Aquatic Botany 134 (2016) 54–60

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Analysis of population structure of *Blysmus sinocompressus* in the Qilian Mountains by ISSR markers

Yan-ping Hu[a], Rui Bao[a], Lin Shi[a,b], Jian-ke Wang[a,b], Jun Wang[a,b], Li Wang[a,*,1], Yi Li[a,*,1]

[a] Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai 810008, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form 11 May 2016
Accepted 25 June 2016
Available online 10 July 2016

Keywords:
Blysmus sinocompressus
Qilian Mountains
Genetic diversity
ISSR
Cyperaceae

ABSTRACT

Blysmus sinocompressus (Cyperaceae) is an edificator and dominant wetland endemic grass of ecological and economical importance to northwestern China. Inter-simple sequence repeats (ISSR) were used to reveal the genetic diversity and population genetic structure in nine populations of *B. sinocompressus* in the Qilian Mountains. The genetic diversity of *B. sinocompressus* was high at species levels (PPB_s = 74.76%, H_s = 0.1668), but relatively low at population levels (PPB_p = 31.01%, H_p = 0.0941). A significant genetic differentiation (G_{st} = 0.4355) and low gene flow (N_m = 0.6480) were found among populations. Analysis of molecular variance (AMOVA) test revealed that 55.52% genetic variation occurred within populations and the remaining 44.48% was found among populations. Using unweighted pair-group method with arithmetic average (UPGMA) dendrogram, the nine populations were classified into three groups: (1) a single population of ZMS from Heihe River; (2) the YHLK population (Heihe River) and a subcluster of populations from Qinghai Lake; (3) the QLHH population (Heihe River) and a subcluster of populations from Daitong River. Similar results were obtained by using Principal coordinates analysis (PCoA) and Bayesian cluster analysis. Moreover, the genetic diversity of *B. sinocompressus* was negatively correlated with latitude, while positively correlated with annual mean temperature. With these observations, we propose a much needed conservation strategy for this plant in the Qilian Mountains.

1. Introduction

The Qilian Mountains, sat on the northeastern margins of the Qinghai-Tibet Plateau, are composed of a string of mountains and valleys in northwestern China. Several inland rivers including the Heihe, Shule, and Shiyang originated from the Qilian Mountains in Qinghai Province have provided valuable water resources for the neighboring lands (Sun et al., 2015; Zhao et al., 2015). Thus, the Qilian Mountains play a critical role in water conservation for regional sustainable development, and serve as important ecological shelters in northwestern China (Dong et al., 2015).

Blysmus sinocompressus Tang & F. T. Wang (Cyperaceae), an edificator and dominant species in hygrophyte communities, is a far-creeping rhizomatous perennial herb endemic to China. Its distribution covers Qinghai, Gansu, Sichuan, Shaanxi, Yunnan, Xizang provinces and northern China at altitudes ranging from 1 000 m to 4 000 m. The habitats in which it often grows include wet places, stream and river margins, riverbeds, grasslands, valleys, swampy meadows and slopes (Wu et al., 2010). With high levels of crude proteins and fats, this species is preferred by livestocks over other plants found on the plateau (Ma and Xu, 2013). Although the asexual vegetative propagation by rhizomes is prevailed, it also flowers and sets seeds during the period from June to September. So far, studies on *B. sinocompressus* have mainly focused on the characterization of growth, development, and its ecological function in wetland plant community (Guo et al., 2007; Tan et al., 2013). Little is known about its breeding system and population genetic diversity. Although widespread, *B. sinocompressus* has been considered to be vulnerable in Zoige plateau and Gannan alpine wetland, due to overgrazing and disappearance of wetland areas (He et al., 2000; Yue et al., 2004). He et al. (2000) compared the changes of plant communities in different degree mire pastures and could hardly find *B. sinocompressure* in overgrazing mire pastures, despite it was the dominant species in the past.

As a powerful tool in measuring plant genetic diversity and population structure, the PCR-based molecular marker inter-simple sequence repeats (ISSR) can reliably produce large numbers of polymorphic bands at low cost. This technique amplifies DNA sequences

* Corresponding authors at: Northwest Institute of Plateau Biology, Chinese Academy of Sciences, 23 Xinning Road, Xining 810008, China.
E-mail addresses: liyi@nwipb.cas.cn (Y. Li) wangli@nwipb.cas.cn (L. Wang),.
[1] Both corresponding authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aquabot.2016.06.011
0304-3770/

Table 1
Sample Information of *B. sinocompressus* in the Qilian Mountains.

Locality	Populations	Latitude (N)	Longitude (E)	Altitute (m)	AMT (°C)	AMP (mm)	Sample size
Qinghaihudong, Haiyan county, Haibei Prefecture	QHHD	36°32′58.6″	100°43′32.4″	3215	1.00	354.13	13
Dayuwugu, Haiyan county, Haibei Prefecture	DYWG	36°51′20″	100°51′44.6″	3236	0.70	342.98	15
Hadengqu, Haiyan county, Haibei Prefecture	HDQ	37°12′50.4″	100°38′45.1″	3363	−0.20	321.51	15
ZhaMaShi, Qilian county, Haibei Prefecture	ZMS	38°12′7.6″	100°1′16.5″	2853	0.70	245.33	15
Youhulukou, Qilian county, Haibei Prefecture	YHLK	38°17′12.3″	99°50′9.7″	3004	−0.20	239.96	16
Heiquanshuiku, Datong county, Xining	HQSK	37°14′39.9″	101°27′36.1″	2904	1.50	321.92	13
Huahaizi, Menyuan county, Haibei Prefecture	HHZ	37°36′13.1″	101°20′56.3″	3208	0.00	306.55	13
Qilianhuahai, Menyuan county, Haibei Prefecture	QLHH	37°59′5.4″	100°49′43.3″	3274	−0.60	280.40	15
Zhamatu, Menyuan county, Haibei Prefecture	ZMT	37°27′51.1″	101°14′11.4″	3143	0.50	312.11	15

AMT: annual mean temperature, based on 1951–1980 year after year; AMP: annual mean precipitation, based on 1971–2000 year after year. AMT and AMP were collected from Scientific Database, Chinese Academy of Sciences.

Table 2
Primers used in ISSR analysis of *B. sinocompressus*.

Primer	Sequences (5′ → 3′)	Annealing temperature (°C)	No. of analyzed bands	No. of polymorphic bands
807	AGAGAGAGAGAGAGAGT	52.0	20	15
808	AGAGAGAGAGAGAGAGC	54.3	14	9
817	CACACACACACACACAA	50.8	16	11
818	CACACACACACACACAG	52.3	28	21
822	TCTCTCTCTCTCTCTCA	48.3	12	7
834	AGAGAGAGAGAGAGAGYT	55.4	19	15
836	AGAGAGAGAGAGAGAGYA	58.0	23	16
857	ACACACACACACACACYG	50.0	24	19
864	ATGATGATGATGATGATG	48.0	28	22
899	CATGGTGTTGGTCATTGTTCCA	50.0	22	19

Y = (C, T).

between two-inverted SSR without prior knowledge of the position of the SSR target sequences in the genome (Zietkiewicz et al., 1994; Hu et al., 2010; Tian et al., 2012). ISSR has been well established as a sensitive and simple approach for detecting species and population genetic diversity and differentiation (Baggio et al., 2013; Jena et al., 2015), in spite of being a dominant marker system (Nybom, 2004).

In the present study, we used ISSR markers to measure the patterns and levels of genetic diversity of *B. sinocompressus* populations from Qilian Mountains. We have also revealed the possible relationships between genetic diversities with geographic and environmental factors. These results are expected to provide basic information for the conservation and breeding strategies in restoring and improving this excellent forage species in northwestern China.

2. Materials and methods

2.1. Plant sampling and DNA extraction

One hundred and thirty individual samples of *B. sinocompressus* were collected from nine populations of Qilian Mountains in Qinghai Province, China (see Supplementary material, Appendix 1 for details). Each population was positioned by GPS, with the location details listed in Table 1. Young leaf tissues were collected from individual plants and then dried in silica gel before DNA extraction. Total genomic DNA was extracted by using the modified Doyle's CTAB method (Doyle and Doyle, 1987). All collected samples were identified by Prof. Xuefeng Lu and voucher specimens were deposited in the Herbarium of Northwest Institute of Plateau Biology (HNWP), Chinese Academy of Sciences.

2.2. ISSR amplification

One hundred primers from the University of British Columbia (UBC set no. 9) were initially screened for PCR amplification. Ten primers that produced distinct, reproducible banding patterns were chosen for final analysis (Table 2). PCR amplifications were carried out in a 20 μL reaction volume consisting of 20 ng of genomic DNA, 1.8 mM $MgCl_2$, 0.1 mM dNTPs, 0.6 μmol of primer, 0.8 U of *Taq* DNA polymerase (*TaKaRa* Biotech Co., Ltd.) and 2.0 μL of 10 × PCR buffer. ISSR-PCR amplifications were performed in a C1000 Touch thermal cycler (Bio-Rad, USA) with the following condition: an initial denaturation at 94 °C for 5 min, followed by 38 cycles of denaturation at 94 °C for 20 s, annealing at primer's specific temperature (see Table 2 for details) for 60 s and extension at 72 °C for 80 s, and a final extension step at 72 °C for 6 min. The negative control consisted of ddH_2O in place of template DNA to test for possible DNA contamination. The amplified products were resolved in 1.2% agarose gels stained with ethidium bromide and photographed with a Chemi Doc™ MP Imaging System (Bio-rad, USA). Molecular weights were estimated using 100 bp and 200 bp DNA ladders (*TaKaRa* Biotech Co., Ltd.), respectively.

2.3. Data analysis

Band profiles generated by ISSR were scored and converted into binary matrices based on the presence (1) or absence (0) of selected loci. The genetic diversity parameters at population and species levels such as the percentage of polymorphic bands (*PPB*), the gene diversity index *H* (Nei, 1973) and Shannon's information index (*I*) were estimated using Popgene 1.32 (Yeh et al., 1999), assuming dominance and Hardy–Weinberg equilibrium. H_s and H_p were the gene diversity at species and population level, respectively. Gene differentiation between populations was estimated by the coefficient of gene differentiation (G_{st}) (($G_{st} = H_s - H_p)/H_s$ Nei, 1973). Gene flow (N_m, the numbers of migrants per generation) was calculated from G_{st} according to McDermott and McDonald (1993), where $N_m = (1 - G_{st})/4G_{st}$. To examine the genetic relationship among populations, original genetic distance and genetic identity (Nei, 1978) were also calculated by Popgene for all pairwise combinations of populations. A dendrogram was constructed from Nei's genetic distance with the unweighted pair-group method of averages (UPGMA) using NTSYSpc software (Rohlf, 2000). Principal coordinates analysis (PCoA) was performed to ordinate

Table 3
Genetic diversity statistics for *B. sinocompressus* populations.

Population	*H*	*I*	H_B	*PPB* %
ZMS	0.0936 ± 0.1692	0.1417 ± 0.2443	0.1546 ± 0.0060	30.58
QHHD	0.1192 ± 0.1778	0.1817 ± 0.2587	0.1824 ± 0.0064	38.35
DYWG	0.1148 ± 0.1791	0.1738 ± 0.2588	0.1720 ± 0.0058	35.92
HDQ	0.0899 ± 0.1584	0.1398 ± 0.2316	0.1496 ± 0.0062	32.52
YHLK	0.0903 ± 0.1645	0.1368 ± 0.2398	0.1533 ± 0.0062	28.16
HQSK	0.1036 ± 0.1706	0.1579 ± 0.2485	0.1761 ± 0.0072	33.01
HHZ	0.0702 ± 0.1471	0.1085 ± 0.2148	0.1431 ± 0.0071	25.24
QLHH	0.0705 ± 0.1509	0.1069 ± 0.2197	0.1311 ± 0.0064	22.82
ZMT	0.0950 ± 0.1679	0.1451 ± 0.2426	0.1674 ± 0.0067	32.52
Average	0.0941	0.1436	0.1588	31.01
Total	0.1668	0.2635	0.2352	74.76

H: Nei's gene diversity (assuming Hardy–Weinberg equilibrium), H_B: Bayesian gene diversity (without assuming Hardy–Weinberg equilibrium), *I*: Shannon's information index, *PPB*: percentage of polymorphic bands.

Table 4
AMOVA analysis of *B. sinocompressus* from nine natural populations with ISSR data.

Source of variation	*d.f.*	SSD	MSD	Variance Component		Fixation index	*P* value*
				Absolute	%		
(i) Total populations							
Among populations	8	1023.11	127.89	8.15	44.48	Φ_{st} = 0.445	<0.001
Within populations	121	1231.74	10.18	10.18	55.52		<0.001
(ii) Three groups according to UPGMA							
Among groups	2	425.66	212.83	2.87	14.90	Φ_{ct} = 0.149	<0.001
Among populations	6	597.45	99.58	6.23	32.31	Φ_{sc} = 0.380	<0.001
Within populations	121	1231.74	10.18	10.18	52.79	Φ_{st} = 0.472	<0.001

SSD: sum of squared deviations; MSD: mean of squared deviation.
* Significance tests after 1 000 permutations.

relationships among populations with Nei's original genetic distance matrix.

To correct for possible bias introduced by the assumption of Hardy–Weinberg equilibrium, Bayesian gene diversity (H_B, analogous to *H*) and population differentiation (θ_B) were also calculated by the Bayesian approach (Holsinger et al., 2002) using Hickory 1.1 (Holsinger and Lewis, 2003). The Bayesian method assumes neither Hardy–Weinberg equilibrium within populations nor treating 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 carried out with default sample parameters (burn-in = 5 000, sample = 100 000, thin = 20) to ensure consistency of the results (Tero et al., 2003). The choice of the best model was based on the Deviance Information Criterion (DIC) (Spiegelhalter et al., 2002). Models with the smaller DICs were chosen (Holsinger and Lewis, 2003).

The degree of differentiation between populations was tested using analysis of molecular variance (AMOVA). Input data files for the AMOVA 1.55 (Excoffier et al., 1992) were generated using AMOVA-PREP (Miller, 1998). The variance components were tested statistically by nonparametric randomization tests using 1000 permutations. The Mantel test was performed using NTSYS (Rohlf, 2000) based on the matrices of genetic and geographic distances (in kilometers).

A Bayesian analysis of ISSR population structure was also run on the data set using the program STRUCTURE (Pritchard et al., 2000) to estimate the number of genetic clusters. This method uses a Markov Chain Monte Carlo (MCMC) algorithm to cluster individuals into populations on the basis of multi-loci genotype data (Falush et al., 2003). STRUCTURE was performed with a burn-in setting of 10,000 followed by 10,000 MCMC iterations under an admixture model with independent allele frequencies. Ten independent runs of *K* = 1–9 were conducted 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).

In addition, Pearson correlation analysis was used for the correlation between genetic diversity and geographic factors, including latitude, longitude, altitude, annual mean temperature and annual mean precipitation. All analyses were calculated with the SPSS 16.0 software (SPSS, 2007).

3. Results

3.1. Analysis of population genetic diversity

A total of 206 ISSR loci were amplified with 10 primers in 130 individual samples. Out of these 206 loci, 154 were polymorphic corresponding to 74.76%. The size of the amplified products ranged from 180 bp to 2 200 bp. Total number of loci ranged from 12 (UBC822) to 28 (UBC818, UBC864) with an average of 20.60 loci per primer. The percentage of polymorphic bands (*PPB*) varied from 22.82% (QLHH) to 38.38% (QHHD), while the average was 31.01% at the population level (Table 3).

The percentage of polymorphic bands (*PPB*), the gene diversity index *H* (Nei, 1973) and Shannon's information index (*I*) and Bayesian gene diversity (H_B, analogous to *H*) were summarized in Table 3. Bayesian gene diversity (H_B) under $f = 0$ model based on the Bayesian procedure was generally higher than Nei's gene diversity (*H*). Nei's gene diversity (*H*) varied from 0.0705 (QLHH) to 0.1192 (QHHD) with the mean values of 0.0941 and 0.1668 at the population and species levels, respectively. The Shannon's information index (*I*) varied from 0.1069 (QLHH) to 0.1817 (QHHD) while the mean estimates were 0.1436 at the population and 0.2635 at the species levels.

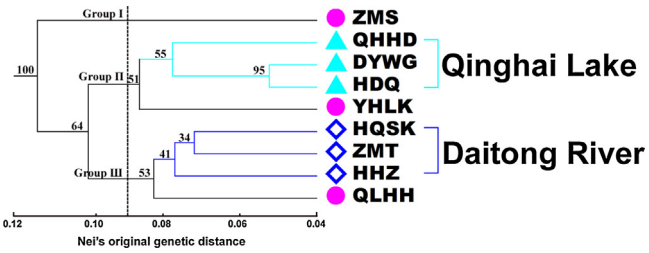

Fig. 1. Dendrogram illustrating the relationships of *B. sinocompressus* populations obtained by UPGMA cluster analysis.

3.2. Population differentiation and genetic structure

Partitioning of genetic variability by AMOVA revealed that most of the ISSR diversity was distributed between individual plants within populations (52.79%), with the remaining diversity distributed between populations within groups (32.31%) and between groups (14.90%) (Table 4). Of the total genetic diversity, 44.48% resided in populations and the rest (55.52%) in individual plants within populations. We also found that 26.26% of the variance was between groups and 73.74% within groups. AMOVA test also showed significant differentiation within and among populations, as well as among groups and within groups ($P < 0.001$, Table 4). According to Nei's gene diversity, the percentages of genetic differentiation among populations were 43.55% ($G_{st} = 0.4355$). A similar result was obtained from the Hickory calculation: population differentiation (θ_B) was 0.3465 under the $f = 0$ model, which had the smallest DIC value (Table 5). Gene flow (N_m) was calculated based on G_{st} and was estimated as 0.6480 for nine populations. The gene flow was also made within groups of populations. The gene flow within Qinghai Lake populations and Daitong River populations was 1.4407 and 1.0681, respectively, whereas the gene flow within Heihe River was 0.6744 which was lower than 1.

The UPGMA dendrogram, based on Nei's original genetic distance, showed all the populations were divided into three groups at 0.09 Nei's original genetic distance (Fig. 1). Group I included only one population (ZMS). In contrast, Group II contained three populations from Qinghai Lake (QHHD, DYWG and HDQ) and YHLK population from Heihe River. Likewise, Group III consisted of populations from Daitong River (HQSK, ZMT and HHZ) and QLHH population from Heihe River. The relationships among populations were further examined by using PCoA (Fig. 2), which produced similar results. In PCoA, the first three axes explained 36.84%, 23.44% and 15.97% of the total variance among populations, respectively, which represented three major groups. Similarly, Bayesian cluster analysis partitioned the populations into three distinct groups (Fig. 3). The highest peak in ΔK revealed the best value for $K = 3$ (Fig. 3A). The Bayesian cluster analysis results were entirely consistent with those of obtained by the UPGMA analysis (Fig. 3B).

The Mantel test was unable to reveal any significant correlations between genetic and geographic distances among populations ($r = 0.2125$, $p = 0.1299$).

3.3. Correlation between genetic diversity and geographic factors

Results of Pearson's correlation analysis indicated that the genetic diversities were significantly decreased with latitudes ($r = -0.687$, $p = 0.041$ for H, Fig. 4; $r = -0.710$, $p = 0.032$ for I; $r = -0.776$, $p = 0.014$ for PPB; $r = -0.693$, $p = 0.039$ for H_B, Table 6), but significantly increased with annual mean temperatures ($r = 0.760$, $p = 0.018$ for H, Fig. 4; $r = 0.758$, $p = 0.018$ for I; $r = 0.728$, $p = 0.026$ for PPB; $r = 0.880$, $p = 0.002$ for H_B, Table 6). Moreover, genetic diversity was negatively correlated with altitude while positively correlated with annual mean precipitation, albeit the correlation was insignificant (Table 6). There was no correlation between genetic diversity and longitude (Table 6).

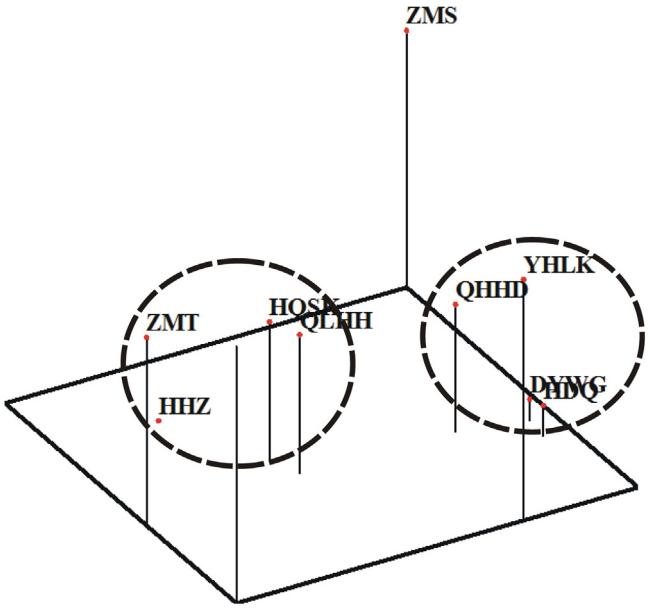

Fig. 2. Principle coordinate analysis based on Nei's (1978) original genetic distance.

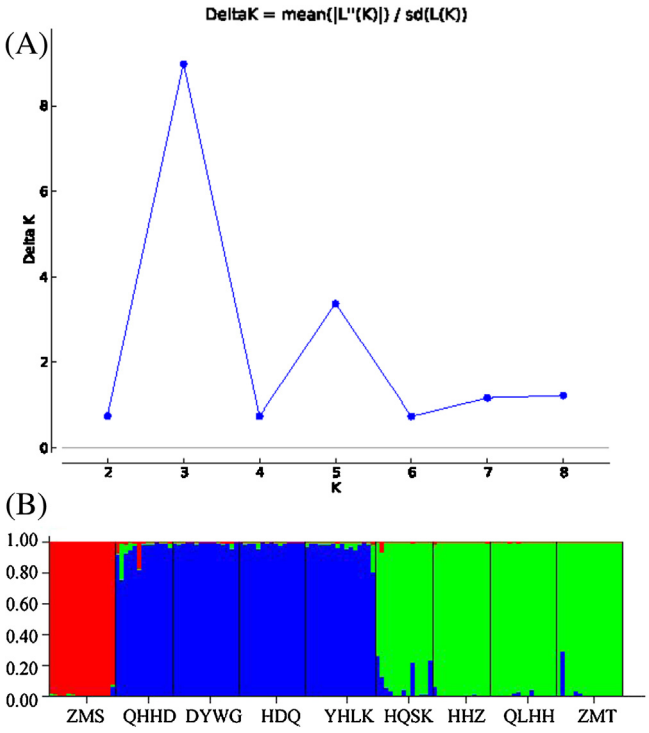

Fig. 3. Graphical representation of genetic structure in nine populations of *B. sinocompressus* using the Bayesian clustering software STRUCTURE ver. 2.3.4. (A) Evanno test results showing estimated delta K; (B) Bar plots showing a relative assignment of *B. sinocompressus* individuals with $K = 3$.

4. Discussion

4.1. Genetic diversity and population differentiation

In the present study, *B. sinocompressus* was found to have high total genetic diversity but relatively low levels of genetic diversity within populations sampled from the Qilian Mountains. For

Table 5
Genetic differentiation (θ_B) of *B. sinocompressus* populations, calculated with different Bayesian models.

Model	θ_B				f				DIC
	Mean	SD	2.5%	97.5%	Mean	SD	2.5%	97.5%	
Full model	0.3526	0.0160	0.3225	0.3849	0.0360	0.0353	0.0009	0.1270	3012.83
f = 0 model	0.3465	0.01502	0.3175	0.3761	0	–	–	–	3011.88
$\theta_B = 0$	0	–	–	–	0.9584	0.0418	0.8441	0.9989	8392.65
f-free model	0.4079	0.0160	0.3764	0.4385	0.5039	0.2902	0.0255	0.9780	3112.82

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

Table 6
Correlation analysis between genetic diversity of *B. sinocompressus* and ecological factors.

Pearson	latitude	longitude	altitude	AMT	AMP
H	−0.687*	−0.040	−0.145	0.760*	0.540
I	−0.710*	−0.023	−0.121	0.758*	0.564
PPB	−0.776*	0.054	−0.013	0.728*	0.645
H_B	−0.693*	0.198	−0.252	0.880**	0.583

H: Nei's gene diversity (assuming Hardy–Weinberg equilibrium), *I*: Shannon's diversity index, *PPB*: percentage of polymorphic bands, H_B: expected Bayesian heterozygosity (without assuming Hardy–Weinberg equilibrium).
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 7
Comparison of genetic diversity of *B. sinocompressus* with other species of Cyperaceae.

Species	PPB_s%	PPB_p%	H_s	H_p	I_s	I_p	Φ_{st}	G_{st}	N_m	marker	References
Kobresia Kansuensis	79.33	63.27	0.2645	0.2266	0.3969	0.3369	0.1429	0.1438	2.9766	RAPD	Zhao et al. (2006)
K. Tibetica	87.70	71.13	0.3106	0.2521	0.4628	0.3772	0.2000	0.1884	2.1534	RAPD	Zhao et al. (2006)
K. setchwanensis	76.67	58.06	0.2527	0.1997	0.3805	0.2998	0.2255	0.2101	1.8801	RAPD	Zhao et al. (2006)
K. humilis	86.60	62.65	0.2662	0.2126	0.3983	0.3185	0.1696	0.1891	2.1445	RAPD	Zhao et al. (2006)
K. royleana	81.82	70.23	0.2738	0.2446	0.4099	0.3662	0.1094	0.1066	4.1906	RAPD	Zhao et al. (2006)
Carex moorcroftii	98.57	58.55	0.2761	0.0988	0.4270	0.1520	0.6237	0.6620	0.2600	ISSR	Liu et al. (2009a)
B. sinocompressus	74.76	31.01	0.1668	0.0941	0.2635	0.1436	0.4450	0.4355	0.6480	ISSR	Present study

PPB_s: Percentage of polymorphic loci at species level; H_s: Nei's gene diversity at species level; I_s: Shannon's information index at species level; PPB_p: percentage of polymorphic loci at population level; H_p: Nei's gene diversity at population level; I_p: Shannon's information index at population level; Φ_{st}: AMOVA-derived genetic differentiation between populations; G_{st}: Nei's coefficient of genetic differentiation among populations; N_m: gene flow.

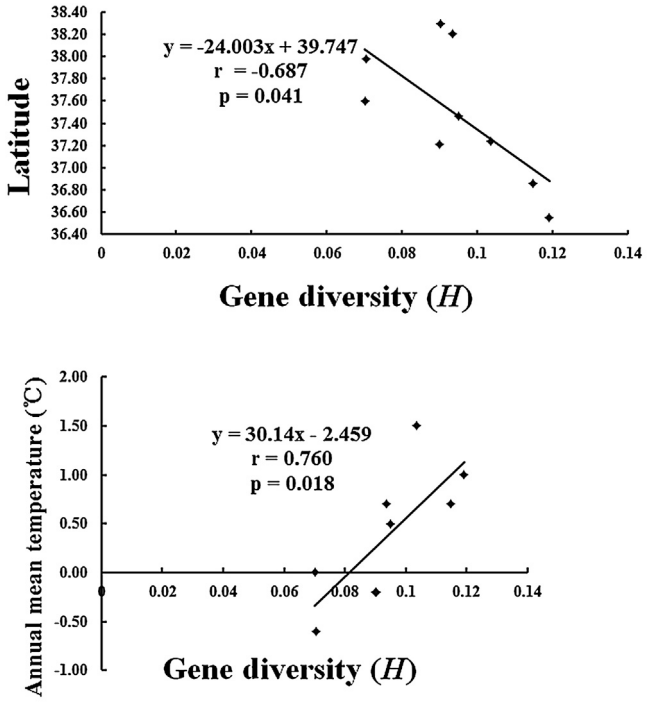

Fig. 4. The correlation between genetic diversity (*H*) and latitude and annual mean temperature.

example, the total genetic diversity of *B. sinocompressus* (PPB_s = 74.76%, H_s = 0.1668) was higher than that of the average value of short-lived perennial herbaceous (PPB_s = 41.30%, H_s = 0.116) and mixed breeding system species (PPB_s = 40.00%, H_s = 0.120) (Hamrick and Godt, 1990). However, the mean value of genetic diversity within populations of *B. sinocompressus* (H_p = 0.0941) was lower than that of the reported average value of long-lived perennial species (H = 0.25) and mixed breeding system species (H = 0.18) (Nybom, 2004). It was also lower than those of other rhizomatous species of Cyperaceae (Table 7). In general, the detection of high levels of polymorphism makes ISSR analysis a powerful tool for assessing genetic diversity in *B. sinocompressus*. None of the individual samples were genetically identical according to ISSR analysis, indicating that the level of resolution in our study was sufficient to distinguish all genotypes.

Our results indicate that *B. sinocompressus* is highly differentiated among populations in the Qilian Mountains. Several possibilities are accounted for this outcome.

Firstly, the mating system has been postulated to be one of the most important factors that determine the genetic diversity in plant species (Hamrick and Godt, 1990). The partition of genetic structure using Nei's gene diversity and AMOVA indicated that genetic diversity in *B. sinocompressus* is distributed equally within and among populations. Since the population genetic structure of a species is affected by multiple evolutionary factors such as mating system, gene flow, genetic drift and natural selection (Hamrick and Godt, 1990), it could be speculated that *B. sinocompressus* might have a mixed mating system, contributed by both outcrossing by pollen and seed dispersal and selfing by underground rhizomes. The outcrossing breeding system would result in a high genetic

diversity at the species level. However, the asexual vegetative propagation by rhizome is so prevailed in the field. The clonality would then diminish genetic diversity within population, while increase genetic differentiation between populations (Liu et al., 2009b).

Secondly, low seed germination rates could lead to poor seedling recruitments. Although these plants set abundant seeds, very few seedling recruitments were observed in the fields (results not shown). This is also supported by a nearly 0% seed germination rate when conducted in laboratory tests without additional treatments. The seed germination rate of *B. sinocompressus* could be enhanced to only 70.60% by cold stratification for 90–120 days in the lab (Jin and Chen, 2014). Therefore, the absence of seedling recruitment would hinder the survival of newly recombined germplasms, which are the main drives for genetic differentiation.

Thirdly, the habitats heterogeneity might be an important factor in determining the pattern of genetic diversity. Although *B. sinocompressus* is distributed widely in the Qilian Mountains where high mountains and deep valleys are abundant, this plant often grows along streams and riverbeds. The complex topography of the region has hindered gene flow ($N_m = 0.6480$) between populations, thus promoted genetic differentiation among populations. The gene flow of populations from Qinghai Lake system (QHHD, DYWG and HDQ) and Daitong River system (HQSK, ZMT and HHZ) is 1.4407 and 1.0681, respectively, whereas the gene flow within Heihe River is below 1. Because there are few mountains between populations in Qinghai Lake and Daitong River, thus the gene flow between populations is greater than 1. In contrast, the gene flow between populations is lower than 1 in the region of Heihe River where high mountains and deep valleys are dominant. This interpretation is also supported by the results of the cluster and principal coordinates analysis. For example, QHHD, DYWG and HDQ populations in Qinghai Lake system and HQSK, ZMT and HHZ in Daitong River system are clustered together, respectively, due to the same river system with no boundaries. This is in sharp contrast with un-clustered populations (ZMS, YHLK and QLHH) in Heihe River system, that are separated by high mountains.

4.2. Correlation between geographic factors and genetic diversity

In *B. sinocompressus*, significant negative correlation is found between genetic diversity and latitude. The relationship between genetic diversity and annual mean temperatures may further explain this phenomenon, as the genetic diversity increases significantly with higher annual mean temperatures. Higher temperatures may enhance seed germination, which helps to increase genetic diversity. This idea is also supported by the results of seed germination experiments conducted in the laboratory. According to Jin and Chen (2014), the germination rate of *B. sinocompressus* was 70.10% under a higher temperature condition (25 °C) and was only 45.20% under a lower temperature condition (15 °C) after 120 days of cold stratification. Finally, we have also found that the genetic diversity is negatively correlated with altitude, although the correlation is statistically insignificant.

4.3. Conservation implication

Genetic diversity is of primary importance for nature conservation and population structure is valuable for proposing conservation strategies. The low levels of genetic variation within populations and the high levels of genetic differentiation among populations of *B. sinocompressus* indicate that the most effective strategy for preserving genetic variation is to conserve as many populations as possible. Because the *B. sinocompressus* populations are highly fragmented in the Qilian Mountains, it is pivotal to preserve the natural habitats. Therefore, the first important step one must take is to reduce the disturbance by human and animal activities. For in situ conservation, preserving only one or two populations of *B. sinocompressus* would not achieve the goal. For example, QHHD population harbors the highest level of genetic diversity and it should be a priority for conservation. Moreover, the three populations in Heihe River should also be important for in situ conservation. For ex situ conservation, a carefully designed and installed germplasm bank is necessary for this species. Transferring the rhizomes from different populations to a more suitable habitat to artificially increase the gene flow should also be considered.

Acknowledgements

This work is financed by International Science and Technology Cooperation program of Qinghai Province, China (2015-HZ-808), National Natural Science Foundation of China (31200245, 31300269) and Youth Innovation Promotion Association, CAS (2014386). We are grateful to Jyan-Chyun Jang (Dept. of Horticulture and Crop Science, Ohio State University) for helping with improvement of the English. The manuscript benefited from the constructive comments of the editor and three anonymous reviewers.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquabot.2016.06.011.

References

Baggio, R.A., Firkowski, C.R., Boeger, M.R.T., Boeger, W.A., 2013. Differentiation within and between river basins of *Podostemum irgangii* (Podostemaceae): a rapid-water macrophyte of southern Brazil. Aquat. Bot. 107, 33–38.

Dong, L., Zhang, M., Wang, S., Qiang, F., Zhu, X., Ren, Z., 2015. The freezing level height in the Qilian Mountains, northeast Tibetan Plateau based on reanalysis data and observations, 1979–2012. Quat. Int. 380, 60–67.

Doyle, J., Doyle, J., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.

Excoffier, L., Smouse, P., Quattro, J., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.

Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587.

Guo, M., Qiang, K., Zhang, X., Long, R., Zhao, L., Liu, Y., 2007. Study on morphological anatomy of leaf in *Blysmus sinocompressus*. J. Gansu Agric. Univ. 42, 82–87.

Hamrick, J.L., Godt, M.J.W., 1990. Allozyme diversity in plant species. In: Brown, A.D.H., Clegg, M.T., Kahler, A.L., Wier, B.S. (Eds.), Plant Population Genetics, Breeding and Genetic Resources. Sinauer Associates Inc., Sunderland, pp. 43–63.

He, C., Zhao, K., Zhao, Z., 2000. Wetlands pasture degeneration mechanism and its sustainable utilzation countermeasure in Roige Plateau. Grassland of China, 11–16.

Holsinger, K.E., Lewis, P.O., 2003. Hickory: A Package for Analysis of Population Genetic Data v1. 1. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, USA.

Holsinger, K.E., Wallace, L.E., 2004. Bayesian approaches for the analysis of population genetic structure: an example from *Platanthera leucophaea* (Orchidaceae). Mol. Ecol. 13, 887–894.

Holsinger, K.E., Lewis, P.O., Dey, D.K., 2002. A Bayesian approach to inferring population structure from dominant markers. Mol. Ecol. 11, 1157–1164.

Hu, Y., Wang, L., Xie, X., Yang, J., Li, Y., Zhang, H., 2010. Genetic diversity of wild populations of *Rheum tanguticum* endemic to China as revealed by ISSR analysis. Biochem. Syst. Ecol. 38, 264–274.

Jena, S.N., Verma, S., Nair, K.N., Srivastava, A.K., Misra, S., Rana, T.S., 2015. Genetic diversity and population structure of the mangrove lime (*Merope angulata*) in India revealed by AFLP and ISSR markers. Aquat. Bot. 120, 260–267.

Jin, L., Chen, Z., 2014. Effects of different treatment methods on germination of *Blysmus sinocompressus* seeds in Qinghai Qilian wetland. Seed 33, 1–2.

Liu, W.S., Wei, W., Dong, M., 2009a. Clonal and genetic diversity of *Carex moorcroftii* on the Qinghai-Tibet plateau. Biochem. Syst. Ecol. 37, 370–377.

Liu, W.S., Dong, M., Song, Z.P., Wei, W., 2009b. Genetic diversity pattern of *Stipa purpurea* populations in the hinterland of Qinghai-Tibet Plateau. Ann. Appl. Biol. 154, 57–65.
Ma, Y.S., Xu, H.S., 2013. Forage Floras in the Source Region of the Yangtze, Yellow and Lantsang Rivers. Science Press, Beijing.
McDermott, J., McDonald, B., 1993. Gene flow in plant pathosystems. Annu. Rev. Phytopathol. 31, 353–373.
Miller, M., 1998. AMOVA-PREP a Program for the Preparation of AMOVA Input Files from Dominant-Marker Raw Data. Northern Arizona University, Flagstaff, USA.
Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. 70, 3321–3323.
Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.
Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol. Ecol. 13, 1143–1155.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Rohlf, F., 2000, NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. New York, USA.
SPSS, I., 2007. SPSS 16.0 for Windows. SPSS, Inc., Chicago.
Spiegelhalter, D.J., Best, N.G., Carlin, B.R., van der Linde, A., 2002. Bayesian measures of model complexity and fit. J. R. Stat. Soc. B—Stat. Method. 64, 583–616.
Sun, F., Lu, Y., Wang, J., Hu, J., Fu, B., 2015. Soil moisture dynamics of typical ecosystems in response to precipitation: a monitoring-based analysis of hydrological service in the Qilian Mountains. Catena 129, 63–75.
Tan, R., Li, W., Yang, Y., Du, F., Wang, J., 2013. Niche characteristics of dominant species in Napahai wetland plant communities under different disturbance regimes. J. Lake Sci. 25, 681–687.
Tero, N., Aspi, J., Siikamaki, P., Jakalaniemi, A., Tuomi, J., 2003. Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. Mol. Ecol. 12, 2073–2085.
Tian, H., Kang, M., Liu, Y., Ye, Q., Yao, X., 2012. High genetic diversity in remnant natural populations of *Myricaria laxiflora*, a species once considered to be extinct in the wild. Aquat. Bot. 103, 48–53.
Wu, Z., Raven, P., Hong, D., 2010, Flora of China. vol. 23. Beijing: Science Press, and St. Louis: Missouri Botanical Garden Press.
Yeh, F., Yang, R., Boyle, T., 1999. POPGENE version 1.32. In: Microsoft Window-Based Freeware for Population Genetic Analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canda.
Yue, D., Li, W., Li, Z., 2004. Analysis of AHP strategic decision for grazing management system and ecological restoration in the alpine wetland at Gannan in Gansu. Acta Bot. Boreal—Occident Sin. 24, 248–253.
Zhang, D., Chen, S., Gao, Q., 2007. Patterns of genetic variation in *Swertia przewalskii*, an endangered endemic species of the Qinghai-Tibet Plateau. Biochem. Genet. 45, 33–50.
Zhao, Q.F., Wang, G., Li, Q.X., Ma, S.R., Cui, Y., Grillo, M., 2006. Genetic diversity of five *Kobresia* species along the eastern Qinghai-Tibet plateau in China. Hereditas 143, 33–40.
Zhao, Y., Wu, F., Fang, X., Yang, Y., 2015. Topsoil C/N ratios in the Qilian Mountains area: implications for the use of subaqueous sediment C/N ratios in paleo-environmental reconstructions to indicate organic sources. Palaeogeogr. Palaeoclimatol. Palaeoecol. 426, 1–9.
Zietkiewicz, E., Rafalski, A., Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain-reaction amplification. Genomics 20, 176–183.