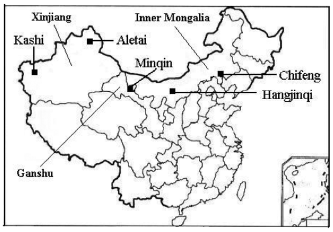

Table 1. Climate characteristics of different study sites of *G. uralensis* in North China (average values of 1980 - 2007).
Tabla 1. Características climáticas de diferentes sitios de estudio de *G. uralensis* en el Norte de China (valores promedio para el período 1980-2007).

Sites	Annual mean temperature (°C)	Annual mean relative humidity (%)	Annual mean precipitation (mm)	Annual sunshine hours (h)	Annual mean highest temperature (°C)	Annual mean lowest temperature (°C)
Chifeng	7.7	49	366.5	2836.9	14.7	1.8
Hangjinqi	7.5	46	261.8	2922.3	14.5	1.3
Minqin	8.7	44	209.0	3135.6	16.3	1.9
Aletai	4.8	59	112.0	2999.7	11.0	-1.0
Kashi	12.2	52	67.3	2766.3	18.6	5.9

Table 2. Soil characteristics of different study sites of *G. uralensis* in North China.
Tabla 2. Características del suelo de diferentes sitios de estudio de *G. uralensis* en el Norte de China.

Sites	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	pH	Organic matter (%)
Chifeng	20.4	1.1	49	8.7	0.383
Hangjinqi	14.1	2.5	104	8.8	0.278
Minqin	66.4	19.2	493	8.7	2.18
Aletai	35.8	8.8	241	8.6	0.976
Kashi	27.4	10.5	128	8.4	0.538

A soil sample 30 cm in depth was taken in each quadrat using a small spade. Thereafter, soil samples were taken to laboratory for chemical analysis. They were air-dried and analyzed in the laboratory for soil pH, organic matter, available N, available P and available K. A 1:2.5 ratio of soil to distilled water suspension was used to measure pH using a Whatman pH sensor meter. Nitrogen was determined following Kjeldahl, and phosphorus was measured via the $HCLO_4$-H_2SO_4 colorimetric method (molybdovanadate method). Potassium was measured using an Atomic Absorption Spectrophotometer, and organic matter was determined using the method of $K_2Cr_2O_7$ – capacitance. Climatic data from the nearest weather station to each site were used.

Measurement of glycyrrhizic acid and liquiritin. *Plant sample preparation*. Dried roots of *Glycyrrhiza uralensis* were crushed. Samples (0.2 g) were extracted with 25mL ethanol (70%) for 30 min by ultrasonic treatment. The residue was filtered using a 0.45 μm microporous membrane.

Equipment and Chemicals. Chromatographic separations were carried out using a HP1100 liquid chromatography (Agilent Technologies). It was equipped with an automatic injector and a Luna C18 (2) column (250 × 4.6 mm, 5.0 mm, Phenomenex, Torrance, CA, USA). An Alltech ELSD2000ES (Alltech, Deerfield, IL, USA) and Agilent 35900E signal transmitter (Agilent Technologies) were also used.

Glycyrrhizae and compound licorice tablets were purchased from a local market. Glycyrrhizic acid monoammonium salt trihydrate ($C_{42}H_{65}O_{16}$·$3H_2O$, MW 894.03, 98%) was purchased from the Acros Organics. Liquiritin ($C_{21}H_{22}O_9$, MW 418.39, 98%) was purchased from the National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine. Acetonitrile, acetic acid, ethanol, and water were of HPLC grade. Other chemicals were of analytical reagent grade (Shen et al., 2006).

Chromatographic Conditions. The optimizing conditions were obtained by a linear gradient elution. The mobile phase was composed of acetonitrile and 3.0% aqueous acetic acid at a flow rate of 1.0 mL/min in a gradient elution. The drift tube temperature of the ELSD was set at 105 °C, and the gas flow rate was 2.8 L/min (Shen et al., 2006).

Calibration Curves and Linearity. Glycyrrhizic acid monoammonium salt (3.1 mg) and liquiritin (4.17 mg) were accurately weighed and dissolved into volumetric flasks with 10 mL ethanol (70%) to make a mixed reference substance solution. It contained 0.0834 mg/mL liquiritin and 0.31 mg/mL glycyrrhizic acid monoammonium salt. Solutions were sonicated for 5 min to ensure complete dissolution. Calibration standards were prepared by diluting the solution with ethanol in appropriate concentrations. The calibration curves were generated by plotting the logarithm of peak areas *versus* the logarithm of the mass of each chemical (Shen et al., 2006).

Precision and Accuracy. Precision was expressed as the percentage relative to the standard deviation (RSD) of the data for five injections for each chemical. The assays to evaluate accuracy and precision were preformed at different mass levels in five replicate injections. All percent mean deviations were calculated to evaluate accuracy; this was determined as the difference between the calculated mean mass (from the calibration curves) and the actual mass. Deviations were 2.08% for liquiritin and 1.47% for glycyrrhizic acid.

Intra-day variations were chosen to determine precision and stability. Approximately 0.2 g of dried and screened *Glycyrrhiza uralensis* licorice were weighed, extracted, and analyzed as described previously. Samples were analyzed in triplicate, six times (at 0, 2, 4, 8, 12, 24h) within one day. The RSD deviations were 2.45% for liquiritin and 1.95% for glycyrrhizic acid.

Contents of liquiritin and glycyrrhizic acid in the extracted licorice root solution were calculated according to their corresponding calibration curves.

RESULTS

Variation between populations. Contents of liquiritin and glycyrrhizic acid varied greatly (Table 3). One-way ANOVA showed that content differences of liquiritin (F = 3.533, $p<0.05$) and glycyrrhizic acid (F = 5.324, $p<0.01$) were significant (Table 4).

Contents of liquiritin varied from 0.83% (Kashi) to 1.99% (Chifeng), and followed the order Chifeng > Hangjinqi > Minqin > Aletai > Kashi. Populations of Chifeng, Hangjinqi and Minqin were comparatively rich in liquiritin (> 1%: the standard content requirements of the China National Formulary); populations of Kashi and Aletai had lower liquiritin contents than this standard.

Contents of glycyrrhizic acid varied from 3.62% (Hangjinqi) to 6.93% (Chifeng), and followed the order Chifeng >

Aletai > Kashi > Minqin > Hangjinqi. All populations were rich in glycyrrhizic acid which exceeded 2%, the content requirement of China National Formulary.

Effects of environmental factors. Populations in different habitats had different environmental conditions which might affect contents of active compounds. For soil variables, contents of liquiritin were significantly correlated with soil N, P, K and organic matter, and those of glycyrrhizic acid were closely related to soil P and K (Table 5). This suggests that physicochemical soil properties are important to the accumulation of active compounds in natural populations of *G. uralensis*. Soil pH had no significant effect on these contents.

Only contents of liquiritin were significantly correlated with annual precipitation (Table 6). This suggests that water level is an important factor affecting contents of that active compound. The remaining climate variables had no effects (p>0.05) on contents of glycyrrhizic acid and liquiritin (Table 6).

Tents of liquiritin and glycyrrhizic acid with environmental variables, linear regression models were established. In the following models, LQ refers to liquiritin; GA to glycyrrhizic acid; and AP to annual precipitation; SN, SP, SK and OM to soil N, P, K and organic matter, respectively.

LQ = 1.879 – 0.470 SN (t = -3.684, p = 0.0006)
LQ = 1.751 – 0.535 SP (t = -4.391, p = 0.0001)
LQ = 1.682 – 0.488 SK (t = -3.871, p = 0.0003)
LQ = 1.677 – 0.442 OM (t = -3.413, p = 0.0013)
GA = 5.236 – 0.327 SP (t = -2.399, p = 0.020)
GA = 5.117 – 0.276 SK (t = -1.989, p = 0.046)
LQ = 0.672 + 0.004 AP (t = -4.481, p = 0.0001)
GA = 3.1032 + 0.0072 AP (t = -1.898, p = 0.0501)

These models might be used to predict the contents of liquiritin and glycyrrhizic acid using the corresponding environmental variables.

Table 3. Content of glycyrrhizic acid and liquiritin on *G. uralensis* populations determined by the HPLC method in North China. Values are mean ± 1 standard deviation of n=10.
Tabla 3. Contenidos de ácido glycyrrhizic y liquiritin en poblaciones de *G. uralensis* determinadas por el método HPLC en el Norte de China. Los valores son promedio ± 1 desviación estándar de n=10.

Sites	Liquiritin (%)	Glycyrrhizic acid (%)
Chifeng	1.99 ± 0.7460a	6.93 ± 1.9363a
Hangjinqi	1.53 ± 0.3145b	3.62 ± 0.7235b
Minqin	1.20 ± 0.6317bc	3.85 ± 0.9877b
Aletai	0.87 ± 0.2113cd	4.29 ± 0.6780c
Kashi	0.83 ± 0.3614d	4.14 ± 1.3116c

Table 4. One-Way ANOVA for contents of glycyrrhizic acid and liquiritin on *G. uralensis* populations in North China. (n=50).
Tabla 4. ANOVA Simple para los contenidos de ácido glycyrrhizic y liquiritin en poblaciones de *G. uralensis* en el Norte de China (n=50).

Compound	Variance	Sum of squares	Degree of freedom	Mean square	F	p
Liquiritin	Inter-populations	5.616	4	1.404	3.533	0.020
	Inner-populations	9.935	25	0.397		
	Total	15.551	29			
Glycyrrhizic acid	Inter-populations	43.507	4	10.877	5.324	0.003
	Inner-populations	51.078	25	2.043		
	Total	94.584	29			

Table 5. Correlation coefficients between contents of glycyrrhizic acid or liquiritin and soil variables for *G. uralensis* populations in North China (n = 50).
Tabla 5. Coeficientes de correlación entre los contenidos de ácido glycyrrhizic o liquiritin y las variables del suelo en poblaciones de *G. uralensis* en el Norte de China (n=50).

Compound	Available N	Available P	Available K	pH	Organic matter
Liquiritin	-0.470***	-0.535***	-0.488***	0.038	-0.442**
Glycyrrhizic acid	-0.159	-0.327*	-0.276*	0.116	-0.172

* p <0 .05, ** p < 0.01, *** p < 0.001.

Table 6. Correlation coefficients between contents of glycyrrhizic acid or liquiritin and climate variables for *G. uralensis* populations in North China (n = 5).
Tabla 6. Coeficientes de correlación entre los contenidos de ácido glycyrrhizic o liquiritin y las variables climáticas en poblaciones de *G. uralensis* en el Norte de China (n=5).

Compound	Mean annual temperature	Mean annual relative humidity	Mean annual precipitation	Annual sunshine hours	Mean annual highest temperature	Mean annual lowest temperature
Liquiritin	-0.188	-0.510	0.987***	-0.141	-0.078	-0.173
Glycyrrhizic acid	-0.114	0.075	0.637	-0.400	-0.106	-0.043

*** p<0.001.

DISCUSSION

The effects of environmental variables on plant growth and development, reproduction, and distribution are well known in plant ecology (Harper, 1977; Begon et al., 1990). The relationships between plant chemical contents and environmental variables have mainly been studied in crops and cultivated species (Zhou, 2003; Hayashi & Sudo, 2009). This information has been used to evaluate the quality of plant products (Zhang, 2003). This paper studied the relationships between the contents of active compounds and environmental variables in natural populations. Results of this research are useful not only in evaluating quality of medicinal products but also in determining the conservation needs of endangered species within those natural populations (Zhang, 2005).

There were significant differences of *Glycyrrhiza uralensis* contents of liquiritin and glycyrrhizic acid between populations in North China. Similar results were found for other compounds (e.g. 1iquiritigenin, isoliquiritigenin, etc.) in the study species (Sun et al., 2003). Results are consistent with those obtained in other studies for other plant species (e.g. *Astragalus membranaceus:* Wang, 2008; *Acorus tatarinowii:* Yin et al., 2004). The difference in compound contents may be due to the influence of the (1) environment, and (2) genetic structure and diversity between populations (Duffy et al., 2009). This suggests that all populations in different habitats are valuable for conservation (Hussain & Hore 2007).

The effects of all soil variables, except pH, were negative and significant on contents of liquiritin. Among these soil variables, P and K were the most important to liquiritin. Furthermore, contents of glycyrrhizic acid were significantly and negatively correlated with soil P and K. This shows that soil P and K are key variables in determining contents of active compounds in *Glycyrrhiza uralensis* (Zhou, 2006). Monitoring of soil P and K has increased the contents of liquiritin and glycyrrhizic acid, and improved the quality of licorice (Ji et al., 2004). These findings do not agree with those obtained for other medicinal plant species which need highly fertilized soils with P and K (e.g. *Astragalus membranaceus***:** Wang, 2008; *Phellodendri cortex:* Park et al., 1999).

Only annual precipitation influenced the contents of liquiritin and glycyrrhizic acid. As precipitation increased, contents of liquiritin and glycyrrhizic acid also increased. This suggests that the eastern regions, such as Chifeng and Hangjinqi, were more suitable for *Glycyrrhiza uralensis* growth and cultivation gardens of licorice for market purposes (Sun et al., 2003; Hussain & Hore, 2007; Hayashi & Sudo, 2009).

The simulation models predicted the contents of liquiritin and glycyrrhizic acid for *Glycyrrhiza uralensis* because their strong correlation with predicting factors (Zhang, 2004). The predicted and measured values were significantly close, and models using precipitation and soil P and K were more precise than others (Yin et al., 2004; Wang et al., 2008). It is suggested that these three variables should be used to predict active compound contents of licorice.

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REFERENCES

Begon, M., L. Harper & C.R., Townsend (1990). Ecology. Blackwell Scientific Publications, Boston, pp. 267-350.

Duffy, K.J., G. Scopece, S. Cozzolino, M.F. Fay, R.J. Smith & J.C. Stout (2009). Ecology and genetic diversity of the dense-flowered orchid, *Neotinea maculata*, at the centre and edge of its range. *Annals of Botany* 104: 507–516.

Harper, J. (1977). Biology of plant populations. Blackwell, London.

Hayashi, H. & H. Sudo (2009). Economic importance of licorice. *Plant Biotrchnology* 26 (Sp. Iss. SI)**:** 101-104.

Hu, J.F. & F.J. Shen (1995). Structure of a new alkaloid from the roots of *Glycyrrhiza yunnanensis. Chemistry Journal of Chinese University* 16: 1245-1247.

Hurst, W.J., J.M. McKim & R.A.J. Martin (1983). High-performance liquid chromatographic determination of glycyrrhizin in licorice products. *Journal of Agricultural and Food Chemistry* 31: 387–389.

Hussain. S. & D.K. Hore (2007). Collection and conservation of major medicinal pants of Darjeeling and Sikkim Himalayas. *Indian Journal of Traditional Knowledge* 6**:** 352-357.

Ji, Y.B., W. Jiang & Y.L. Fan (2004). Advance in studies on flavonoids of licorice. *Chinese Traditional Herb Drugs* 35: 5-6.

Park, J-I, J-K Shim, J-W Do, S-Y Kim, E-K Seo, H-J Kwon, T-K Lee, J-K Kim, D-Y Choi & C-H Kim (1999). Immune-stimulating properties of polysaccharides from *Phellodendri cortex* (Hwangbek). *Glycoconjugate Journal* 16: 247–252.

Pan, B.R. & Y.M. Zhang (2002). Characteristics and conservation of biodiversity in Xinjiang. *Science in China Series D – Earth Sciences* 45 (Suppl.): 174-179.

Sabbioni, C., R. Mandrioli, A. Ferranti, F. Bugamelli, M.A. Saracino, G.C. Forti, S. Fanali & M.A. Raggi (2005). Separation and analysis of glycyrrhizin, 18beta-glycyrrhetic acid and 18alpha-glycyrrhetic acid in liquorice roots by means of capillary zone electrophoresis. *Journal of Chromatography A* 1081: 65–71.

Shen, S., Z. Chang, J. Liu, X. Sun, X. Hu & H. Liu (2006). Simultaneous determination of glycyrrhizic acid and liquiritin in *Glycyrrhiza uralensis* extract by HPLC with ELSD detection. *Journal of Liquid Chromatography & Related Technologies* 29: 2387–2397.

Shen, T., T. Fu & G. Yang (2003). Relationship between Hybrid Performance and Genentic Diversity Based on SSRs and ISSRs in *Brassicanapus*. *Agricultural Science in China* 2: 1083-1090.

Sun, G., Y. Wang & Y. Sun (2003). The quantitative determinations of glycyrrhizic acid, glycyrrhetinic acid, morphine, and sodium benzoate in compound liquorice tablets by HPCE. *Journal of Liquid Chromatography & Related Technologies* 26: 43–51.

Wang, W.L. (2008). Study on the Nutrient Absorption Characteristics and Secondary Metabolism Regulation of Astragalus Membranaceus (Fisch.) Bge. PhD Thesis, Northwest Agriculture University, Yangling.

Yin, A.P., Q.Chen, E.F.Cheng, B. Bi & P.R. Li (2004). Cultivation experiment of wild medicinal plant *Acorus tatarinowii*. *Yunnan Forestry Science and Technology* 15: 2-25.

Zhang, J.H., J. Yao & Y.L. Yang (2001). Analysis and menstruate contents of alkaloid in licorice. *Acta Botanica Boreal- Occidenta Sinica* 21: 1259-1262.

Zhang, J-T (2003). Applied ecology. Science Press, Beijing. (in Chinese).

Zhang, J-T (2004). Quantitative ecology. Science Press, Beijing. (in Chinese).

Zhang, J-T (2005). Succession analysis of plant communities in abandoned croplands in the Eastern Loess Plateau of China. *Journal of Arid Environments* 63: 458-474.

Zhang, J-T & T. Chen (2007). Effects of mixed *Hippophae rhamnoides* on community and soil in planted forests in the Eastern Loess Plateau, China. *Ecological Engineering* 31: 115–121.

Zhang, J-T, W.M. Ru & B. Li (2006). Relationships between vegetation and climate on the Loess Plateau in China. *Folia Geobotanica* 41: 151–163.

Zhang, Q.Y. & M. Ye (2009). Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). *Journal of Chromatography A* 1216: 1954–1969.

Zhou, C.M. (2003). The outline of the normative Cultivation Techniques for *Glycyrrhiza uralensis*. *Research and Information on Traditional Chinese Medicine* 5: 25-28.

Zhou, C.M. (2006). Cultivation Techniques for *Glycyrrhiza uralensis*. *Xinjiang Farmland Reclamation Science and Technology* 15: 14-15.