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Genetic diversity and population structure of *Stipa bungeana*, an endemic species in Loess Plateau of China, revealed using combined ISSR and SRAP markers

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ABSTRACT. Sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeat (ISSR) markers were used to assess the genetic diversity within and among 15 natural populations of *Stipa bungeana* from the Loess Plateau of China. Using 15 SRAP primers, 504 (99.80%) polymorphic loci were detected, and 372 polymorphic loci (96.12%) were identified using 15 ISSR primers. At the species level, the *S. bungeana* populations showed relatively low levels of genetic diversity (H_E = 0.2017 for SRAP; H_E = 0.2066 for ISSR). The results of analysis of molecular variance indicated that genetic variation within populations (42.02% for SRAP and 38.40% for ISSR) is lower than that among populations (57.98% for SRAP and 61.60% for ISSR). The genetic distance was significantly correlated with geographical distance by the Mantel test (r = 0.3978, P = 0.002). Our results demonstrated that both SRAP and ISSR markers are effective and reliable for assessing

the genetic diversity of *S. bungeana*. In addition, these data inform conservation and breeding strategies for *S. bungeana*.

Key words: *Stipa bungeana*; SRAP; ISSR; Genetic diversity

INTRODUCTION

Stipa bungeana Trin. is a perennial grass that grows mainly in western China, including Tibet, Gansu, Ningxia, Xinjiang, Qinghai, Shaanxi, Shanxi, and Inner Mongolia. It grows with typical vegetative propagation by repeatedly producing tiller ramets from the shoot base, and is considered as a dominant and constructive species in the typical steppe of the Loess Plateau (Cheng et al., 2011). In addition, *S. bungeana* is also an important pasture species due to its high level of nutrients and excellent drought tolerance. To date, studies on *S. bungeana* have focused on its biological traits and ecological significance, while information on its genetic diversity and population structure has not yet been reported. Analyses of genetic diversity and structure at the intraspecific level are particularly important for species conservation, exploration of plant genetic resources, and future breeding programs of wild plants (Hamrick and Godt, 1996).

Molecular markers are effective tools for revealing genetic diversity and population structure. Among the various DNA marker-assisted techniques, inter-sequence simple repeat (ISSR), a newly developed modification of the simple sequence repeat-based marker system, is useful for detecting genetic polymorphisms among accessions by generating a large number of markers that target multiple microsatellite loci distributed across the genome (Godwin et al., 1997). Sequence-related amplified polymorphism (SRAP), another proven molecular marker technique, is a simple and reliable polymerase chain reaction (PCR)-based marker system that is designed to detect mostly coding sequence polymorphisms (Li and Quiros, 2001). Combinations of the two types of marker systems have been successfully used to analyze genetic diversity and population structure in some plant species (Ferriol et al., 2003; Shao et al., 2010; Song et al., 2010; Wu et al., 2010b). To date, the genetic diversities of certain plants belonging to the *Stipa* genus have been reported, including *Stipa grandis*, *Stipa purpurea*, and *Stipa krylovii* (Wang et al., 2006; Zhao et al., 2006, 2008; Liu et al., 2009; Wu et al., 2010a).

In the present study, a combination of ISSR and SRAP procedures were used to assess the genetic diversity within and among 15 *S. bungeana* populations from the Loess Plateau of China. The objectives of this study were to: 1) investigate the level of genetic diversity in *S. bungeana* populations, 2) analyze the relationship between genetic diversity and environmental factors, and 3) provide information for the development of conservation practices for *S. bungeana*.

MATERIAL AND METHODS

Study area and population sampling

In August 2010, 15 sampling sites (A1 to A15) were selected in the semi-arid area of the Loess Plateau of China, which is located between northern latitudes 33°48' to 38°06' and eastern longitudes 105°01' to 109°45'. These sites included Ningxia, Inner Mongolia, Gansu,

and Shaanxi Provinces (Figure 1; Table 1). Twenty individuals were sampled randomly from each *S. bungeana* population, and the distance between the plants was at least 10 m in the same population to avoid collecting ramets from the same genet. Young and fresh leaves were collected from *S. bungeana* individuals and immediately stored on silica gel for transportation to the laboratory for DNA extraction.

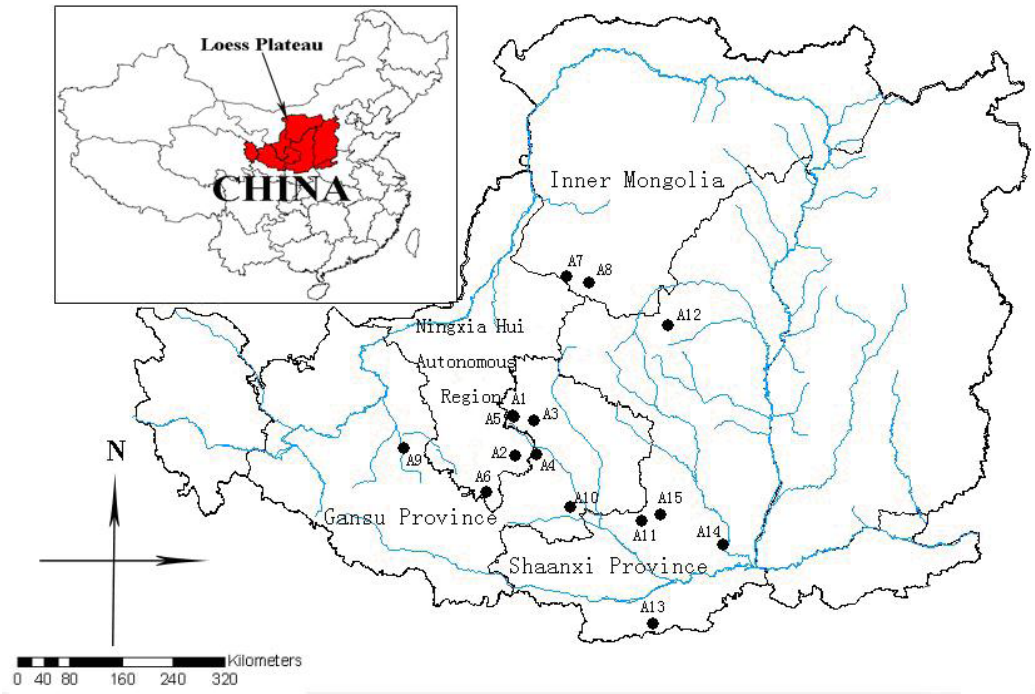

Figure 1. Map of research sites in the Loess Plateau of China.

Table 1. Locations and the habitat characteristics of sampling sites.

Location	Code	Altitude (m)	Longitude	Latitude	Habitat
Nanshan, Yunwu Mountain, Ningxia	A1	2049	106°37'	36°25'	Typical Steppe
Xiji, Ningxia	A2	2081	106°40'	35°56'	Typical Steppe
Huangmao Mountain, Ningxia	A3	1772	106°56'	36°21'	Typical Steppe
Hechuan, Ningxia	A4	1650	106°59'	35°57'	Typical Steppe
Deteriorated grassland, Yunwu Mountain, Ningxia	A5	1980	106°37'	36°27'	Typical Steppe
Liupan Mountain, Ningxia	A6	1760	106°16'	35°29'	Typical Steppe
Chengchuan, Inner-Mongolia	A7	1364	107°23'	38°06'	Desert Steppe
Machangjie, Inner-Mongolia	A8	1354	107°43'	38°02'	Desert Steppe
Huining, Gansu	A9	1726	105°01'	35°58'	Typical Steppe
Jingchuan, Gansu	A10	1305	107°31'	35°19'	Forest Steppe
Tong Chuan, Shaanxi	A11	1200	108°34'	35°10'	Forest Steppe
Jing Bian, Shaanxi	A12	1390	108°56'	37°32'	Desert Steppe
Huxian, Shaanxi	A13	450	108°44'	33°48'	Forest Steppe
Pucheng, Shaanxi	A14	430	109°45'	34°53'	Forest Steppe
Binxian, Shaanxi	A15	1161	108°50'	35°15'	Forest Steppe

Genomic DNA extraction

Genomic DNA was extracted from young leaves by the modified cetyltrimethylammonium bromide method (Zhao et al., 2006). DNA concentration and quality were evaluated by UV-VIS spectrophotometry and on 0.8% (w/v) agarose gels, respectively. Samples were diluted to 20 ng/μL and stored at -20°C for PCR analysis.

SRAP analysis

A set of 38 primers, including 17 forward primers and 21 reverse primers, were synthesized by the Beijing Aoke Biological Engineering Technology Co., Ltd., as reported in previous studies (Ferriol et al., 2003; Budak et al., 2004; Guo and Luo, 2006). Four individual plants from different populations were used for primer screening. Finally, 15 primer combinations (Table 2) were selected for subsequent studies, based on clarity, reproducibility of amplified bands, and high rates of polymorphism. For PCR amplification, SRAP amplifications were performed in 20-μL reaction volumes containing 3 μL 20 ng/μL DNA, 0.2 μL Taq DNA polymerase (TaKaRa Biotechnology, Dalian, China), 1.4 μL 2.5 mM dNTPs, 3 μL 10 μM primer, 2 μL 25 mM Mg^{2+}, 2.5 μL 10X buffer, and 7.9 μL ddH_2O. PCR amplification was carried out on an Eppendorf PCR instrument and comprised the following profile: 5 min of denaturing at 94°C, five cycles of three steps: 1 min of denaturing at 94°C, 1 min of annealing at 35°C, and 1 min of extension at 72°C. In the following 35 cycles, the annealing temperature was increased to 50°C, with a final elongation step of 10 min at 72°C. Amplified products were electrophoresed on 6% denaturing polyacrylamide gel, which was silver stained and photographed.

Table 2. Primer combinations used in sequence-related amplified polymorphism (SRAP) analysis.

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
Me1+Em2	TGA GTC CAA ACC GGATA GAC TGC GTA CGA ATT TGC	Me12+Em8	TGA GTC CAA ACC GGAGA GAC TGC GTA CGA ATT CAC
Me1+Em4	TGA GTC CAA ACC GGATA GAC TGC GTA CGA ATT TGA	Me12+Em14	TGA GTC CAA ACC GGAGA GAC TGC GTA CGA ATT CTT
Me1+Em17	TGA GTC CAA ACC GGATA GACTGCGTACG AATT GAG	Me13+Em12	TGA GTC CAA ACC GGAAG GAC TGC GTA CGA ATT CTC
Me4+Em1	TGA GTC CAA ACC GGACC GAC TGC GTA CGA ATT AAT	Me13+Em14	TGA GTC CAA ACC GGAAG GAC TGC GTA CGA ATT CTT
Me5+Em4	TGA GTC CAA ACC GGAAG GAC TGC GTA CGA ATT TGA	ME6-X+Em7	TGA GTC CTT TCC GG TAA GAC TGC GTA CGA ATT CAA
Me5+Em11	TGA GTC CAA ACC GGAAG GAC TGC GTA CGA ATT CTA	ME6-X+Em8	TGA GTC CTT TCC GG TAA GAC TGC GTA CGA ATT CAC
Me8+Em19	TGA GTC CAA ACC GGACT GACTGCGTACG AATT TCA	ME6-X+EM3-X	TGA GTC CTT TCC GG TAA GAC TGC GTA CGA ATT CGA
Me11+Em9	TGA GTC CAA ACC GGAAC GACTGCGTACG AATT TCA		

ISSR analysis

According to the primer sequences published by the University of British Columbia, 96 ISSR primer sequences were synthesized by Beijing Aoke Biological Technology Co., Ltd. Four individual plants from each population were used for primer screening. Finally, 15 primers (Table 3) were selected for ISSR analysis. The PCR amplification comprised a total volume of 20 μL containing 3 μL 20 ng/μL DNA, 0.2 μL Taq DNA polymerase (TaKaRa Biotechnology), 1.4 μL 2.5 mM dNTPs, 3 μL 10 μM primer, 1.4 μL 25 mM Mg^{2+}, 2.5 μL 10X buffer, and 8.3 μL ddH_2O. PCR amplification was carried out on the Eppendorf PCR instrument and the reaction program comprised an initial 5 min at 94°C; 35 cycles of 45 s at 94°C, 45 s annealing at 50°C (varying for different primers), and a 90-s extension at 72°C; ending with a final extension of 5 min at 72°C and storage at 4°C. Amplified products were electrophoresed on 6% denaturing polyacrylamide gel, which was silver stained and photographed.

Table 3. Primers used in inter-simple sequence repeat (ISSR) analysis.

Primer	Sequence (5'-3')	Optimal annealing temperature (°C)	Primer	Sequence (5'-3')	Optimal annealing temperature (°C)
UBC806	$(TA)_8G$	41.5	UBC840	$(GA)_8YT$	54.1
UBC813	$(CT)_8T$	48.4	UBC864	$(ATG)_5$	46.7
UBC814	$(CT)_8A$	50.0	UBC868	$(GAA)_5$	42.6
UBC820	$(GT)_8C$	57.7	UBC880	$(GGAGA)_3$	51.0
UBC822	$(TC)_8A$	55.7	UBC886	$VDV(CT)_7$	57.7
UBC823	$(TC)_8C$	57.7	UBC887	$VDV(TC)_7$	54.5
UBC827	$(AC)_8G$	56.4	UBC891	$HVH(TG)_7$	47.1
UBC834	$(AG)_8YT$	56.0			

Data analysis

Clear and reproducible bands between 200-2000 bp were selected for statistical analysis. Amplified fragments were scored as 1 for presence or 0 for absence. The DCFA1.1 program was used to build the original document for data analysis (Zhang and Song, 2002). POPGENE1.32 (Yeh and Boyle, 1997) was used to calculate the following genetic diversity parameters: the percentage of polymorphic bands (PPB), Shannon's information index (I), Nei's gene diversity (H), the effective number of alleles (N_E) and observed number of alleles (N_A), genetic differentiation (G_{ST}), and Nei's genetic distance. The average level of gene flow (N_M) among populations was indirectly calculated using the formula: $N_M = 0.5 (1 - G_{ST}) / G_{ST}$ (McDermott and McDonald, 1993). The unweighted pair-group method with arithmetic averages (UPGMA) dendrograms were constructed based on the matrix of Nei's genetic distances using NTSYS-pc (Rohlf, 2000). In addition, analysis of molecular variance (AMOVA) was used to estimate the coefficient of genetic variation among and within populations (Excoffier et al., 1992). The variance components were tested statistically by nonparametric randomization tests using 1000 permutations. Pearson's correlation analysis was used to detect correlations between genetic diversity parameters and environmental factors, including altitude, longitude, latitude, annual mean temperature, and annual mean precipitation. All of these analyses were performed with SPSS11.0 (SPSS 2001). Finally, the relationship between genetic distance and corresponding geographical distance among all populations was tested with the Mantel test (Mantel, 1967).

RESULTS

Genetic diversity detected by SRAP and ISSR

For the 300 individuals, the 15 selected SRAP primers generated 504 polymorphic bands, and the PPB varied from 17.82 (A7) to 45.54% (A5), with a mean of 29.12%. The *H* value ranged from 0.0485 in the A7 population to 0.1131 in the A5 population, with a mean of 0.0848. The *I* values were between 0.0755 (A7) and 0.1748 (A5), with a mean of 0.1299. At the species level, the *H* and *I* values were 0.2017 and 0.3273, respectively (Table 4).

Table 4. Genetic diversity of *Stipa bungeana* within populations by sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeat (ISSR) analyses.

Groups	Polymorphic bands		Percentage of polymorphic bands		Nei's gene diversity		Shannon's information index	
	SRAP	ISSR	SRAP	ISSR	SRAP	ISSR	SRAP	ISSR
A1	137	109	27.13	18.17	0.0778	0.0800	0.1198	0.1240
A2	173	202	34.26	52.20	0.1011	0.1511	0.1550	0.2322
A3	116	136	34.85	35.14	0.0989	0.1026	0.1526	0.1571
A4	171	134	33.86	34.63	0.0870	0.0989	0.1364	0.1530
A5	230	157	45.54	40.57	0.1131	0.1108	0.1748	0.1723
A6	161	101	31.88	26.10	0.0826	0.0704	0.1285	0.1093
A7	90	101	17.82	26.10	0.0485	0.0834	0.0755	0.1260
A8	116	74	22.97	19.12	0.0725	0.0588	0.1092	0.0895
A9	141	105	27.92	27.13	0.0864	0.0878	0.1308	0.1329
A10	135	84	26.73	21.71	0.0881	0.0613	0.1321	0.0940
A11	140	87	27.72	22.48	0.0860	0.0646	0.1310	0.0989
A12	113	52	22.38	12.40	0.0645	0.0400	0.0989	0.0613
A13	149	71	29.50	18.35	0.0884	0.0608	0.1333	0.0899
A14	98	48	19.41	13.44	0.0675	0.0437	0.1004	0.0647
A15	176	93	34.85	24.03	0.1090	0.0757	0.1705	0.1133
Mean	183.1	103.6	29.12	26.10	0.0848	0.0793	0.1299	0.1212
Species	504	372	99.80	96.12	0.2017	0.2066	0.3273	0.3342

For ISSR analysis, 372 polymorphic bands were detected using 15 ISSR primers. The PPB varied from 12.40% in the A12 population to 52.20% in the A2 population. The *H* value varied from 0.0400 (A12) to 0.1511 (A2). The *I* values ranged from 0.0631 (A12) to 0.1548 (A2). At the species level, the *H* and *I* values were 0.2066 and 0.3342, respectively. Among the 15 populations, both SRAP and ISSR analyses indicated the highest levels of genetic diversity in the A5 and A2 populations, and the lowest levels in the A7 and A12 populations.

Population genetic differentiation and gene flow

Genetic diversity parameters at the species and population levels are shown in Table 5. For SRAP and ISSR analysis, the *H* values were 0.0848 and 0.0793 within populations (H_S), and at the species level (H_T) they were 0.2017 and 0.2066, respectively. The *I* values were 0.1299 and 0.1175 at the population level (H_{POP}), and at the species level (H_{SP}) they were 0.3273 and 0.3342, respectively.

Table 5. Genetic differentiation within and among populations of *Stipa bungeana* by sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeat (ISSR) analyses.

Genetic diversity parameters	Nei's gene diversity		Genetic diversity parameters	Shannon's information index	
	SRAP	ISSR		SRAP	ISSR
H_S	0.0848	0.0793	H_{POP}	0.1299	0.1175
H_T	0.2017	0.2066	H_{SP}	0.3273	0.3342
H_S / H_T	0.4204	0.3838	H_{POP} / H_{SP}	0.3969	0.3516
G_{ST}	0.5798	0.6160	$(H_{SP} - H_{POP}) / H_{SP}$	0.6031	0.6484
N_M	0.3623	0.3117			

POPGENE 1.32 was used to analyze genetic differentiation from SRAP and ISSR data. For the SRAP analysis, 57.98% of genetic variation existed among populations and 41.02% of the genetic variation was within populations. For ISSR analysis, the genetic variation among populations was 61.60% (Table 5). The level of gene flow (N_M) was estimated to be 0.3623 individuals per generation among populations (Table 5). The results of ISSR were similar to results of SRAP, with G_{ST} = 0.6160, N_M = 0.3117, and (H_{SP} - H_{POP}) / H_{SP} = 0.6484. Both SRAP and ISSR analyses indicated high inter-population genetic differentiation and moderate intra-population genetic differentiation.

Genetic identity (I) and genetic distance (D) may be used to further illustrate the degree of genetic differentiation (Wang et al., 2011). According to the Mantel test, there was a significant correlation between the I values generated by the two marker systems (r = 0.6241, P = 0.001). Therefore, POPGENE 1.32 was used to calculate D values among the populations using combined SRAP and ISSR markers (Table 6). Based on the results of two markers, the I values of the 15 populations of *S. bungeana* ranged from 0.7613 (between A8 and A14) to 0.9870 (between A3 and A4), with a mean of 0.8749. The D values varied from 0.0192 (between A14 and A15) to 0.2882 (between A8 and A12), with a mean of 0.1365.

Table 6. Nei's unbiased genetic distances of *Stipa bungeana* populations.

Groups	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
A1	0	0.906	0.825	0.824	0.844	0.816	0.825	0.8	0.876	0.819	0.825	0.785	0.838	0.793	0.835
A2	0.099	0	0.955	0.946	0.952	0.93	0.868	0.848	0.927	0.87	0.875	0.83	0.895	0.839	0.884
A3	0.192	0.047	0	0.987	0.956	0.937	0.816	0.804	0.874	0.829	0.832	0.789	0.844	0.793	0.834
A4	0.193	0.056	0.013	0	0.968	0.95	0.815	0.802	0.872	0.822	0.825	0.782	0.843	0.788	0.829
A5	0.17	0.049	0.045	0.033	0	0.979	0.836	0.822	0.892	0.836	0.838	0.794	0.864	0.803	0.845
A6	0.203	0.072	0.065	0.051	0.022	0	0.802	0.792	0.86	0.808	0.809	0.769	0.832	0.777	0.816
A7	0.192	0.142	0.204	0.205	0.179	0.22	0	0.801	0.874	0.818	0.823	0.784	0.847	0.798	0.839
A8	0.223	0.165	0.218	0.221	0.196	0.233	0.222	0	0.864	0.794	0.792	0.75	0.816	0.761	0.801
A9	0.132	0.076	0.135	0.137	0.115	0.151	0.135	0.146	0	0.906	0.91	0.872	0.91	0.881	0.926
A10	0.2	0.139	0.187	0.196	0.18	0.214	0.201	0.231	0.099	0	0.979	0.967	0.869	0.946	0.957
A11	0.192	0.133	0.184	0.192	0.177	0.212	0.195	0.234	0.094	0.022	0	0.978	0.875	0.956	0.965
A12	0.242	0.186	0.238	0.246	0.23	0.263	0.244	0.288	0.137	0.034	0.022	0	0.835	0.948	0.949
A13	0.177	0.111	0.17	0.171	0.146	0.184	0.167	0.203	0.094	0.14	0.134	0.18	0	0.847	0.886
A14	0.232	0.175	0.232	0.238	0.219	0.253	0.226	0.273	0.127	0.055	0.045	0.054	0.166	0	0.981
A15	0.181	0.123	0.182	0.188	0.168	0.203	0.175	0.222	0.077	0.044	0.036	0.053	0.121	0.019	0

Genetic relationships

Genetic relationships among the populations were constructed by UPGMA cluster analysis based on Nei's unbiased genetic distance matrix using combined SRAP and ISSR markers

(Figure 2). The A1, A2, A3, A4, A5, and A6 populations grouped together initially, then clustered with the A9, A10, A11, A12, A13, A14, and A15 populations, and lastly, they clustered with the A7 and A8 populations. The UPGMA dendrogram indicated that populations of *S. bungeana* clustered together randomly, and did not form clear geographical distributions.

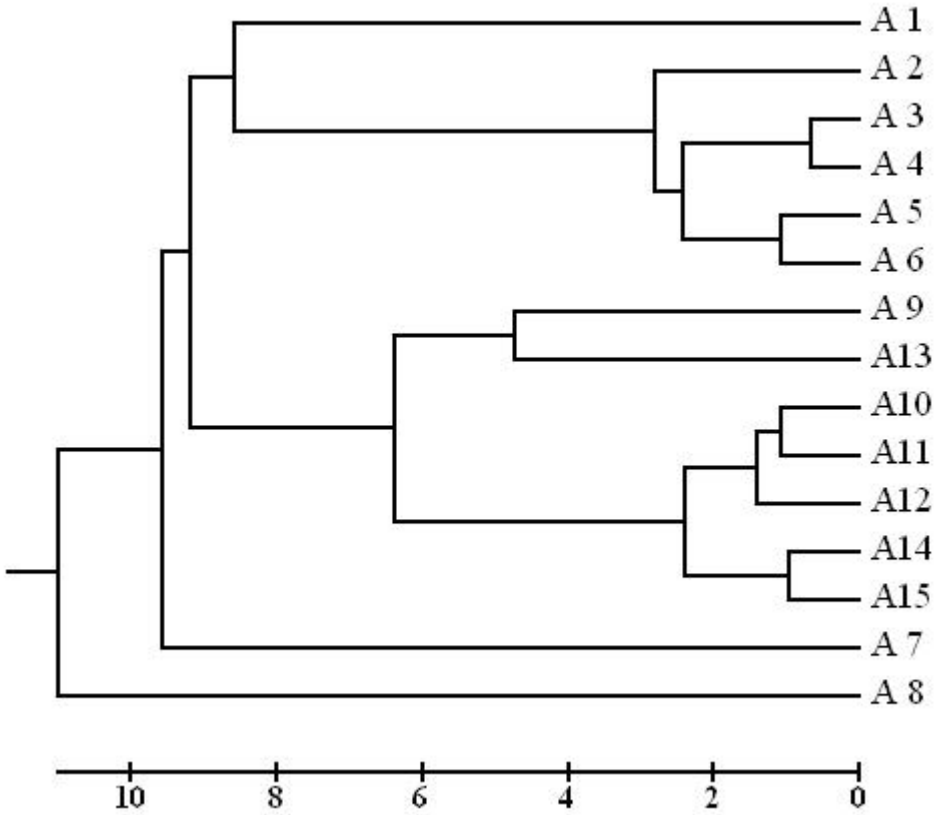

Figure 2. UPGMA dendrogram of *Stipa bungeana* populations based on SRAP and ISSR data.

The Mantel test performed using the R language showed a significant correlation between genetic distance and geographical distance ($r = 0.3978$, $P = 0.01$) for the 15 populations.

DISCUSSION

Genetic diversity of *S. bungeana*

Genetic diversity refers to the level of genetic differentiation within a species. It also reflects the ability of a species to adapt to environmental changes and its potential for transformation and exploitation (Wang et al., 2011). Therefore, evaluation of genetic diversity is an essential component in germplasm characterization and collection (Wu et al., 2010a). Geographically isolated populations of plant species tend to accumulate genetic variations during

the course of environmental adaptations (Sarwat et al., 2008). In the present study, based on ISSR markers, the *H* value was 0.2066 and the *I* value was 0.3342 at the species level. These results were higher than those obtained using SRAP markers (*H* = 0.2017, *I* = 0.3273). Our results were similar to those of Song et al. (2010) who reported that SRAP markers only amplified target regions (the functional regions) of open reading frames, whereas ISSR markers are scattered throughout the genome, thus revealing the entire genomic diversity. Therefore, both ISSR and SRAP markers were effective and reliable for the accurate assessment of genetic variability of *S. bungeana*.

Compared with *S. grandis* (*H* = 0.1952, *I* = 0.3035) (Wu et al., 2010a), our study revealed a relatively high level of genetic diversity (*H* = 0.2017, *I* = 0.3273, SRAP markers; and *H* = 0.2066, *I* = 0.3342, ISSR markers) in *S. bungeana*. One reason may be that the *S. grandis* populations were close to each other within a typical steppe, whereas our populations were collected from four provinces and different steppes. However, the observed genetic diversity was lower than that reported for *S. krylovii* (*H* = 0.2392, *I* = 0.3818) (Wang et al., 2006) and *S. purpurea* (*H* = 0.2204, *I* = 0.3517) (Liu et al., 2009). The genetic diversity was also lower than the reported values for long-lived perennial species (*H* = 0.25, N = 37) and widespread species (*H* = 0.22, N = 20) (Nybom, 2004). Sun (1996) showed that the scope of a population has significant relevance to its genetic diversity. The lower genetic diversity of *S. bungeana* may be caused by human activities, such as diverse land use and the fragmented habitat formed in the Loess Plateau of China.

During the field survey and sampling, we found that the populations with low genetic diversity were located near human residential areas or traffic arteries. In contrast, populations with high genetic diversity were located far from residential areas. These areas have favorable environmental conditions, little human disturbance, and the populations are large. Genetic theory indicates that genetic variation levels are positively correlated with effective population size (Frankham, 1996). In addition, larger and older populations often have higher intra-population genetic variation than do younger populations (Hamrick and Godt, 1996). Therefore, we suggest that the low level of human disturbance and large effective population size have led to relatively higher genetic diversity in *S. bungeana*.

Population genetic structure of *S. bungeana*

Population genetic structure is defined as the nonrandom distribution pattern of genetic variation of one species or population in space and time. To a large extent, it represents the evolutionary potential of a species or population (Sun, 1996). Genetic differentiation and gene flow are important indices for assessing the population genetic structure of a species. In the present study, the values of G_{ST} for *S. bungeana* were 0.5798 and 0.6160 based on the SRAP and ISSR markers, which were higher than the values reported for long-lived perennial species (Φ_{ST} = 0.25, N = 60), out-crossing species (Φ_{ST} = 0.25, N = 73), mixed breeding species (Φ_{ST} = 0.40, N = 18), and widespread species (Φ_{ST} = 0.34, N = 32) (Nybom, 2004). Wright (1951) pointed out that genetic differentiation was strong when the coefficient was greater than 0.25. Therefore, results of the present study showed that *S. bungeana* populations have high genetic differentiation.

Gene flow, the movement of genes within and among populations, is negatively correlated with genetic differentiation, and is essential for population transfer and plant evolution (Slatkin, 1985). Previous studies demonstrated that if $N_M < 1$, the rare communication among

populations cannot compensate for the genetic diversity lost by genetic drift and inbreeding depression, both of which reduce genetic diversity within populations and eventually enhance genetic differentiation among populations (Slatkin, 1985; Apostol et al., 1996; Lenormand et al., 1998). In this study, the N_M values of *S. bungeana* were 0.3623 and 0.3117 using SRAP and ISSR markers, respectively. This level of migration will not prevent divergence among populations. Habitat fragmentation and diverse land use are factors that affect genetic structure, but further evidence is needed to explain whether these factors have had such an impact on the genetic structure of *S. bungeana* (Yu et al., 2012). In addition, Billings (1973) indicated that alpine plants are capable of asexual reproduction through clonal growth, which is considered to be the result of adaptation to the harsh conditions where pollinators are sparse and seedling survival is relatively low. In our study, the most adverse environmental condition is drought, and it is hard for seedlings to establish themselves in the Loess Plateau. The seed germination rate of *S. bungeana* is relatively low, and it grows mainly by clonal reproduction to expand the population. This special reproduction increases mating opportunities among similar individuals and blocks effective gene flow among different populations.

Hamrick and Godt (1989) reported that genetic distance among populations was correlated with geographical distance. However, our study showed the opposite trend. We found low levels of correlation between geographic and genetic distances after merging the SRAP and ISSR marker data ($r = 0.3978$, $P = 0.01$). These results are similar to an analysis in *Angiopteris chauliodonta* Copel. in the Pitcairn islands (Kingston et al., 2004), for *Rheum tanguticum* Maxim. ex Balf. in China (Hu et al., 2010), and for *Tetraena mongolica* Maxim. in China (Ge et al., 2003), which indicated that geographical distance might not be the main factor affecting genetic differentiation among populations of *S. bungeana*. Volis et al. (2001) suggested that if natural selection was environmentally induced, the genetic similarity between population groups must reflect the similarity between their environments and must be independent of geographical distance. Therefore, we suggest that the correlation between geographical distance and genetic diversity cannot be a general rule; the link between different geomorphologic and historical situations to genetic diversity should be considered.

Conservation considerations

S. bungeana is one of the main wild forage plants in the grassland of northwest China. It is also important for controlling the loss of water and soil erosion in the Loess Plateau because of its fibrous roots and highly developed root system (Cheng et al., 2011). Wild invasion by exotic species and human disturbance have reduced and fragmented the population's habitats and driven the species toward extinction (Kölliker et al., 1998). If the environmental degradation caused by natural and human-induced factors exceeds the maximum limit, the result will be loss of genetic diversity for a species or population (Yan et al., 2010). Successful management and conservation of populations of endangered species rely on a good understanding of the distribution of genetic variation of the species. Our study provides important genetic information for developing collection strategies for *S. bungeana*.

The relatively low genetic diversity and limited gene flow of *S. bungeana* populations indicated an endangered status of *S. bungeana* under current conditions. Considering the intensive utilization and increased fragmentation of the *S. bungeana*-dominated steppe in the Loess Plateau, more rational land use practices should be adopted so that the grassland

is conserved. If necessary, natural reserves of *S. bungeana* should be established, this could protect the species and its habitat, especially for the low genetic diversity populations. In addition, gene flow among populations could be improved by artificial means, such as deliberate pollination, extensive seed collection, distribution of seeds into different populations, and transplanting individuals from one habitat to another.

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