Plant Syst Evol (2014) 300:2217–2227
DOI 10.1007/s00606-014-1039-3

ORIGINAL ARTICLE

Genetic variation in cultivated rhubarb (*Rheum tanguticum* Maxim. ex Balf.) and the relationship with their wild relatives in China revealed by ISSR markers

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Received: 20 November 2013 / Accepted: 7 March 2014 / Published online: 4 April 2014

Abstract *Rheum tanguticum* is a widely used Chinese medicinal plant. Recently, because of the great demand, the wild populations have been declining rapidly. In this study, the levels of genetic variation of 11 wild and five cultivated populations of *R. tanguticum* were investigated by ISSR markers. The 13 selected ISSR primers amplified 306 polymorphic bands out of a total of 326 (93.87 %). Based on Nei's gene diversity and Shannon's index, the genetic diversity in cultivated populations of *R. tanguticum* ($H = 0.2490$; $I = 0.3812$; $H_B = 0.3033$) was relatively lower than that of wild populations ($H = 0.2666$; $I = 0.4124$; $H_B = 0.3115$), although no significant differences were identified. Assignment was performed with AFLPOP program, and XGM was the most likely source population of HM. The origins of the rest cultivated populations were admixture. UPGMA and PCoA analyses showed that wild and cultivated populations were not separated into two groups, indicating that a large number of wild genotypes were maintained in the cultivated gene pool. The coefficient of genetic differentiation between wild and cultivated populations was 0.0305 (G_{st}), which was in good agreement with the results of analysis of molecular variance (AMOVA), in which, only 1.85 % of the total variance existed between groups of wild and cultivated populations, while 70.91 % occurred within populations and 27.24 % among populations. Together, these results indicated that cultivated populations were not genetically differentiated from wild populations. On the basis of this study, we have made some suggestions for the conservation and efficient management of the genetic resources of this important medicinal herb.

Keywords *Rheum tanguticum* Maxim. ex Balf. · Polygonaceae · ISSR · Genetic variation · Cultivated populations

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Introduction

Rheum tanguticum Maxim. ex Balf. (Dahuang in Chinese) is a widely used medicinal herb in China and other Asian countries (Wang and Ren 2009; Yang et al. 2001). Its root and rhizome officially listed in the Chinese Pharmacopoeia have been traditionally used for over 2,000 years to treat various syndromes caused by the obstruction of blood circulation (such as dysmenorrhoea, hypermenorrhea, hematemesis, lower abdominal pain, etc.,), jaundice, diarrhea, as well as constipation (Li et al. 2006; Yang 1991; Chinese Pharmacopoeia Commission 2010; Komatsu et al. 2006).

Rheum tanguticum belongs to the family Polygonaceae, and along with *R. palmatum* and *R. officinale* are three official species of rhubarb (Chinese Pharmacopoeia Commission 2010). Compared to the other two species, the roots and rhizome (rhubarb) from *R. tanguticum* have the best medicinal quality. As a result of the increasing demand for rhubarb, most of the which is still met by the wild resources, the natural resources of *R. tanguticum* have been declining dramatically due to overharvesting and deterioration of its habitat by humans over the past several decades (Hu et al. 2010; Wang 2010). So *R. tanguticum* is listed in the list of key protected plants of Qinghai Province by the government in 2009 (The People's Government of Qinghai Province

2009). It is an endangered perennial herbaceous plant endemic to China, mainly distributed in Qinghai, Gansu Provinces and West Tibetan Autonomous Region at altitudes between 2,300 and 4,200 m above sea level and can be found on margins of forests, in valleys or shrubs (Wang and Ren 2009; Yang 1991; Li 2003; Liu 1997). The breeding system of *R. tanguticum* is outcrossing. It is probably pollinated by both wind and insects (Chen et al. 2009). Large panicles produce a great many winged seeds spread by wind. Seed set and germination rates of *R. tanguticum* are both very high (Xie 2009).

Since 1960s, *R. tanguticum* was extensively cultivated in its original producing area—Qinghai Province. The species predominantly propagate sexually by seeds. Farmers usually collected mature seeds directly from wild populations of local or different areas randomly, mixed them together, and planted them in the fields. Sometimes, they exchanged seeds with their relatives or friends, thus the germplasm was dispersed to other places. The cultivation practices of *R. tanguticum*, which may decrease genetic diversity, were primitive, irregular and sparse (Zhang 2004). However, little is known about whether the domestication and cultivation has impacted the extent and distribution of genetic variation in populations of *R. tanguticum*.

As one of the most important Chinese medicinal herbs, *R. tanguticum* has been extensively studied. To date, most studies on *R. tanguticum* have focused on cultivation, cell biology, medical phytochemistry and pharmacology (Komatsu et al. 2006; Jin et al. 2007; Liu et al. 2009; Hu et al. 2011). *R. tanguticum* is diploid ($2n = 22$) and no polyploidy was found with the karyotype analysis (Hu et al. 2011). Previous reports have provided preliminary assessments on genetic variation of wild *R. tanguticum* by SSR and ISSR analysis (Chen et al. 2009; Hu et al. 2010; Wang et al. 2012b); however, little is known regarding the genetic variation between wild and cultivated populations. Information on the genetic diversity is critical for the development of conservation strategies, exploration of plant genetic resources and cultivation of wild plants (Hamrick and Godt 1996; Yao et al. 2012; Martins et al. 2013; de Oliveira et al. 2010; Buzatti et al. 2012; Alves et al. 2013; Chen et al. 2013; Yue et al. 2012; Zheng et al. 2012).

Inter-simple sequence repeats (ISSR) have been widely used to determine evolutionary relationships and levels of genetic variation among wild and cultivated populations (Yang et al. 2011; Verma and Rana 2013; He et al. 2007; Qiu et al. 2009; Shi et al. 2008; Satya et al. 2013; Kumchai et al. 2013). The objectives of this study were to: (1) Assess the present patterns of genetic diversity in wild and cultivated populations of *R. tanguticum*. (2) Evaluate the possible impact of cultivation practices without deliberate artificial selections on the intraspecific genetic diversity. (3) Determine the relationships among cultivated

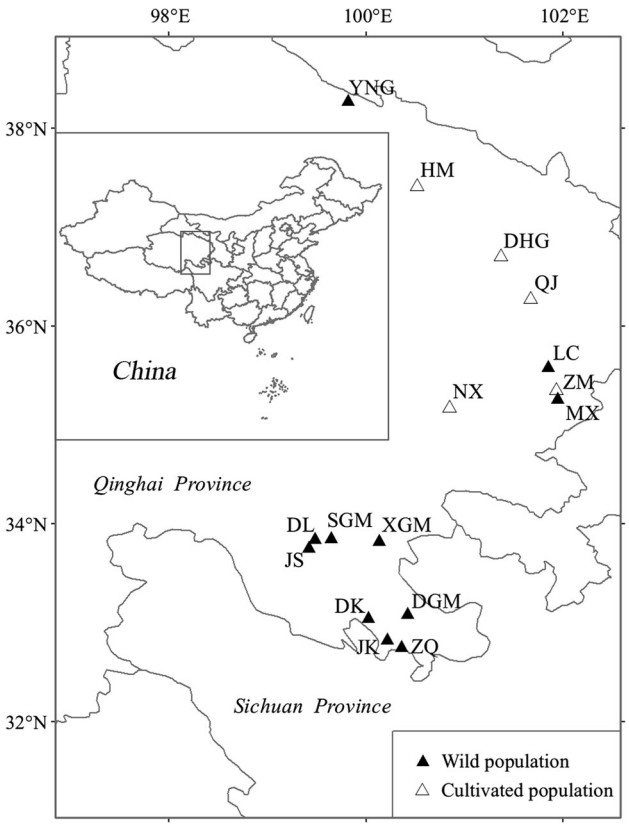

Fig. 1 Locations of the 16 *Rheum tanguticum* populations sampled in the present study from Qinghai Province

populations and their origin of wild populations. (4) Provide useful information to maintain genetic diversity in the future cultivation of this medicinal plant.

Materials and methods

Plant materials

A total of 208 individuals of *R. tanguticum* were sampled from 11 wild populations and five cultivated populations in Qinghai Province, China (Fig. 1). Each population was positioned by GPS with location details listed in Table 1. Young leaf tissues were collected from each sampled individual plants located at least 10 m apart and dried in silica gel for subsequent DNA extraction. All the materials were 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 CTAB method described by Doyle and Doyle (Doyle and Doyle

Table 1 Sample information of 16 populations of *Rheum tanguticum* in the present study

Population	Locality	Longitude (E)	Latitude (N)	Sample size	Vouchers	Altitude (m)
Wild population						
ZQ	Zhiqin, Banma county, Golog Prefecture	100°22′3.2″	32°46′2.7″	14	200608262	3,782
DGM	Duogongma, Banma county, Golog Prefecture	100°25′31.9″	33°5′54.8″	12	200608285	3,811
DK	Daka, Banma county, Golog Prefecture	100°1′59.5″	33°3′40.9″	17	200608273	4,010
JS	Jianshe, Dari county, Golog Prefecture	99°25′51.9″	33°46′24.6″	17	200609018	3,976
DL	Dangluo, Maqin county, Golog Prefecture	99°29′27.7″	33°51′27.5″	15	200609029	4,063
SGM	Shanggongma, Gande county, Golog Prefecture	99°39′17.4″	33°52′12.4″	14	200608306	4,061
XGM	Xiagongma, Gande county, Golog Prefecture	100°8′23.9″	33°50′29.2″	14	200608317	3,926
JK	Jika, Banma county, Golog Prefecture	100°12′50.8″	32°51′2.4″	14	200608274	3,858
YNG	Yeniugou, Qilian county, Haibei Prefecture	99°49′35.9″	38°17′22.9″	12	2006091310	3,074
MX	Maxiu, Zeku county, Huangnan Prefecture	101°56′38.1″	35°16′35.6″	16	2006091913	3,128
LC	Lancai, Tongren county, Huangnan Prefecture	101°51′8.8″	35°35′44.4″	5	2006092014	2,848
Cultivated populations						
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

1987). 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 for ISSR amplification.

One hundred primers from the University of British Columbia (UBC set no. 9 http://www.michaelsmith.ubc.ca/services/NAPS/Primer_Sets/Primers_Oct2006.pdf) were initially screened for PCR amplification, and 13 primers (Table 2) that produced clear and reproducible banding patterns were chosen for final analysis. PCR amplifications were performed in a 20-μL reaction volume consisting of 30-ng genomic DNA, 3.0-mM $MgCl_2$, 0.1-mM dNTP, 10-pmol primer, 0.75 U *Taq* DNA polymerase (*TaKaRa* Biotech Co., Ltd.) and 2.0 μL 10× PCR buffer, which were determined after comparison and optimization. ISSR–PCR amplifications were performed in a PTC-221 thermocycler (MJ Research, Bio-Rad, USA) under the following program: an initial step of 5 min at 94 °C followed by 20 s at 94 °C, 60 s at appropriate annealing temperature (see Table 2 for details) and 80 s at 72 °C for 38 cycles, ending with a final extension of 6 min at 72 °C. The negative control was conducted by replacing template DNA with ddH_2O to test for the possibility of contamination. The amplification products were separated in 1.5 % agarose gels stained with ethidium bromide and photographed with 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). Smeared 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. The following genetic diversity parameters including the percentage of polymorphic bands (PPB), Nei's (Nei 1973) gene diversity (H) and Shannon's information index (I) were obtained at both species level and population level. 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 evaluated from G_{st} according to McDermott and McDonald (McDermott and McDonald 1993), where $N_m = 0.5(1 - G_{st})/G_{st}$. To examine the genetic relationship among populations, Nei's (Nei 1978) unbiased genetic distance and genetic identity 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). Nevertheless, taking into account a possible bias induced by the assumption of Hardy–Weinberg equilibrium, Bayesian gene diversity (H_B) and population differentiation (θ_B) was also calculated by Bayesian approach (Holsinger et al. 2002) using HICKORY, version 1.1 (Holsinger and Lewis

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

Primers	Sequence 5′ → 3′	T_m (°C)	No. of bands studied	No. of polymorphic bands
809	$(AT)_8T$	53.2	23	20
811	$(GA)_8C$	52.5	25	24
825	$(AC)_8T$	52.0	27	27
834	$(AG)_8YT$	53.0	29	28
836	$(AG)_8YA$	53.0	24	22
840	$(GA)_8YT$	51.0	25	22
841	$(GA)_8YC$	53.2	23	22
842	$(GA)_8YG$	53.2	25	25
868	$(GAA)_6$	51.2	25	25
888	$BDB(CA)_7$	58.5	25	21
889	$DBD(AC)_7$	55.0	25	21
890	$VHV(GT)_7$	59.0	23	23
891	$HVH(TG)_7$	56.2	27	26
Total			326	306

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

2003). The Bayesian method takes full advantage of the information provided by dominant markers, allowing us to incorporate uncertainty about the magnitude of the within-population inbreeding coefficient into estimates of F_{st} (Holsinger and Wallace 2004; Zhang et al. 2007). Several runs were conducted with default sampler parameters (burnin = 5,000, sample = 100,000, thin = 20) to ensure consistency of results (Tero et al. 2003). Model selection was based on the Deviance Information Criterion (DIC) (Spiegelhalter et al. 2002). Models with smaller DICs are preferred (Holsinger and Lewis 2003).

The 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. Significant differences between wild and cultivated populations were quantified using Kruskal–Wallis test with SPSS 16.0 software (SPSS 2007). Principal Coordinates analysis (PCoA) was performed using the program NTSYSpc (Rohlf 2000) based on Nei's (Nei 1978) unbiased genetic distance matrix of populations. To test the correlation between genetic and geographic distances (in kilometers) among populations, Mantel test was performed with TFPGA software (Miller 1997).

The assignment testing to identify the possible origin of cultivated populations was done by the likelihood-based AFLPOP program (Duchesne and Bernatchez 2002).

The software AFLPOP can do assignment testing with dominant data, such as ISSRs. Each individual is allocated to the population showing the highest likelihood for that genotype (Duchesne and Bernatchez 2002; He et al. 2004). For each of simulation run, this was repeated 1,000 times. Average assignments to a given site were subsequently calculated as a percent value on ten repeats of these 1,000 iterations. The AFLPOP program allows the user to set a log-likelihood threshold for each assignment. A minimum threshold value of log-likelihood difference (MLD) between populations showing the highest and the second-highest likelihood was imposed to the test to increase the reliability of the assignment.

Results

Genetic diversity

A total of 326 ISSR bands were generated in 208 individuals of 16 populations of *R. tanguticum* with the 13 selected primers, 306 (93.87 %) of which were polymorphic (Table 3). The bands ranged in size from 200 to 2,800 bp. The total number of bands varied from 23 (UBC809, UBC841, UBC890) to 29 (UBC834) with an average of 25.08 fragments per primer. The percentage of polymorphic bands (PPB) ranged from 25.77 % in LC population to 58.90 % in ZQ population (Table 3). The wild populations had a slightly higher PPB (92.33 %) than that of cultivated populations (82.19 %).

Measurements of genetic diversity within wild, cultivated populations and all populations are summarized in Table 3. The estimate of heterozygosity (H_B) under $f = 0$ model based on the Bayesian procedure was generally higher than Nei's gene diversity (H). The value of Nei's gene diversity (H) was 0.2670, and Shannon's Information index (I) was 0.4137 at species level while at populations level 0.1774 and 0.2622, respectively. Among all the populations investigated, the highest and lowest levels of genetic variability occurred in population ZQ ($H = 0.2104$, $I = 0.3127$) and population LC ($H = 0.1026$, $I = 0.1499$), respectively. When compared to wild populations (species level, $H = 0.2666$, $I = 0.4124$; population level, $H = 0.1772$, $I = 0.2620$), the cultivated populations (species level, $H = 0.2490$, $I = 0.3812$; population level, $H = 0.1689$, $I = 0.2491$) exhibited lower genetic diversity. The Kruskal–Wallis test was used to reveal if significant differences existed between wild and cultivated populations for the percentage of polymorphic bands, Nei's gene diversity, Shannon's information index and expected Bayesian heterozygosity. Kruskal–Wallis test failed to reject the null hypothesis ($P > 0.05$), indicating that wild and cultivated populations do not differ significantly in any of the genetic diversity measures (Table 4).

Table 3 Genetic diversity indices of the 16 populations of *Rheum tanguticum*

Population	*H* (SE)	H_B (SD)	*I* (SE)	PPB (%)
Wild populations				
ZQ	0.2104 (0.2045)	0.2562 (0.0049)	0.3127 (0.2912)	58.90
DGM	0.1651 (0.2026)	0.2256 (0.0052)	0.2460 (0.2878)	47.85
DK	0.1741 (0.2003)	0.2363 (0.0051)	0.2595 (0.2879)	48.77
JS	0.1926 (0.2020)	0.2479 (0.0049)	0.2879 (0.2879)	56.13
DL	0.1974 (0.2079)	0.2499 (0.0049)	0.2917 (0.2963)	53.37
SGM	0.2019 (0.2094)	0.2523 (0.0050)	0.2969 (0.2994)	52.76
XGM	0.1843 (0.2107)	0.2448 (0.0051)	0.2704 (0.2999)	48.16
JK	0.1605 (0.2074)	0.2354 (0.0052)	0.2354 (0.2949)	42.64
YNG	0.1700 (0.2044)	0.2454 (0.0055)	0.2515 (0.2920)	46.63
MX	0.1898 (0.2098)	0.2450 (0.0047)	0.2796 (0.2982)	50.92
LC	0.1026 (0.1829)	0.2302 (0.0066)	0.1499 (0.2624)	25.77
Mean at population level	0.1772	0.2456	0.2620	48.35
Total at species level	0.2666	0.3115	0.4124	92.33
Cultivated populations				
HM	0.1850 (0.2067)	0.2435 (0.0052)	0.2735 (0.2951)	50.31
NX	0.1738 (0.2045)	0.2433 (0.0055)	0.2570 (0.2929)	46.93
ZM	0.1640 (0.2081)	0.2563 (0.0062)	0.2400 (0.2968)	42.02
DHG	0.1913 (0.2080)	0.2527 (0.0051)	0.2825 (0.2965)	51.84
QJ	0.1757 (0.2018)	0.2342 (0.0053)	0.2610 (0.2902)	48.47
Mean at population level	0.1689	0.2460	0.2491	45.18
Total at species level	0.2490	0.3033	0.3812	82.19
Mean (overall)	0.1774	0.2437	0.2622	48.22
Total (overall)	0.2670	0.3078	0.4137	93.87

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, *SD* standard deviation

Genetic differentiation

The genetic differentiation among populations (G_{st}), estimated by Nei's method, was 0.3362 which indicated that 33.62 % of total genetic diversity was distributed among populations, whereas 66.38 % occurs within populations (Table 5). Furthermore, the level of gene flow (N_m) was measured to be 0.9873 individual per generation between populations. Generally, cultivated populations (G_{st} = 0.2742) exhibited lower genetic differentiation than wild populations (G_{st} = 0.3350). The level of genetic differentiation (G_{st}) between the wild group (a single group consisting of all wild individuals) and cultivated group (a single group consisting of all cultivated individuals) was 0.0305, indicating only about 3.05 % genetic variation resided between the two groups (wild and cultivated). The level of gene flow (N_m) between cultivars and their wild progenitors was 15.8777 individuals per generation (Table 5). AMOVA analysis further revealed a similar pattern of genetic differentiation among and within the populations. Of the total variation 28.33 % was attributed to among-populations differences, a value much lower than the within-population proportion (71.67 %). Among- and within-population results were statistically significant ($P < 0.001$) (Table 6). AMOVA analysis also revealed a low level of genetic differentiation, but this was significant ($P = 0.0180$) between the wild and cultivated populations. Of the total variation, 1.85 % was attributable to among-groups, while 27.24 % and 70.91 % were among-populations, within-groups and within-populations (Table 6). A similar result was obtained from the Hickory calculation: population differentiation (θ_B) was 0.2226 under the $f = 0$ model, which had the smallest DIC value (Table 7).

Table 4 Kruskal–Wallis test for differences in genetic variation measures between wild and cultivated populations

Y	χ^2	*df*	χ^2-prob
H	0.157	1	0.692
I	0.157	1	0.692
PPB	0.388	1	0.533
H_B	0.157	1	0.692

H Nei's gene diversity, *I* Shannon's diversity index, PPB percentage of polymorphic bands, *df* degrees of freedom, H_B expected Bayesian heterozygosity (without assuming Hardy–Weinberg equilibrium)

Genetic relationships of cultivated *R. tanguticum* and their origin wild relatives

The UPGMA dendrogram, based on Nei's (Nei 1978) unbiased genetic distance, was shown in Fig. 2. All the populations were divided into three groups. The group I included most populations of Golog Tibetan Autonomous Prefecture (ZQ, DGM, DK, JK, JS, DL, SGM and XGM) and three cultivated populations (HM, DHG and QJ). YNG, NX and ZM populations formed the group II, while MX and LC made up group III. It appeared that the three groups were divided similar with the three Prefectures that the populations were collected from except cultivated populations. The relationships among 16 populations were further examined by performing a PCoA (Fig. 3). In this analysis, the first three axes explained 27.62, 17.79 and 15.32 % of the total variance among populations, respectively, and revealed three major groups. The wild and cultivated populations were not separated in the UPGMA and PCoA analysis.

Mantel test analysis showed that genetic distance was significantly correlated with geographic distance ($r = 0.5311$, $P = 0.0470$) in wild populations; however, this pattern was not recovered in cultivated populations ($r = 0.1176$, $P = 0.3686$).

Assignment tests using the AFLPOP procedure reallocated 203 individuals (97.60 %) to their population of sampling origin under the minimal log-likelihood difference (MLD) set at 2. This suggested considerable genetic homogeneity within populations and significant heterogeneity among populations. The probable source populations of cultivated individuals were analyzed with the allocation procedure (Table 8). Choice of zero frequency replacement value and the MLD were set to 0.001 and 0, respectively. 69 % of HM individuals were allocated to XGM wild population and 31 % were assigned to JS, indicating that the possible origin of HM cultivated population is XGM. But for NX, ZM, DHG and QJ, they were allocated to different wild populations, indicating the origins of these cultivated populations were admixture.

Table 5 Comparisons of the genetic structure within wild, cultivated and all the populations

Populations	*n*	H_t	H_s	G_{st}	N_m	Φ_{st}
Wild	11	0.2664	0.1772	0.3350	0.9925	0.290
Cultivated	5	0.2498	0.1813	0.2742	1.3236	0.239
Wild vs. cultivated	2	0.2967	0.2876	0.0305	15.8777	0.057
All samples	16	0.2673	0.1774	0.3362	0.9873	0.283

n number of populations, H_t total genetic diversity, H_s genetic diversity within populations, G_{st} Nei's coefficient of genetic differentiation among populations, Φ_{st} AMOVA-derived Φ_{st}, N_m gene flow

Discussion

Genetic diversity

In the present study, the population genetic diversity parameters ($H = 0.1774$, Table 3), indicated that *R. tanguticm* has moderately high levels of variation, when compared to those of allied species of Polygonaceae, such as *R. 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). Besides, high levels of genetic diversity in *R. tanguticum* were previously reported by SSR markers ($H = 0.5150$) (Chen et al. 2009). Population genetic diversity is largely influenced by factors such as breeding system, seed dispersal, genetic drift, evolutionary history as well as life form. Life form and breeding system have highly significant influences on genetic diversity. In general, long-lived and outcrossing species tend to be more

Table 6 Analysis of molecular variance (AMOVA) for wild, cultivated and all the populations of *Rheum tanguticum*

Source of variation	*df*	Sum of squares	Mean squares	Variation components	Total variation (%)	*P* value[a]
Among populations	15	2,622.86	174.86	11.30	28.33	<0.0010
Within populations	192	5,486.30	28.57	28.57	71.67	<0.0010
Wild vs. cultivated	1	230.91	220.91	0.74	1.85	0.0180
Among populations	14	2,391.95	170.85	10.96	27.24	<0.0010
Within populations	192	5,486.30	28.57	28.57	70.91	<0.0010
Among wild populations	10	1,852.59	185.26	11.57	29.03	<0.0010
Within wild populations	139	3,933.37	28.30	28.30	70.97	<0.0010
Among cultivated populations	4	539.36	134.84	9.21	23.91	<0.0,010
Within cultivated populations	53	1,552.93	29.30	29.30	76.09	<0.0010

df Degrees of freedom, *SSD* sum of squares, *MSD* mean squared deviation

[a] Significance tests after 1,000 permutations

Table 7 Genetic differentiation calculated between populations of *Rheum tanguticum* under different Bayesian approach

Model	θ_B				f				DIC
	Mean	SD	2.5 %	97.5 %	Mean	SD	2.5 %	97.5 %	
Full model	0.2260	0.0067	0.2133	0.2392	0.0242	0.0206	0.0008	0.0776	12,964.2
$f = 0$ model	0.2226	0.0060	0.2109	0.2344	0	–	–	–	12,959.5
$\theta_B = 0$	0	–	–	–	0.1600	0.0442	0.1585	0.2518	24,396.4
f free model	0.2794	0.0087	0.2638	0.2983	0.5008	0.2895	0.0221	0.9765	13,383.9

θ_B analogous to Wright's F_{st}; f analogous to Wright's F_{is}; DIC deviance information criterion; *SD* standard deviation

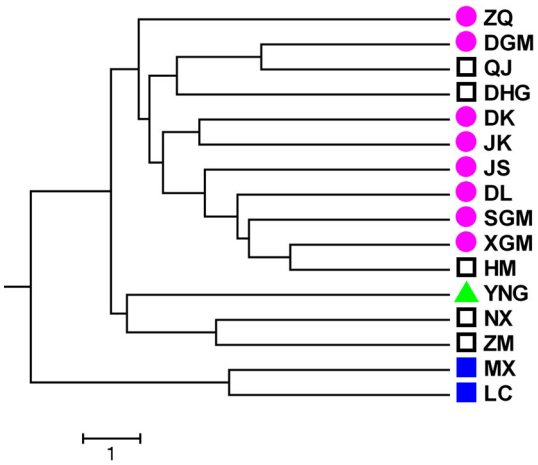

Fig. 2 Dendrogram illustrating the relationships of wild and cultivated *Rheum tanguticum* obtained by UPGMA cluster analysis

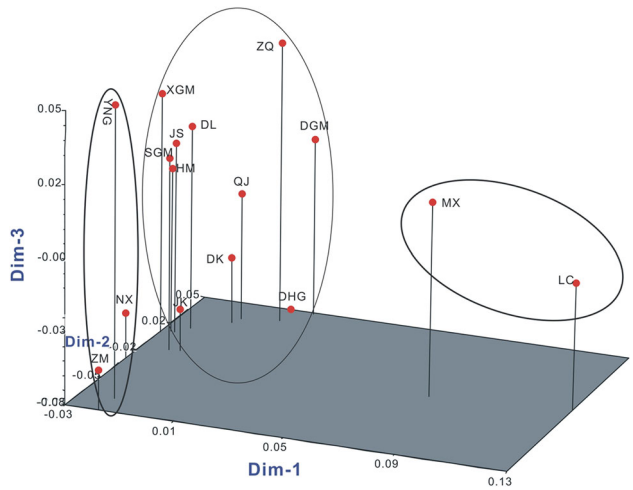

Fig. 3 Principle coordinate analysis based on Nei's (1978) unbiased genetic distances

genetically diverse (Hamrick and Godt 1996). The moderately high genetic diversity observed in *R. tanguticum* may be attributed to its outcrossing and long-lived herbaceous nature.

Numerous studies have shown that cultivated populations maintain a subset of the total genetic diversity occurring in their wild ancestors because of genetic bottleneck and artificial selection (Wright et al. 2005; Xu et al. 2008; Hernandez-Verdugo et al. 2001; Li et al. 2009). Whereas, no significant differences between wild and cultivated *R. tanguticum* populations were detected with the Kruskal–Wallis test (Table 4). AFLPOP uses ISSR presence/absence data to calculate log-likelihood values for any individuals' membership in a reference population, based upon their banding patterns. With the AFLPOP assignment, the possible origin of HM cultivated population was XGM. There was no loss of genetic diversity in HM relative to its source population XGM (Table 3). It was likely reflected the relatively small reduction in diversity that accompanied the cultivation of *R. tanguticum*. The coefficient of genetic differentiation ($G_{st} = 0.0305$) between wild and cultivated populations, as well as AMOVA analysis (Tables 5, 6) indicated low genetic differentiation between wild and cultivated populations. The result was further confirmed by UPGMA and PCoA analyses, in which the results (Figs. 2, 3) did not separate the cultivated populations from the wild populations. This indicated that a large number of wild genotypes were introduced from the wild gene pool to the cultivated gene pool in *R. tanguticum*. Therefore, the influence of a "cultivation bottleneck or founder effect" is not evident. This may be due to a transient cultivation history, weak artificial selection pressure and extensive seed exchange among different geographical areas. Traditionally, farmers collect and preserve *R. tanguticum* seeds randomly without deliberate selection and mix them together before planting. Sometimes, they exchange seeds with their relatives or friends. And these would reserve more genetic diversity as the wild species. For example, DHG and QJ cultivated populations were planted by the researchers of our institute. The seeds of these two places were collected from different places in Golog Prefecture. The results of the assignment test (Table 8) in this study further confirmed this fact. Most of the individuals in DHG and QJ were assigned to different wild populations in Golog Prefecture. Furthermore,

Table 8 Assignment of cultivated individuals to source populations using the AFLPOP program

Cultivated population	Assigned to source populations										
	ZQ	DGM	DK	JS	DL	SGM	XGM	JK	YNG	MX	LC
HM	0.00	0.00	0.00	0.31	0.00	0.00	0.69	0.00	0.00	0.00	0.00
NX	0.09	0.00	0.00	0.46	0.09	0.27	0.00	0.09	0.00	0.00	0.00
ZM	0.14	0.00	0.00	0.00	0.14	0.14	0.00	0.14	0.29	0.14	0.00
DHG	0.36	0.00	0.36	0.14	0.07	0.07	0.00	0.00	0.00	0.00	0.00
QJ	0.15	0.46	0.15	0.08	0.08	0.08	0.00	0.00	0.00	0.00	0.00

the origin seeds of these two populations were mixed from different places in Golog Prefecture.

In some perennial plants, cultivated populations may harbor a relatively higher percentage of genetic diversity of their wild ancestors in comparison to annual plants (Miller and Schaal 2006; He et al. 2009; Otero-Arnaiz et al. 2005). Similarly, our findings are in consistence with several other long-lived cultivated plants where genetic diversity is as high as wild relatives (He et al. 2007; Shi et al. 2008). Compared with cultivated populations of other endangered Chinese medicinal plants (Table 9) (He et al. 2007; Qiu et al. 2009; Shi et al. 2008; Song et al. 2010; Wu et al. 2006), cultivated *R. tanguticum* were found to have moderate levels of genetic diversity. Therefore, the influence of a "cultivation bottleneck or founder effect" is not evident.

Comparison of genetic structure between wild and cultivated populations

It has been shown that long-lived and outcrossing species typically allocate greater genetic variability within populations than among populations (Hamrick and Godt 1996; Nybom 2004). The results obtained in this study revealed that more genetic variance was retained within populations for both wild (70.97 %) and cultivated (76.09 %) *R. tanguticum*. When compared to other plants, the values of genetic differentiation in this plant ($G_{st} = 0.3362$) and its wild group ($G_{st} = 0.3350$) were a slightly higher than the means of long-lived perennial ($G_{st} = 0.19$) and outcrossing ($G_{st} = 0.22$) species (Nybom 2004). This indicated that a higher amount of diversity existed among populations. Several factors should be considered for the observed genetic structure. First, *R. tanguticum* is an outcrossing and self-incompatible species. Second, because the rhizomes are traditionally used for medicinal purposes, excessive excavation of the herb has caused a tremendous decrease of population size in the wild. Finally, wild *R. tanguticum* are mainly distributed in forests slopes or valleys of Qinghai-Tibetan Plateau where high mountains and deep valleys are dominant. The complex topography of the region may have hindered gene flow via pollen and seeds among populations. These factors lead to notably limited gene flow ($N_m = 0.9925$) and contribute to the genetic differentiation among wild *R. tanguticum*.

Table 9 Comparison of genetic diversity of cultivated *Rheum tanguticum* with other important herbs in cultivated populations

Species	Genetic diversity values	Markers	References
R. tanguticum	$PPB_s = 82.19$ %; $H_s = 0.2490$; $PPB_p = 45.18$ %; $H_p = 0.1689$	ISSR	Present study
Salvia miltiorrhiza	$PPB_s = 100$ %; $H_s = 0.1951$; $PPB_p = 65.00$ %; $H_p = 0.1591$	ISSR	(Song et al. 2010)
Coptis chinensis	$PPB_s = 89.01$ %; $H_s = 0.2040$; $PPB_p = 65.20$ %; $H_p = 0.1600$	ISSR	(Shi et al. 2008)
Gastrodia elata	$PPB_s = 61.04$ %; $H_s = 0.1280$; $PPB_p = 35.71$ %; $H_p = 0.1000$	ISSR	(Wu et al. 2006)
Corydalis yanhusuo	$PPB_s = 71.54$ %; $H_s = 0.1630$; $PPB_p = 25.32$ %; $H_p = 0.0940$	ISSR	(Qiu et al. 2009)
Paris polyphylla	$PPB_s = 83.27$ %; $H_s = 0.1860$; $PPB_p = 57.24$ %; $H_p = 0.1530$	ISSR	(He et al. 2007)

PPB_s Percentage of polymorphic loci at species level; H_s Nei's gene diversity at species level; PPB_p percentage of polymorphic loci at population level; H_p Nei's gene diversity at population level

Similar to the result of AMOVA, UPGMA and PCoA analyses indicated that populations from wild and cultivated groups did not form independent clades (Figs. 2, 3). The high gene flow ($N_m = 15.8777$) between the wild and cultivated populations may have prevented genetic differentiation between cultivated *R. tanguticum* and their wild progenitors. A high level of gene flow between wild and cultivated *R. tanguticum* might have resulted from a combination of different effects, including exchange of pollen by pollinators, transplantation of wild individuals to

cultivated populations, and using the seeds from wild progenitors to establish new cultivated populations.

UPGMA and PCoA analysis proved that the cultivated populations were grouped with the wild population closest to its source. The probable origin of HM was XGM, which was further approved by AFLPOP assignment. For NX, ZM, DHG and QJ populations, they were allocated to different wild populations, indicating the origins of these cultivated populations were admixture and from different places rather than local places. The results of the assignment of these four cultivated populations were consistent with the records of local growers.

Implication for conservation and cultivation

Measuring of genetic diversity plays a very important role in the conservation program of plant genetic resources. Current study indicates that *R. tanguticum* has maintained a relatively high level of genetic diversity both in the wild and cultivated populations. The cultivated populations, retained 89 % of the genetic variation of wild populations, may play a crucial role as native populations has been declining. The primitive agricultural practices, i.e., random collecting, preserving and planting seeds without deliberate selection, do not seem to cause too much change in genetic diversity of *R. tanguticum*, considering that the populations are large enough and the cultivation history is short. Such practices might be an effective way to maintain and conserve gene pools of wild plants (Song et al. 2010; Guo et al. 2007). Furthermore, given that the genetic diversity of DHG and QJ cultivated populations of *R. tanguticum* was relatively higher than that of wild populations at the population level, the present planting of these two sites' populations can be seen as a prior step for the ex situ conservation. Nevertheless, it will still be beneficial to introduce additional germplasms from other wild localities to retain as much genetic diversity as possible.

Acknowledgments This work was supported by National Key Technology R&D Program of China (2012BAC08B04), National Nature Science Foundation of China (31200245) and West Light Foundation of the Chinese Academy of Sciences for Doctors (2011).

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