



Genetic variation in cultivated *Rheum tanguticum* populations

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Abstract

To examine whether cultivation reduced genetic variation in the important Chinese medicinal plant *Rheum tanguticum*, the levels and distribution of genetic variation were investigated using ISSR markers. Fifty-eight *R. tanguticum* individuals from five cultivated populations were studied. Thirteen primers were used and a total of 320 DNA bands were scored. High levels of genetic diversity were detected in cultivated *R. tanguticum* ($PPB = 82.19$, $H = 0.2498$, $H_b = 0.3231$, $I = 0.3812$) and could be explained by the outcrossing system, as well as long-lived and human-mediated seed exchanges. Analysis of molecular variance (AMOVA) showed that more genetic variation was found within populations (76.1%) than among them (23.9%). This was supported by the coefficient of gene differentiation ($G_{st} = 0.2742$) and Bayesian analysis ($\theta_b = 0.1963$). The Mantel test revealed no significant correlation between genetic and geographic distances among populations ($r = 0.1176$, $p = 0.3686$). UPGMA showed that the five cultivated populations were separated into three clusters, which was in good accordance with the results provided by the Bayesian software STRUCTURE ($K = 3$). A short domestication history and no artificial selection may be an effective way of maintaining and conserving the gene pools of wild *R. tanguticum*.

Keywords: cultivated populations, genetic variation, ISSR, Polygonaceae, *Rheum tanguticum* Maxim. ex Balf.

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Introduction

Rheum tanguticum Maxim. ex Balf. (Dahuang in Chinese) is an endangered perennial herbaceous plant endemic to China, with a distribution mainly in Qinghai, Gansu Provinces and west Tibetan Autonomous Region at altitudes of 2,300-4,200 m above sea level; this plant is found along forest edges and in valleys (Yang, 1991; Liu, 1997; Li, 2003; Wang and Ren, 2009). The roots and rhizomes of this plant officially listed in the Chinese Pharmacopoeia have been used in traditional medicine for over 2,000 years to treat various syndromes caused by circulatory problems (e.g., dysmenorrhoea, hypermenorrhoea, hematemesis, lower abdominal pain, etc), jaundice, diarrhea and constipation (Yang, 1991; Komatsu *et al.*, 2006; Li *et al.*, 2006; Chinese Pharmacopoeia Commission, 2010).

As a result of over-harvesting and destruction of its habitat by humans in recent decades, natural populations of *R. tanguticum* have declined dramatically (Hu *et al.*, 2010; Wang, 2010). This led to *R. tanguticum* being listed as a key protected plant of Qinghai Province by the government in 2009 (The People's Government of Qinghai Province, 2009). The breeding system of *R. tanguticum* is outcrossing, with pollination probably involving wind and insects

(Chen *et al.*, 2009). Large panicles produce many winged seeds that are dispersed by wind. The seed set and germination rates of *R. tanguticum* are very high (Xie X (2009) PhD thesis, Graduate University of the Chinese Academy of Sciences, Xining).

Since the 1960s, *R. tanguticum* has been extensively cultivated in its original area of production (Qinghai Province). The species is propagated predominantly by sexual reproduction involving seeds. Farmers usually randomly collect mature seeds directly from wild populations in local or distant areas, mix them together, and plant them in the fields. Sometimes, the seeds are exchanged with relatives or friends, an activity that disperses the germplasm to other places. To date, most studies of *R. tanguticum* have focused on cultivation, tissue culture and analysis of the plant's chemical constituents (Zhang, 2004; Che *et al.*, 2006; VanMen *et al.*, 2012). Although previous reports have provided preliminary assessments of the genetic diversity of wild *R. tanguticum* (Chen *et al.*, 2009; Hu *et al.*, 2010; Wang *et al.*, 2012b), there has been no report on the genetic diversity of cultivated populations.

Inter-simple sequence repeats (ISSR) are molecular markers with primers designed based on repeat motifs (microsatellites) of eukaryotic genomes that require no prior knowledge of DNA sequence (Zietkiewicz *et al.*, 1994). These markers have good stability and large polymorphism. In recent years, ISSR has been successfully

used to investigate the genetic diversity and relationships at species, population and cultivar levels in many plants, including some medicinal species (Tacuata *et al.*, 2012; Chen *et al.*, 2013; Kumchai *et al.*, 2013; Thriveni *et al.*, 2013; Verma and Rana, 2013). In this study, we used ISSR markers to: (1) detect the level of genetic diversity and structure in five cultivated populations and (2) evaluate the possible impact of cultivation practices on the genetic diversity of *R. tanguticum*. The results obtained should be useful in developing strategies for efficient management of the genetic resources of *R. tanguticum* and for future breeding programs.

Materials and Methods

Plant materials

Fifty-eight individuals of *R. tanguticum* were sampled from five cultivated populations in Qinghai Province, China (Figure 1). Each population was positioned by GPS, with the location details listed in Table 1. Young leaf tissues were collected from individual plants located at least 10 m apart and then dried in silica gel. All of the material collected was identified by Dr. Xuefeng Lu and voucher specimens were deposited in the Qinghai-Tibetan Plateau Museum of Biology, Northwest Institute of Plateau Biology, Chinese Academy of Sciences.

DNA extraction and ISSR amplification

Genomic DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method described by Doyle and Doyle (1987). The DNA concentration was determined by comparing the sample with known standards of lambda DNA in 0.8% (w/v) agarose gels. The

isolated genomic DNA was diluted to 30 ng/μL and stored at -20 °C until ISSR amplification.

One hundred primers from the University of British Columbia (UBC set no. 9) were initially screened for PCR amplification and 13 primers that produced clear, reproducible banding patterns were chosen for final analysis (Table 2). PCR amplifications were done in a 20 μL reaction volume consisting of 30 ng of genomic DNA, 3.0 mM MgCl₂, 0.1 mM dNTP, 10 pmol of primer, 0.75 U of *Taq* DNA polymerase (*TaKaRa* Biotech Co., Ltd.) and 2.0 μL of 10 PCR buffer. ISSR-PCR amplifications were done in a PTC-221 thermocycler (MJ Research, Bio-Rad, USA) using the following program: an initial step of 5 min at 94 °C followed by 20 s at 94 °C, 60 s at the appropriate annealing temperature (see Table 2 for details) and 80 s at 72 °C for 38 cycles, with a final extension of 6 min at 72 °C. The negative control consisted of replacing template DNA with ddH₂O to test for contamination. The amplification products were separated in 1.5% agarose gels stained with ethidium bromide and photographed with a GDP-8000 System (UVP Inc., USA). Molecular weights were estimated using a 200 bp DNA ladder (*TaKaRa* Biotech Co., Ltd.).

Data analysis

Only unambiguously and reproducibly amplified ISSR bands were scored as present (1) or absent (0). Smear and weak bands were excluded. The resulting binary data matrix was analyzed using POPGENE version 1.32 (Yeh *et al.*, 1999) to estimate the level of genetic diversity under the assumption of Hardy-Weinberg equilibrium. Genetic diversity parameters, including the percentage of polymorphic bands (PPB), the gene diversity

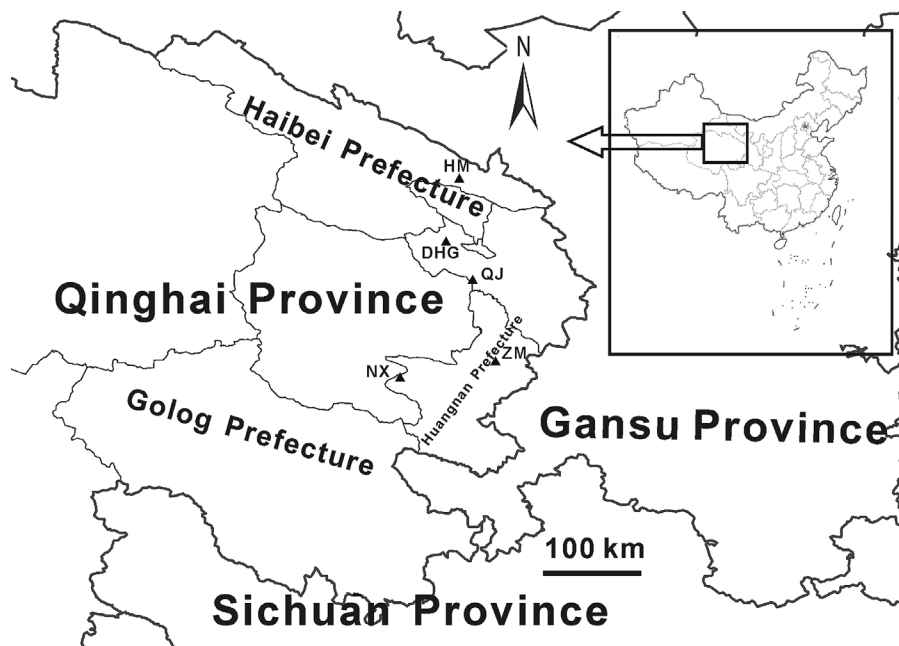


Figure 1 - Locations of the five *R. tanguticum* populations sampled from Qinghai Province in this study.

Table 1 - Sample information for the five cultivated populations of *R. tanguticum* used in this study.

Population	Locality	Longitude (E)	Latitude (N)	Sample size	Vouchers	Altitude (m)
HM	Haomen, Menyuan county, Haibei Prefecture	100°31'15.2"	37°26'13.3"	13	2006091611	2,997
NX	Ningxiu, Zeku county, Huangnan Prefecture	100°51'6.4"	35°11'27.3"	11	2006091812	3,352
ZM	Zhamao, Tongren county, Huangnan Prefecture	101°55'46.3"	35°22'23.6"	7	2006092115	3,099
DHG	Daheigou, Huangyuan county, Xining City	101°22'18.3"	36°43'29.8"	14	2006092516	2,897
QJ	Qunjia, Huangzhong county, Xining city	101°40'25.3"	36°17'30.7"	13	2006092617	2,818

Table 2 - Primers used for ISSR amplification and bands amplified in all individuals sampled.

Primers	Sequence 5' → 3'	T _m (°C)	No. of bands studied	No. of polymorphic bands
809	(AT) ₈ T	53.2	23	20
811	(GA) ₈ C	52.5	24	20
825	(AC) ₈ T	52.0	27	25
834	(AG) ₈ YT	53.0	29	24
836	(AG) ₈ YA	53.0	22	20
840	(GA) ₈ YT	51.0	24	18
841	(GA) ₈ YC	53.2	23	21
842	(GA) ₈ YG	53.2	24	20
868	(GAA) ₆	51.2	25	20
888	BDB(CA) ₇	58.5	25	19
889	DBD(AC) ₇	55.0	25	17
890	VHV(GT) ₇	59.0	23	21
891	HVH(TG) ₇	56.2	26	18
Total			320	263

Y = (C, T); B = (C, G, T); D = (A, G, T); H = (A, G, T); V = (A, C, G).

index H (Nei, 1973) and Shannon's information index (I), were obtained at the species and population levels. Gene differentiation between populations was estimated by the coefficient of gene differentiation (G_{st}) and gene flow (N_m , the numbers of migrants per generation) was calculated from G_{st} according to McDermott and McDonald (1993), where $N_m = 0.5(1 - G_{st})/G_{st}$. To examine the genetic relationship among populations, unbiased genetic distance and genetic identity (Nei, 1978) were also calculated for all pairwise combinations of populations by POPGENE and a dendrogram was constructed from Nei's genetic distance with the unweighted pair-group method of averages (UPGMA) using NTSYSpc software (Rohlf, 2000).

To correct for possible bias introduced by the assumption of Hardy-Weinberg equilibrium, Bayesian gene diversity (H_B) and population differentiation (θ_B) were also calculated by the Bayesian approach (Holsinger *et al.*, 2002) using HICKORY, version 1.1 (Holsinger and Lewis, 2003). The Bayesian method does not assume Hardy-Weinberg equilibrium within populations and does not treat multilocus ISSR phenotypes as haplotypes, but takes full advantage of the information provided by dominant

markers. This allows the incorporation of uncertainty regarding the magnitude of the within-population inbreeding coefficient into estimates of F_{st} (Holsinger and Wallace, 2004; Zhang *et al.*, 2007). Several runs were done with default sample parameters (burn-in = 5 000, sample = 100 000, thin = 20) to ensure consistency of the results (Tero *et al.*, 2003). Model selection was based on the Deviance Information Criterion (DIC) (Spiegelhalter *et al.*, 2002) in which models with smaller DICs are preferred (Holsinger and Lewis, 2003).

An additional measurement for partitioning genetic variation was obtained with the hierarchical analysis of molecular variance analysis (AMOVA), using AMOVA 1.55 (Excoffier *et al.*, 1992). The variance components were tested statistically by nonparametric randomization tests using 1,000 permutations. The Mantel test was used in conjunction with TFPGA software to examine the correlation between genetic and geographic distances (in kilometers) among populations (Miller, 1997).

A Bayesian analysis of ISSR population structure was run on the data set using the program STRUCTURE (Pritchard *et al.*, 2000) to estimate the number of genetic clusters and to evaluate the degree of admixture among them. This method uses a Markov Chain Monte Carlo (MCMC) algorithm to cluster individuals into populations on the basis of multilocus genotype data (Falush *et al.*, 2003). STRUCTURE was run with a burn-in setting of 10,000 followed by 10,000 MCMC iterations using the admixture model with allele frequencies independent among populations. Ten independent runs of $K = 1-5$ were done to ensure consistent results. The most likely value for K was calculated with Structure Harvester (Earl and vonHoldt, 2012) by predicting from plots of *ad hoc* posterior probability models of ΔK . The ΔK statistic was more appropriate than the highest LnPr (X/K) method for inferring the population number (Evanno *et al.*, 2005). Once the number of clusters was determined, individuals were assigned to respective populations based on proportional membership (q) for which an arbitrary threshold value of $q = 0.90$ was used. Individuals with $q > 0.90$ were regarded as members of this cluster, or otherwise as an admixture.

Results

The 13 selected primers generated 320 ISSR bands in 58 individuals from five populations of *R. tanguticum*, 263

(82.2%) of which were polymorphic (Table 3). The bands ranged in size from 200 bp to 2,800 bp. The total number of bands varied from 22 (UBC836) to 29 (UBC834), with an average of 24.6 fragments per primer. The percentage of polymorphic bands (PPB) ranged from 42.8% in the ZM population to 51.8% in the DHG population (Table 3). In terms of the presence of alleles within the 320 alleles detected, there were 57 common alleles in the five populations but no unique allele was found for any of the five populations studied.

The measurements of genetic diversity are summarized in Table 3. The value of Nei's gene diversity (H) was 0.2498 and Shannon's Information index (I) was 0.3812 at the species level while at the populations level the corresponding values were 0.1813 and 0.2677, respectively. The estimates of heterozygosity (H_B) in the full model (which had the lowest DIC, Table 4) based on the Bayesian procedure were generally higher than Nei's gene diversity (H). The expected Bayesian heterozygosity (H_B) was 0.3231 at the species level and 0.2712 at the population level. Among all the populations investigated, the highest and lowest levels of genetic variability occurred in populations DHG ($H = 0.1949, I = 0.2878$) and ZM ($H = 0.1671, I = 0.2445$), respectively.

The genetic differentiation among populations (G_{st}), estimated by Nei's method, was 0.2742, which indicated that 27.4% of the total genetic diversity was distributed among populations, whereas 72.6% occurred within pop-

ulations. Furthermore, the level of gene flow (N_m) was estimated to be 1.3236 individuals per generation between populations, suggesting that gene exchange between populations was high. AMOVA analysis further revealed a similar pattern of genetic differentiation among and within the populations (Table 5). Of the total variation, 23.9% was attributed to among-population differences, a value much lower than the within-population proportion (76.1%). AMOVA also showed that differentiation among populations and within populations was significant ($p < 0.0010$).

The results for θ_B (analogous to Wright's F_{st}) estimated by the Bayesian analysis are shown in Table 4. The best model, which had the smallest DIC (3876.31), was the full model, with $\theta_B = 0.1963$ and $f = 0.0886$.

The UPGMA dendrogram, based on Nei's (1978) unbiased genetic distance, is shown in Figure 2. The populations were separated into three groups: HM and QJ formed group I, DHG formed group II and group III contained NX and ZM. The Mantel test revealed no significant correlation between genetic and geographic distances among populations ($r = 0.1176, p = 0.3686$).

The highest peak in ΔK revealed the best value for $K = 3$ indicated that three clusters were detected ($\Delta K = 9.10$, Figure 3A). These clusters were entirely consistent with those of the UPGMA clustering results (Figure 3B). Ten individuals were considered admixtures, with $q < 0.90$ (Figure 3B).

Table 3 - Genetic diversity indices of the five populations of *R. tanguticum*.

Population	H (SE)	H_B (SD)	I (SE)	PPB (%)
HM	0.1885 (0.2071)	0.2700 (0.0085)	0.2786 (0.2955)	51.25
NX	0.1770 (0.2050)	0.2674 (0.0085)	0.2618 (0.2935)	47.81
ZM	0.1671 (0.2088)	0.2807 (0.0096)	0.2445 (0.2977)	42.81
DHG	0.1949 (0.2082)	0.2789 (0.0080)	0.2878 (0.2967)	51.81
QJ	0.1790 (0.2022)	0.2590 (0.0082)	0.2659 (0.2907)	49.38
Mean at population level	0.1813	0.2712	0.2677	48.61
Total at species level	0.2498	0.3231	0.3812	82.19

H - Nei's gene diversity (assuming Hardy-Weinberg equilibrium), H_B - expected Bayesian heterozygosity (without assuming Hardy-Weinberg equilibrium); I - Shannon's diversity index; PPB - percentage of polymorphic bands, SE - standard error and SD - standard deviation.

Table 4 - Genetic differentiation calculated among populations of *R. tanguticum* using different Bayesian approaches.

Model	θ_B				f				DIC
	Mean	SD	2.5%	97.5%	Mean	SD	2.5%	97.5%	
Full model	0.1963	0.0135	0.1719	0.2244	0.0886	0.0646	0.0051	0.0761	3876.31
f = 0 model	0.1862	0.0108	0.1656	0.2083	0	-	-	-	3883.16
$\theta_B = 0$	0	-	-	-	0.2801	0.0690	0.1547	0.4241	5971.85
f-free model	0.2490	0.0150	0.2199	0.2784	0.4948	0.2896	0.0257	0.9777	3980.26

θ_B - analogous to Wright's F_{st} , f - analogous to Wright's F_{is} , DIC - deviance information criterion and SD - standard deviation.

Table 5 - Analysis of molecular variance (AMOVA) for wild, cultivated and all populations of *R. tanguticum* examined in this work.

Source of variation	d.f.	Sum of squares	Mean squares	Variation components	Total variation (%)	p value ^a
Among populations	4	539.36	134.84	9.21	23.91	< 0.0010
Within populations	53	1552.93	29.30	29.30	76.09	< 0.0010

d.f. - degrees of freedom. ^aSignificance tests after 1,000 permutations.

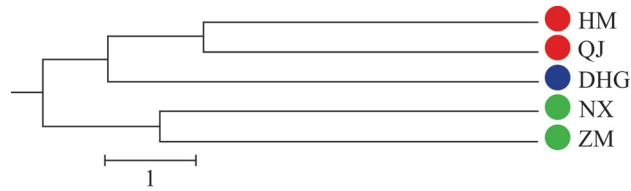


Figure 2 - Dendrogram of cultivated *R. tanguticum* obtained by UPGMA cluster analysis. The three clusters obtained in this analysis are identified by different colored circles.

was similar to that of wild populations ($H = 0.1724$) (Hu *et al.*, 2010), as assessed with ISSR markers. However, the genetic diversity was lower than with SSR markers ($H = 0.5150$) in wild *R. tanguticum* (Chen *et al.*, 2009). When compared to those of allied species of Polygonaceae, such as *Rheum officinale* ($H = 0.1008$) (Wang *et al.*, 2012a), *Polygonum viviparum* ($H = 0.1227$) (Lu *et al.*, 2008) and *Eriogonum shockleyi* var. *shockleyi* ($H = 0.1620$) (Smith and Bateman, 2002), the genetic diversity in cultivated populations of *R. tanguticum* was high.

Discussion

In this study, the genetic diversity parameter for cultivated populations of *R. tanguticum* ($H = 0.1813$, Table 3)

The genetic diversity of plant populations is largely influenced by factors such as breeding system, seed dispersal, genetic drift and evolutionary history, as well as life form. Life form and breeding system have highly signifi-

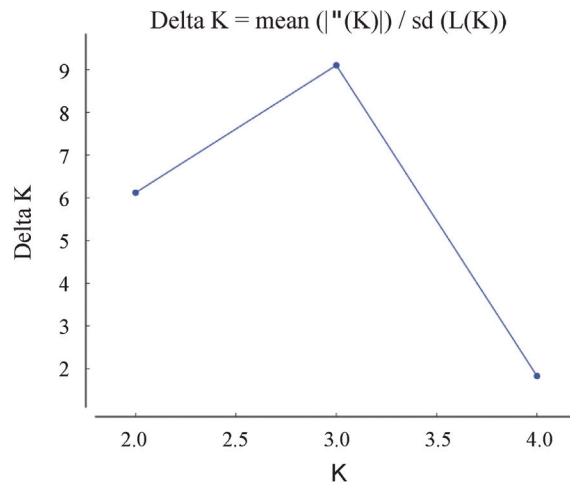


Figure A

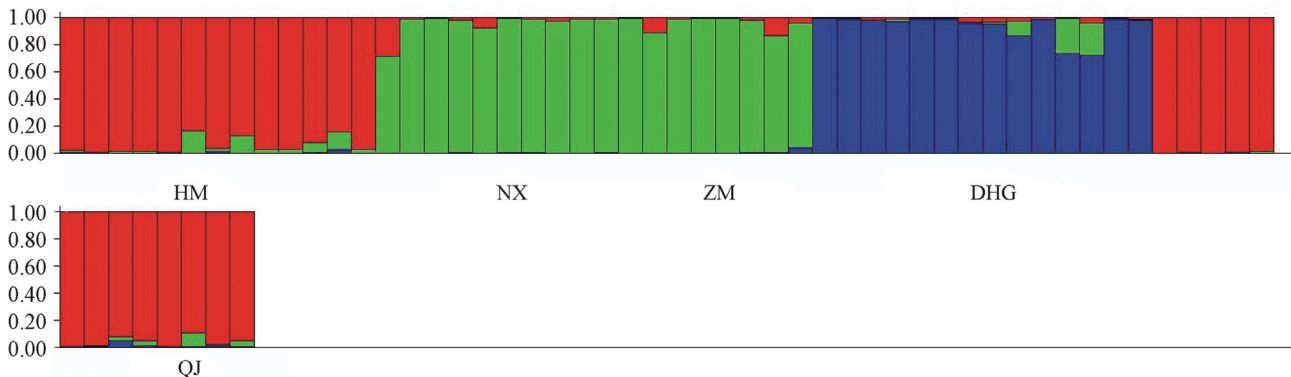


Figure B

Figure 3 - Bayesian inference of the number of clusters (K) in *R. tanguticum*. (A) K was estimated from plots of *ad hoc* posterior probability models of ΔK . (B) Bayesian admixture proportions (q) of individuals of cultivated *R. tanguticum* for $K = 3$. Each individual is represented by a single line broken into K colored segments, with lengths proportional to each of the K -inferred clusters.

cant influences on genetic diversity. In general, long-lived and outcrossing species have higher levels of genetic diversity than selfing and clonal plants (Hamrick and Godt, 1996). Bayesian analysis revealed that the inbreeding coefficient ($f = 0.0886$) in *R. tanguticum* was small, a finding that confirmed the outcrossing breeding system of this species. Outcrossing and elevated longevity contribute to the moderately high level of genetic diversity in *R. tanguticum*. Notably, the current high level of genetic diversity in cultivated *R. tanguticum* may be markedly influenced by the traditional, irregular and sparse agricultural practices of this plant. Traditionally, growers collect and preserve *R. tanguticum* seeds randomly without deliberate selection and mix them together before planting. This practice would preserve more genetic diversity than in wild plants.

R. tanguticum has been planted in Qinghai Province since the 1960s. The domestication history is short, there is no artificial selection and cultivation apparently does not influence genetic diversity. In cultivated populations, genetic diversity is partly determined by the way genetic material is passed from one cultivated generation to the next (Miller and Schaal, 2006; Yao *et al.*, 2012). For *R. tanguticum*, seeds are the main genetic material used to establish the cultivated population. Cultivated *R. tanguticum* may have been established from the seeds of a large number of wild progenitors. According to local growers, *R. tanguticum* are collected randomly and mixed together before planting. This was confirmed by the proportional membership (q) calculated with Bayesian analysis. The value of q in 10 individuals was < 0.90 (Figure 3B), indicating that the origins of these 10 individuals differed from that of other individuals in the same population.

The germplasm can occasionally be dispersed to other places by farmers' relatives or friends. For example, the QJ population was planted by researchers of our institute. The seeds of this population were collected from different places in Golog Prefecture and mixed together. This was confirmed by Bayesian analysis with STRUCTURE software. Figure 3B shows that there were five individuals with a proportional membership < 0.90 . The frequent exchange of seeds further improves the

maintenance of genetic diversity (Guo *et al.*, 2007; Yao *et al.*, 2012). Consequently, traditional agricultural practice applied to this plant was another important factor that influenced the abundant genetic diversity of cultivated *R. tanguticum*.

Compared to annual plants, cultivated populations of some perennial plants may harbor a relatively higher percentage of genetic diversity than their wild ancestors (Otero-Arnaiz *et al.*, 2005; Miller and Schaal, 2006; He *et al.*, 2009). Our findings were similar to several other long-lived cultivated plants in which genetic diversity is as high as in the wild relatives (He *et al.*, 2007; Shi *et al.*, 2008). Cultivated *R. tanguticum* had moderate levels of genetic diversity compared with cultivated populations of other endangered Chinese medicinal plants (Table 6) (Wu *et al.*, 2006; Guo *et al.*, 2007; Qiu *et al.*, 2009; Song *et al.*, 2010; Li *et al.*, 2011; Yao *et al.*, 2012), *i.e.*, there was no evidence that a "cultivation bottleneck or founder effect" affected the genetic diversity.

Long-lived and outcrossing species retain most of their genetic variability within populations. In contrast, annual and selfing species allocate most of their genetic variability among populations (Hamrick and Godt, 1996; Nybom, 2004). Our study was consistent with this trend. AMOVA results revealed that in cultivated *R. tanguticum* 76.1% of the genetic variance was retained within populations while 23.9% was among populations (Table 5). In the long-lived tree *Spondias purpurea*, the proportion of genetic variation distributed among populations was greater in cultivated populations than in wild populations, a reflection of the relative amounts of vegetative propagation (Miller and Schaal, 2006). The opposite was observed in cultivated *R. tanguticum*. Cultivated populations ($G_{st} = 0.2742$, $\Phi_{st} = 0.239$) had a lower proportion of their genetic variability distributed among populations than wild populations ($G_{st} = 0.3585$, $\Phi_{st} = 0.290$) (Hu *et al.*, 2010). Bayesian analysis revealed that the inbreeding coefficient ($f = 0.0886$) in *R. tanguticum* was small. Based on the records of local growers, these cultivated *R. tanguticum* were grown from seeds rather than being propagated vegeta-

Table 6 - Comparison of genetic diversity of cultivated *R. tanguticum* with other important herbs in cultivated populations.

Species	Genetic diversity values	Markers	References
<i>R. tanguticum</i>	$PPB_s = 82.19\%$; $H_s = 0.2498$; $PPB_p = 48.61\%$; $H_p = 0.1813$	ISSR	Present study
<i>Salvia miltiorrhiza</i>	$PPB_s = 100\%$; $H_s = 0.1951$; $PPB_p = 65.00\%$; $H_p = 0.1591$	ISSR	Song <i>et al.</i> (2010)
<i>Panax ginseng</i>	$PPB_s = 98.96\%$; $H_s = 0.2886$; $PPB_p = 58.01\%$; $H_p = 0.1890$	ISSR	Li <i>et al.</i> (2011)
<i>Gastrodia elata</i>	$PPB_s = 61.04\%$; $H_s = 0.1280$; $PPB_p = 35.71\%$; $H_p = 0.1000$	ISSR	Wu <i>et al.</i> (2006)
<i>Corydalis yanhusuo</i>	$PPB_s = 71.54\%$; $H_s = 0.1630$; $PPB_p = 25.32\%$; $H_p = 0.0940$	ISSR	Qiu <i>et al.</i> (2009)
<i>Codonopsis pilosula</i>	$PPB_s = 87.23\%$; $H_s = 0.2992$; $PPB_p = 73.43\%$; $H_p = 0.2367$	RAPD	Guo <i>et al.</i> (2007)
<i>Eucommia ulmoides</i>	$PPB_p = 51.23\%$; $H_p = 0.1572$	AFLP	Yao <i>et al.</i> (2012)

H_p - Nei's gene diversity at the population level, H_s - Nei's gene diversity at the species level, PPB_p - percentage of polymorphic loci at the population level and PPB_s - percentage of polymorphic loci at the species level.

tively. Thus, the mating system was outcrossing in cultivated populations, the same as in wild populations.

STRUCTURE analysis showed that 10 individuals were admixtures with $q < 0.90$ (Figure 3B), indicating that cultivated *R. tanguticum* had been introduced to cultivation from the seeds of a large number of wild progenitors. The planting records revealed that the germplasm had been dispersed to other places by the growers, with mixing and exchanging of seeds before planting. The frequent exchange of seeds enhanced the gene flow from wild populations into cultivated populations. Subsequently, the genetic variation within populations increased while that among populations decreased in cultivated populations.

The findings of this study indicate that *R. tanguticum* has maintained a relatively high level of genetic diversity in cultivated populations that may play a crucial role in conserving this species in the face of declining wild populations. A short cultivation history and no artificial selection do not decrease the genetic diversity of *R. tanguticum* and no special cultivar is formed. The primitive agricultural practices, *i.e.*, random collecting, preserving and planting of seeds without deliberate selection may be an effective way of maintaining and conserving the gene pools of wild plants (Guo *et al.*, 2007; Song *et al.*, 2010). Furthermore, given that the genetic diversity of the QJ cultivated populations of *R. tanguticum* was relatively higher than that of wild populations at the population level, the current planting at this site can be seen as a preliminary step for *ex situ* conservation. Additionally, other effective approaches, such as Good Agricultural Practice (GAP), also need to be adopted to meet the market demand and achieve future sustainable use of this medicinal plant.

Acknowledgments

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