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Full Length Research Paper

Genetic diversity of populations of an endangered medicinal plant species (*Glycyrrhiza uralensis)* in different environments of North China

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Glycyrrhiza uralensis **is an endangered and national-protected medicinal plant species distributed in semi-arid and arid areas of North China. This study addresses the genetic diversity and relationship between populations in different habitats by amplified fragment length polymorphism (AFLP). The plant materials consisted of 50 individuals from 5 different populating areas of Chifeng (Inner Mongolia), Hengjinqi (Inner Mongolia), Minqin (Ganshu), Aletai (Xinjiang) and Kashi (Xinjiang). Eight AFLP primer combinations generated a total of 1025 bands with 52.7% polymorphism. Unweighted pair group method based on arithmetic average (UPGMA) analysis was performed on Jaccard's similarity coefficient matrix. According to results, the genetic resources and diversity in wild populations of *G. uralensis* were rich (polymorphism = 54.3%, *He* = 0.1932). The polymorphism among populations was Chifeng > Hangjinqi > Minqin > Aletai > Kashi and the genetic diversity varied from 0.1794 - 0.2061 and was in the order of Minqin > Chifeng > Hangjinqi > Aletai > Kashi. Genetic diversity was significantly correlated with annual mean precipitation and soil pH. Aletai and Kashi populations had close genetic relationship, and so Minqin and Chifeng populations. Conservational efforts have to be strengthened for all populations of the plant species in different habitats.**

Key words: Genetic characteristic, plant population, liquorice, semi-arid and arid area, biodiversity conservation.

INTRODUCTION

Medicinal plants are significant to urban and rural populations in many countries and regions, such as China, India, Japan, Korea, Taiwan etc. (Hussain and Hore, 2007; Nautiyal et al., 2009). Most medicinal plant species are harvested from the wild and extent of its use has led to endangered or even extinct some species (Schippmann et al., 2002; Larsen and Olsen, 2007). Conservation of medicinal plants is important content of biodiversity conservation (Kate and Laird, 1999). Genetic diversity is a part of biodiversity, and hence it is important in conservation. *Glycyrrhiza uralensis* is one of the most useful Chinese herbal medicinal plants which have been widely used in medicine, food, tobacco, chemical industries etc (Zhou, 2006; Zhang and Chen, 2007). Because of great market demand, the wild resources of this species are much reduced and the conservation for wild populations becomes urgent (Zhang et al., 2006; Zhang and Ru, 2010). Conservation of medicinal plants is a priority feature of environmental policies in many countries and regions. Genetic diversity is total number of genetic characteristics in the genetic makeup of a species or a population. Populations in different environments may have different genetic characteristics (Duffy et al., 2009; Karimi et al., 2009). Species that occur over a large geographical area often have populations that occupy a variety of habitats (Caughley et al., 1988; Zhang,

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Abbreviations: AFLP, Amplified fragment length polymorphism; **UPGMA,** unweighted pair group method based on arithmetic average; **CTAB,** hexadecyl trimethyl ammonium bromide.

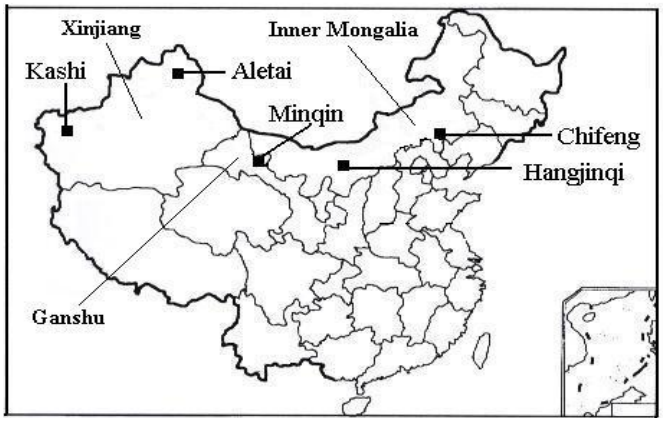

Figure 1. Locations of sampling sites for populations of *G. uralensis* in North China. Chifeng and Hangjinqi were in Inner Mongolia autonomous region, Minqin in Ganshu province and Aletai and Kashi in Xingjiang autonomous region.

Duffy et al., 2009). However, not all habitats provide populations with equal ecological advantages. Differences in abiotic and biotic factors may affect the abundance of populations and individuals within populations (Caughley et al., 1988; Lawton, 1993). *G. uralensis* is widely distributed from east to west in north China and its habitat conditions varied greatly. Study on genetic relationships of populations in different environment is the basis for conservation of this species. Development of biotechnology makes it easy to analyze genetic characteristics of a specific species in varying environmental conditions. Over the past decades, a number of PCR-based marker systems have been developed for DNA analysis and AFLP is one of the powerful techniques in detecting polymorphism among them (Duffy et al., 2009; Karimi et al., 2009). The AFLP marker, needs no cloning and sequencing, and gives a very large number of scorable fragments, which enhance its power to detect polymorphism. No comprehensive study is available about molecular phylogenetic analysis on *G. uralensis* and its populations. This paper reports its genetic diversity for the first time. The objective of this study is to test the hypothesis that populations in different environments have different genetic diversity and to elucidate the genetic relationships between populations of *G. uralensis*. Additionally, the efficient of the AFLP marker technique will be tested in this study.

MATERIALS AND METHODS

Sampling

Based on a general survey of distribution of *G. uralensis*, five study sites, Chifeng (in Inner Mongolia Autonomous Region), Hengjinqi (in Inner Mongolia Autonomous Region), Minqin (in Ganshu Province), Aletai (in Xinjiang Autonomous Region) and Kashi (in Xinjiang Autonomous Region), were selected as sampling sites in North China (Figure 1). These sites represented different populations in different climate and soil conditions (Tables 1 and 2). The climate data were collected from local meteorological stations. Five soil samples of 30 cm in depth in each site were thoroughly mixed and then one quarter was collected and taken to laboratory for chemical analysis. The area of each sampling site was about 40 ha. The density of *G. uralensis* varied from 4 200 - 5 600 plants per ha and its frequency varied from 39% to 61% among the sites. Ten quadrants of 1 × 1 m were established randomly at each site. The whole plant of one individual of *G. uralensis* was collected and stored in zip-lock plastic bags. Total, 50 samples from five sites were collected for this study.

DNA extraction

Leaf samples (ten leaves for each individual) were used for DNA extraction and genomic DNA was extracted by the CTAB method with minor modification (Kafkas et al., 2006; Karimi et al., 2009). One gram leaf samples were ground in liquid nitrogen and mixed with 6 ml of CTAB buffer (100 mM Tris–HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 2% PVP, 0.2% *b*-mercaptoethanol, 0.1% $Na_2S_2O_5$). Then samples were incubated at 65°C for 1h, followed by extraction with an equal volume of chloroform-isoamylalcohol (24/1). The aqueous phase was recovered and mixed with an equal volume of cold isopropanol and left at -20°C for 24 h. The precipitated nucleic acids were recovered by centrifugation at 1,000 rpm for 2 min, washed with ammonium acetate (10 mM) in 76% ethanol, dried and resuspended in double distilled water. DNA concentration was estimated by comparing with known DNA concentrations, after 0.8% agarose gel electrophoresis and ethidium bromide staining, and concentration was adjusted to 50 ng/µl for AFLP analysis (Karimi et al., 2009).

AFLP reaction

A total of 50 ng of genomic DNA was used. The restriction/ ligation was performed using 5 units of *Eco*RI and 1 unit of *Mse*I enzymes, 5 pmol of each *Eco*RI and *Mse*I adaptors, 1 unit of T4 DNA ligase, 1X ligase buffer, 1.1 µl of 0.5 M NaCl and 0.55 µl BSA (1 ng/µl), in a final volume of 11 µl (Karimi et al., 2009).

The preselective amplification reaction mixture contained 4 µl restricted–ligated DNA as PCR template, 5 pmol of each preselective amplification primers (*Eco*RI + A and *Mse*I + C), 3 mM dNTPs, 2 µl PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 0.4 mM $MgCl_2$ and 1 unit of Taq DNA polymerase. Thermocyclic conditions were according to Kafkas (2006). The selective amplification reaction was conducted in a final volume of 20 µl containing 4 µl of diluted (1/20) preselective amplification products as template, 5 pmol each of specific *Eco*RI and *Mse*I primers, 3 mM dNTPs, 1X PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 3 mM $MgCl_2$ and 1 unit of Taq DNA polymerase. The AFLP program included one cycle for 2 min at 94°C, followed by ten cycles of denaturation at 94°C for 45 s, gradient annealing at 65°C (-1°C/cycle) for 45 s and extension at 72°C for 2 min. Additional 20 cycles of denaturation, annealing and extension at 94°C for 45 s, 56°C for 45 s and 72°C for 2 min were applied followed by a final incubation for 30 s at 60°C (Kafkas et al., 2006; Karimi et al., 2009).

Data analysis

The PCR products were separated on an ABI 377 DNA Sequencer. The polymorphic bands were scored as present (1) or absent (0). The percentage of polymorphic bands was Calculated by $p = (k / n) \times 100\%$, where k is the number of polymorphic bands; n is

the total number of measured bands. The AFLP-SURVEY 1.0 software was

Table 1. Climatic characteristics of different distribution area of *G. uralensis* populations in North China (average values of 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

The climate data were collected from local meteorological stations.

Table 2. Soil characteristics of different distribution area of *G. uralensis* populations in North 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

used to calculate Nie's gene diversity (*He*) (Nie, 1978) for individual and population. The Jaccard's similarity matrix was calculated using numerical taxonomy and multivariate analysis system NTSYSpc Ver 2.11 (Rohlf, 2004) and the dendrogram produced using the Unweighted pair-group method with arithmetic average (UPGMA) (Zhang, 004).

One-way ANOVA was used to test the difference of genetic diversity between *G. uralensis* populations, and Pearson correlation was used to analyse the relationships between genetic diversity and environmental variables.

RESULTS

AFLP polymorphism

Eight primer combinations selected from 64 combinations of 8 *Eco*RI and 8 *Mse*I and their sequences are listed in Table 3. These eight selected primer combinations were used in this study that generated total 1025 scorable bands, with an average of 128 bands per primer pair, of which 540 bands were polymorphic, that is, percentage of polymorphic bands was 52.7%. The number of polymorphic bands varied from 56 (E-AAG/M-CTT) to 80 (E-AAC/M-CAG). The average polymorphic bands per primer pair were 67.5 (Table 3).

Fingerprint construction

Among eight pairs of primer combinations, E-AAC/M-CAG amplified a total of 144 bands and amplified DNA fragments between 50 - 450 bp. There were 80 polymorphic bands and the percentage of polymorphic bands reached to 55.6%, the highest one among the primer combinations. It detected 8 specific bands, 6 of which at 74, 95, 113, 167, 258, 300 bp were detected in Minqin population, 5 at 98, 106, 113, 171, 184 bp and 2 at 89, 357 bp were detected in Aletai and Kashi populations, respectively. Therefore, this primer combination with a strong detection of genetic variability was used to construct fingerprint of *G. uralensis* (Figure 2).

Genetic diversity of populations

The populations of *G. uralensis* in different environments were different in genetic diversity (Table 4). Their polymorphic bands varied from 55 to 73 bands, and the percentage of polymorphic bands from 48.4% (Kashi) to 58.6% (Chifeng). Their genetic diversity varied between 0.1794 and 0.2061 with the order of Minqin > Chifeng > Hangjinqi > Aletai > Kashi. The average genetic diversity of populations was 0.1932 (Table 4).

Genetic relationships among populations

The AFLP generated data in this study were analyzed using Jaccard's similarity coefficient. According to similarity matrix and UPGMA clustering, 50 individuals

Table 3. The base sequence of the 8 primer combinations for AFLP analysis and their results of AFLP analysis for *G. uralensis* populations in North China.

Primer combination	Prime sequence (5′-3′)	No of total bands	No of polymorphic bands	% of polymorphism
E-AAC/M-CAA	GACTGCGTACCAATTCAAAC GATGAGTCCTGAGTAACAA	129	68	52.7
E-AAC/M-CAG	GACTGCGTACCAATTCAAAC GATGAGTCCTGAGTAACAG	144	80	55.6
E-AAC/M-CTC	GACTGCGTACCAATTCAAAC GATGAGTCCTGAGTAACTC’	137	69	50.4
E-AAG/M-CAA	GACTGCGTACCAATTCAAAG GATGAGTCCTGAGTAACAA	111	59	53.2
E-AAG/M-CAG	GACTGCGTACCAATTCAAAG GATGAGTCCTGAGTAACAG	143	75	52.4
E-AAG/M-CTC	GACTGCGTACCAATTCAAAG GATGAGTCCTGAGTAACTC	138	72	52.2
E-AAG/M-CTT	GACTGCGTACCAATTCAAAG GATGAGTCCTGAGTAACTT	113	56	49.6
E-ACT/M-CTC	GACTGCGTACCAATTCAACT GATGAGTCCTGAGTAACTC	110	61	55.4
Total		1025	540	--
Mean		128	67.5	52.7

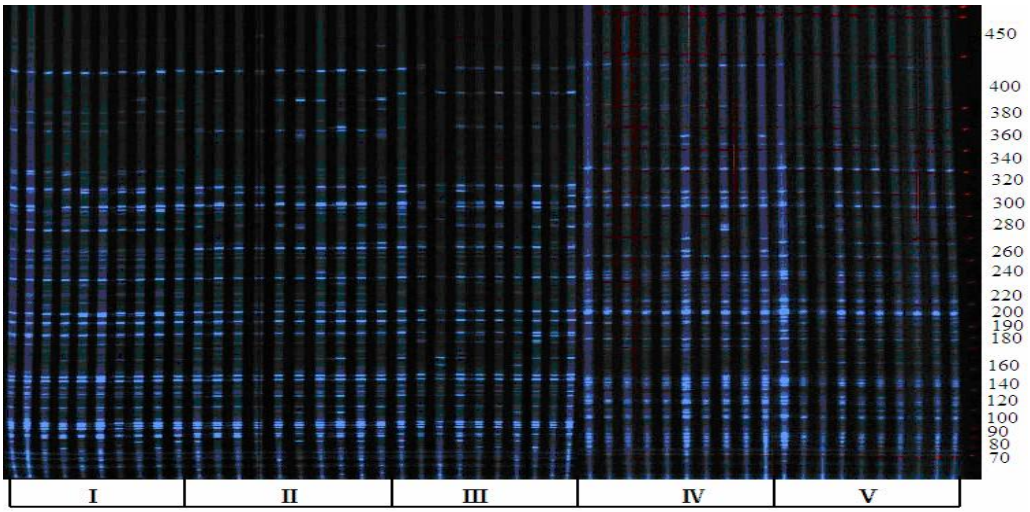

Figure 2. AFLP fingerprinting amplified with primer E-AAC/M-CAG of the five populations in different environments in North China. I - Hangjinqi, II- Altai, III – Kashi, IV- Minqin and V- Chifeng.

second branch contained Hangjinqi, Aletai and Kashi populations. Minqin and Chifeng populations were separated at coefficient of 0.59; Hangjinqi population was separated from Aletai and Kashi populations at coefficient of 0.61; and Aletai and Kashi populations were separated at coefficient of 0.65. The genetic relationships among these populations were clear (Figure 3). The individuals within a population were clustered into a group at different

coefficient levels, thaat is, Minqin at 0.60, Chifeng 0.62, Hangjinqi 0.69, Aletai 0.72 and Kashi 0.73, which

Table 4. Genetic diversity of populations of *G. uralensis* in North China.

Populations (sites)	Number of sample	No of total bands	No of polymorphic bands	% of polymorphic bands	Gene diversity (*He*) (±standard error)
Chifeng	10	133	78	58.6	0.1993 (±0.0182)
Hangjinqi	10	129	73	56.6	0.1952 (±0.0175)
Minqin	10	129	71	55.2	0.2061 (±0.0186)
Aletai	10	128	67	52.5	0.1858 (±0.0179)
Kashi	10	114	55	48.4	0.1794 (±0.0184)
Total	50	633	344	271.3	0.9658
Mean	10	126.6	68.8	54.3	0.1932

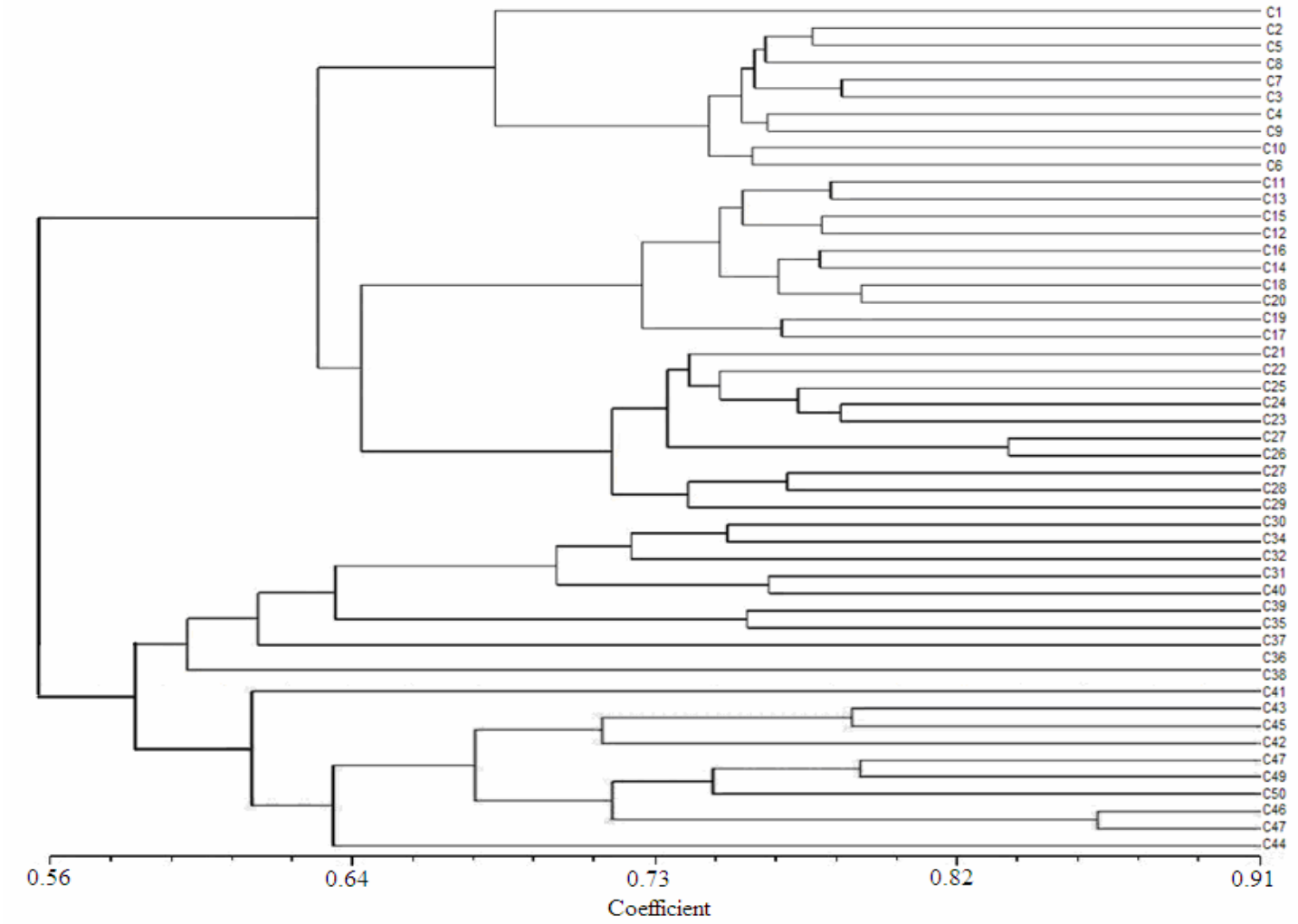

Figure 3. Dendrogram of UPGMA cluster analysis of 50 samples of *Glycyrrhiza uralensis* populations based on the AFLP data illustrating the genetic relationships among the 50 individuals and 5 populations in different environments in North China. c1-c10 were samples from Hangjinqi, c11-c20 from Aletai, c21-c30 from Kashi, c31-c40 from Minqin and c41-c50 from Chifeng.

also reflected the genetic diversity of a population. The correlation analysis showed that the genetic diversity of *G. uralensis* populations was significantly correlated with annual mean precipitation and soil pH (Table 5).

DISCUSSION

Morphological and molecular markers are important to genetic analysis of related plant species and populations

Table 5. Correlation coefficients between genetic diversity and environmental variables for *G. uralensis* populations in North China.

Genetic diversity	Annual mean temperature	Annual mean relative humidity	Annual mean precipitation	Annual sunshine hours	Annual mean highest temperature	Annual mean lowest temperature	Available N	Available P	Available K	pH	Organic matter
Percentage of polymorphic bands	-0.492	-0.504	0.958***	0.267	-0.347	-0.512	-0.123	-0.422	-0.091	0.903**	-0.041
Gene diversity	-0.247	-0.743	0.736	0.623	-0.052	-0.336	0.429	0.166	0.447	0.785*	0.499

Note: *P < 0.5; **P < 0.01; ***P < 0.001.

(Helm et al., 2009). DNA markers are the most powerful techniques because they are not influenced by environmental factors and developmental stages of plant (Karimi et al., 2009). AFLP marker is one of the powerful techniques to analyze genetic diversity in different herbaceous species. This method needs no plants sequence information prior to application and has high multiplex ratio, generating larger number of amplified products in a single reaction (Kafkas, 2006).

In this study, AFLP marker was used to analyze genetic diversity of populations in different environments. Eight primer combinations generated 52.7% polymorphism. This proved that these primer combinations were suitable and AFLP parker was very effective in this study (Karimi et al., 2009; Duffy et al., 2009). The level of genetic diversity in wild populations of *G. uralensis* obtained in this study (polymorphism = 54.3%, *He* = 0.1932) is close to the average values for widespread distributed species (polymorphism = 58.9%, *He* = 0.202) and long-lived perennial herbs (polymorphism = 41.3%, *He* = 0.116) (Hamrick and Godt, 1989). It is a little less than populations of *G. uralensis* collected only from Inner Mongolia (polymorphism = 66.5%, *He* = 0.217) (Yao et al., 2008). Comparatively, *G. uralensis* had rich genetic resources and diversity. The percentage of polymorphic bands of populations was great with a variation of 10.2%. The polymorphism was decreased from east (Chifeng, eastern Inner Mongolia) to west (Kashi, western Xinjiang). The genetic diversity among populations also varied greatly, from 0.1794 - 0.2061 and their order was Minqin > Chifeng > Hangjinqi > Aletai > Kashi. The polymorphism was significantly correlated with annual mean precipitation ($r = 0.958$, $P < 0.001$) and soil pH ($r = 0.903$, $P < 0.01$), and the gene diversity was significantly correlated with soil pH ($r = 0.785$, $P < 0.05$) and near significantly correlated with annual mean precipitation ($r = 0.736$, $P > 0.05$) (Table 5). This suggests that annual mean precipitation and soil pH were important factors affecting genetic diversity of populations of *G. uralensis* because this species mainly distributed in semi-arid and arid areas in North China (Zhang et al., 2006). Similar results for other species in arid area were reported (Zhao et al., 2006; Lattoo et al., 2008; Kumar et al., 2009). Other environmental factors did not show significant relationships with genetic diversity of *G. uralensis* populations. The genetic relationship between Aletai and Kashi populations was the closest, and they were close to Hangjinqi. Minqin and Chifeng populations were close in genetic structure. According to the relations of genetic diversity and environmental factors, we deduced that the genetic relationships among the five populations were also correlated with annual mean precipitation and soil pH (Zhang et al., 2006; Zhao et al., 2006). The geographical distance might have some effects on genetic relationships, but it was not the key factor. Aletai and Kashi are short in distance, but they are far away from Hangjinqi. The five populations were different in genetic resources and diversity with some specials, and therefore they all need special conservation (Schippmann et al., 2002; Zhang et al., 2007). For the conservation purpose, the management of wild *G. uralensis* populations must be legally and effectively, e.g., digging must be strictly controlled (Zhou, 2006; Nautiyal et al., 2009) and grazing in the study sites should be limited (Zhang et al.,2006).

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REFERENCES

Caughley G, Grice D, Barker R, Brown B (1988). The edge of the range. J. Anim. Ecol. 57: 771-785.

Duffy KJ, Scopece G, Cozzolino S, Fay MF, Smith RJ, Stout JC (2009). Ecology and genetic diversity of the dense-flowered orchid, Neotinea maculata, at the centre and edge of its range. Ann. Bot. 104: 507–516.

Hamrick JL, Godt MJW (1989). Allozyme diversity in plant species. - In: Brown AH., Clegg MT, Kahler AL, Weir BS (eds): Plant Population Genetics, Breeding and Genetic resources. Sinauer Associates, Massachusetts, pp. 43-46.

Helm A, Oja T, Saar L, Takkis K, Talve T, Partel M (2009). Human influence lowers plant genetic diversity in communities with extinction debt. J. Ecol. 97: 1329-1336.

Hussain S, Hore DK (2007). Collection and conservation of major medicinal pants of Darjeeling and Sikkim Himalayas. Indian J. Tradition. Knowl. 6: 352-357.

Kafkas S (2006). Phylogenetic analysis of the genus Pistacia by AFLP markers. Pl. Syst. Evol. 262: 113–124.

Kafkas S, Ozkan H, Acar I, Atli HS, Koyuncu S (2006). Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm: Comparison of AFLP, ISSR and PAPD markers. J. Am. Soc. Hort. Sci. 131: 522–529.

Karimi HR, Kafkas S, Zamani Z, Ebadi A (2009). Genetic relationships among Pistacia species using AFLP markers. Plant Syst. Evol. 279: 21–28.

Kate KT, Laird SA (1999). The commercial use of biodiversity. Earthscan Publications Ltd., London.

Kumar J, Verma V, Goyal A, Shahi AK, Sparoo R, Sangwan RS, Qazi GN (2009). Genetic diversity analysis in Cymbopogon species using DNA markers. Plant OMICS 2: 20-29.

Larsen HO, Olsen CS (2007). Unsustainable collection and unfair trade? Uncovering and assessing assumptions regarding Central Himalayan medicinal plant conservation. Biodivers. Conserv. 16: 1679-1697.

Lattoo SK, Rekha SD, Khan S, Bamotra S, Bhan MK, Dhar AK, Gupta KK (2008). Comparative analysis of genetic diversity using molecular and morphometric markers in Andrographis paniculata (Burm. f.) Nees. Genet. Resour. Crop. Evol. 55: 33-43.

Nautiyal BP, Nautiyal MC, Khanduri VP, Rawat N (2009). Floral Biology of Aconitum heterophyllum Wall: A Critically Endangered Alpine Medicinal Plant of Himalaya, India. Turk. J. Bot. 33: 13-20.

Niei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. - Genetics 89: 583-590.

Rohlf JF (2004). NTSYS-pc: 2.11 Numerical Taxonomy and Multivariate Analysis System, Version 2.11. Exeter Publishing Ltd, New York.

Schippmann U, Leaman DJ, Cunningham AB (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Satellite event on the occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture. Inter-Departmental Working Group on Biological Diversity for Food and Agriculture, Rome.

Yao H, Zhao Y, Chen DF, Chen JK Zhou TS (2008). ISSR primer screening and preliminary evaluation of genetic diversity in wild populations of *Glycyrrhiza uralensis.* Biol. Plantarum 52: 117-120.

Zhang JT (2004). Quantitative ecology. Science Press, Beijing. (in Chinese).

Zhang JT (2005). Succession analysis of plant communities in abandoned croplands in the Eastern Loess Plateau of China. J. Arid Environ. 63(2): 458-474.

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

Zhang JT, Meng DP, Ru WM (2007). The ecological characteristics of the endangered species *Paeonia suffruticosa* var. *spontanea* in China. Nat. Area. J. 27: 145-154.

Zhang JT, Ru WM (2010). Population characteristics of endangered species *Taxus chinensis* var. *mairei* and its conservation strategy in Shanxi, China. Popul. Ecol. In press. DOI 10.1007/s10144-009-0192-y.

Zhang JT, Ru WM, Li B (2006). Relationships between vegetation and climate on the Loess Plateau in China. Folia Geobot. 41: 151-163.

Zhao NX, Gao YB, Wang JL (2006). RAPD diversity of *Stipa grandis* populations and its relationship with some ecological factors. Acta Ecol. Sinica 26: 1312-1319.

Zhou CM (2006). Cultivation Techniques for *Glycyrrhiza uralensis*. Xinjiang Farmland Reclamation Sci. Technol. 15 (1): 14-15.