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Genetic diversity of a dominant species *Stipa bungeana* and its conservation strategy in the Loess Plateau of China

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ABSTRACT

In order to investigate the genetic diversity and population structure of *Stipa bungeana* under the background of grassland utilization and grazing exclusion, ten *S. bungeana* populations were selected from different steppe type in the Loess Plateau of China. Sequence-related amplified polymorphism (SRAP) marker was used to assess the genetic diversity. Fifteen SRAP primer combinations generated a total of 482 amplification bands, 418 (86.72%) were polymorphic bands. A relatively high level of genetic diversity (PPB = 89.80%, $h = 0.1972$, $H = 0.3154$) was detected at the species level, but the genetic diversity was low at the population level (PPB = 17.01–33.33%, $h = 0.0438$–0.0967, $H = 0.0361$–0.1447). AMOVA analysis revealed a high level of genetic differentiation among populations ($\Phi_{ST} = 0.6757$), and a limited among-population gene flow ($N_m = 0.1200$). There was no significant correlation between genetic distance and geographic distance by Mantel test ($r = 0.1126$, $P = 0.204$). Conservation implications were proposed for *S. bungeana*.

1. Introduction

The *Stipa* genus includes 300–400 annual and perennial species. They usually grow in arid and dry areas, and some species with primitive morphological characteristics grow in semi-arid areas. *Stipa* species are especially relevant to restoration studies in arid environments due to the fact that they dominate large parts of the Eurasian zonal vegetation. Owing to their strong and long-term association with human activities, *Stipa* steppes are considered to be highly important model systems in arid land ecology (Hassan et al., 2009).

Stipa bungeana is a perennial C_3 tussock grass with the clonal growth by tillering. It distributes in typical steppe of semi-arid areas that belong to warm temperate zones. *S. bungeana* appears as the accompanying species or dominant species in west arid and semi humid areas of China. *S. bungeana* is a main wild forage in northwest natural grassland of China, and it has low water content and loses water quickly. It is useful for the stabilization and conservation of grassland because of its fibrous roots and highly developed root system. *S. bungeana* plays an important role in the maintenance of soil and water because it can quickly form community and become the dominant and constructive species with its strong ability to compete for growth (Cheng et al., 2010a,b).

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A number of studies have reported on *S. bungeana* ecology (Cheng et al., 2010a,b; Huang et al., 2001a,b). Apart from these, no studies have reported about its genetic diversity, breeding and growth system. The genetic diversity of a few *Stipa* species have been reported that including *Stipa grandis*, *Stipa purpurea*, and *Stipa tenacissima* (Zhao et al., 2006; Zhao et al., 2008; Wu et al., 2010; Mohamed et al., 2010; Liu et al., 2009). Sequence-related amplified polymorphism (SRAP) is recognized as one of most used molecular marker first introduced by Li and Quiros (2001). Owing to its simplicity, reproducibility, high polymorphic rate, SRAP has been applied extensively in genetic diversity analysis (Li et al., 2010; Huang et al., 2011).

The objective of this study is to evaluate the genetic diversity and population structure among and within populations of *S. bungeana* in the Loess Plateau of China, in order to improve the management of genetic resources. For this purpose, we analyzed the genetic variation of natural populations in different steppe types. The relationship between genetic diversity index and geographic distance was also analyzed.

2. Materials and methods

2.1. Study area

The study areas were located in the different steppe of the Loess Plateau, China (Fig.1; Table 1). In September of 2010, ten sites were selected based on different steppe types and utilization status in four provinces that including Shaanxi, Inner Mongolia, Ningxia and Gansu.

2.2. Plant sampling

S. bungeana is a perennial C_3 grass, and it is the dominant species in the Loess Plateau of China. Thirty plants were sampled randomly from per population, and the distance between individuals was at least 30 m. Fresh leaves were collected from *S. bungeana* individuals and immediately stored in zip-lock bags with silica gel and brought back to laboratory and stored at −80 °C for later DNA extraction.

2.3. DNA extraction

Genomic DNA from silica gel-dried leaves was extracted using a modification of the Protocol of standard CTAB method (Zhao et al., 2006). DNA quality was detected by electrophoresis on 0.8% (w/v) agarose gel. DNA concentration was measured by a UV–VIS spectrophotometer, and adjusted to 40 ng μL^{-1} and then stored at −20 °C for SRAP-PCR analysis.

2.4. SRAP-PCR amplification

SRAP markers were used to detect the genetic diversity among 10 populations according to previously established protocols by Li and Quiros (2001). The primer sequences were synthesized by Beijing Aoke Biological Technology and Service Co. Ltd. 225 primer combinations were screened among four plants from four provinces. From these, 15 primer combinations were selected for the present study based on reproducibility, clarity of bands, and their highly polymorphic (Table 2).

PCR amplification reactions were carried out in 20 μL volume, containing 40 ng of template DNA, 10 × PCR buffer (100 Mm Tris-HC, pH 8.3; 500 mM KCl), 0.18 mM of each dNTP, 0.75 mM of each primer, 1.87 mM of $MgCl^2$, 1 units of Taq DNA polymerase (TaKaRa Biotechnology Dalian Co., Ltd., China). PCR amplification was performed as follows: initial 5 min at 94 °C,

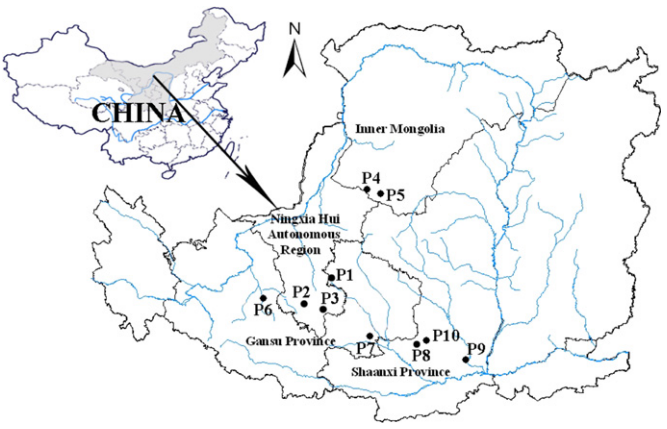

Fig. 1. Locations of research site in the Loess Plateau, China.

Table 1
Locations and the habitat characters.

Location	Population	Sample code	Altitude (m)	Longitude	Latitude	Vegetation type	Constructive species	Utilization status
Yunwu Mountain, Ningxia Hui Autonomous Region	P1	1–30	2049	106 °37′	36°25′	Typical steppe	*Stipa bungeana* + *S. grandis*	Fencing
Piancheng village, Xiji County, Ningxia Hui Autonomous Region	P2	31–60	2081	105°58′	35°57′	Typical steppe	*Stipa bungeana* + *Artemisia sacrorum*	Grazing
Liupan Mountain, Ningxia Hui Autonomous Region	P3	61–90	1760	106°27′	35°49′	Typical steppe	*Stipa bungeana*	Fencing
Chengchuan village, Etuoke City, Inner Mongolia Autonomous Region	P4	91–120	1364	107°23′	38°06′	Desert steppe	*Achnatherum splendens*	Grazing
Machangjie village, Etuoke City, Inner Mongolia Autonomous Region	P5	121–150	1354	107°43′	38°02′	Desert steppe	*Stipa bungeana*	Grazing
Huining County, Gansu Province	P6	151–180	1726	105°01′	35°58′	Typical steppe	*Stipa bungeana*	Grazing
Jingchuan County, Gansu Province	P7	181–210	1305	107°31′	35°19′	Forest steppe	*Stipa bungeana* + *Bothriochloa ischaemum*	Fencing
Jinhua Mountain, Tongchuan City, Shaanxi Province	P8	211–240	1200	108°34′	35°10′	Forest steppe	*Bothriochloa ischaemum* + *Stipa bungeana*	Mowing
Manquanhe Village, Pucheng County, Shaanxi Province	P9	241–270	430	109°45′	34°53′	Forest steppe	*Bothriochloa ischaemum* + *Artemisia sacrorum*	Mowing
Bai Village, Bin County Shaanxi Province	P10	271–300	1161	108°50′	35°15′	Forest steppe	*Stipa bungeana* + *Artemisia sacrorum*	Mowing

followed by 5 cycles of 1 min at 94 °C, 1 min at 36 °C, and 1 min at 72 °C. In the following 30 cycles, the annealing temperature was increased to 51 °C, and a final 7 min extension at 72 °C. PCR products were separated on 6% denatured polyacrylamide gels and detected by silver staining. Then clear and reproducible distinguished bands were recorded and used in the following analysis. DL2000 DNA ladder (TaKaRa Biotechnology Dalian Co., Ltd., China) was used as DNA markers.

Table 2
Primer combination used in this study and their polymorphism and diversity analysis.

Primer combination	Sequence	*t*	*p*	P %
Me1 + Em2	F: TGA GTC CAA ACC GGATA R: GAC TGC GTA CGA ATT TGC	32	26	81.25
Me1 + Em4	F: TGA GTC CAA ACC GGATA R: GAC TGC GTA CGA ATT TGA	26	23	88.46
Me1 + Em11	F: TGA GTC CAA ACC GGATA R: GAC TGC GTA CGA ATT CTA	31	27	87.10
Me4 + Em1	F: TGA GTC CAA ACC GGACC R: GAC TGC GTA CGA ATT AAT	27	23	85.19
Me5 + Em4	F: TGA GTC CAA ACC GGAAG R: GAC TGC GTA CGA ATT TGA	35	34	97.14
Me5 + Em11	F: TGA GTC CAA ACC GGAAG R: GAC TGC GTA CGA ATT CTA	32	30	93.75
Me8 + Em19	F: TGA GTC CAA ACC GGACT R: GAC TGC GTA CGA ATT TCA	38	36	94.77
Me11 + Em19	F: TGA GTC CAA ACC GGAAC R: GAC TGC GTA CGA ATT TCA	32	28	87.50
Me12 + Em8	F: TGA GTC CAA ACC GGAGA R: GAC TGC GTA CGA ATT CAC	28	24	85.71
Me12 + Em14	F: TGA GTC CAA ACC GGAGA R: GAC TGC GTA CGA ATT CTT	34	27	79.41
Me13 + Em12	F: TGA GTC CAA ACC GGAAG R: GAC TGC GTA CGA ATT CTC	36	31	86.11
Me13 + Em14	F: TGA GTC CAA ACC GGAAG R: GAC TGC GTA CGA ATT CTT	26	17	65.38
ME6-X + Em7	F: TGA GTC CTT TCC GGTAA R: GAC TGC GTA CGA ATT CAA	33	29	87.88
ME6-X + Em8	F: TGA GTC CTT TCC GGTAA R: GAC TGC GTA CGA ATT CAC	37	34	91.89
ME6-X + EM3-X	F: TGA GTC CTT TCC GGTAA R: GAC TGC GTA CGA ATT CGA	35	29	82.86
Total		482	418	86.72
Average		32.13	27.87	86.72

Abbreviation: *t*, Number of total loci; *p*, Number of polymorphic loci; P %, the percentage of polymorphic loci.

2.5. Data analysis

In SRAP analysis, each clear, reproducible, amplified DNA polymorphic bands between 100 bp to 2000 bp were scored as 1 for presence or 0 for absence to form a binary matrix for further analysis. POPGENE version 1.32 (Francis et al., 2000) was used to compute the number of effective loci, the percentage of polymorphic loci, observed number of alleles (na), effective number of alleles (ne), Nei's genetic diversity (h) (Nei, 1973), and Shannon's information index (H). H was calculated at two levels: the genetic diversity within the species (H_{sp}) and the mean diversity within the population (H_{pop}). Genetic diversity was partitioned by Shannon's information index (H), where the portion of variation within a population is H_{pop}/H_{sp} and the portion of variation among populations is $(H_{sp} - H_{pop})/H_{sp}$ (Lewontin, 1972). In addition, an analysis of molecular variance (AMOVA) was carried out using AMOVA program version 1.5 (Excoffier et al., 1992). Φ_{ST} was used to show genetic differentiation values between populations. Based on Φ_{ST}, the number of migrants per generation (Nm) was estimated using $Nm = (1 - \Phi_{ST})/4\Phi_{ST}$. Relationships between the genetic distance matrix and the geographic distance matrix were estimated with the Mantel test (Mantel, 1967) in the NTSYS-pc program (Rohlf, 2000) by 1000 permutations of bootstrapping.

3. Results

3.1. Genetic diversity of S. bungeana

Fifteen SRAP primer combinations generated a total of 482 bands varied in size from 100 to 2000 bp, and 418 bands were polymorphic with a polymorphic ratio of 86.72%. Each primer combination generated an average of 32.13 bands (Table 2).

The genetic diversity parameters of *S. bungeana* populations were shown in Table 3. The Nei's genetic diversity (h) was 0.1972 and Shannon's information index (H) was 0.3154 at the species level. Within each population, the number of polymorphic loci ranged from 75 (P4) to 147 (P2), with a mean of 117. The percentage of polymorphic band (PPB) ranged from 17.01% (P4) to 33.33% (P2) with an average 26.53%. The observed number of alleles (na) varied from 1.1701 (P4) to 1.3333 (P2) with an average of 1.2653. The effective number of alleles (ne) ranged from 1.0716 (P4) to 1.1664 (P2) with a mean of 1.1348. Nei's genetic diversity (h) varied from 0.0438 (P4) to 0.0967 (P2) and the mean value was 0.0800. Shannon's information index (H) ranged from 0.0361 (P4) to 0.1447 (P2), with an average value of 0.1118. Results showed that Population 2 from Piancheng village with the highest level of genetic diversity, whereas the lowest genetic diversity showed in population 4 from Chengchuan village.

3.2. Genetic differentiation among populations

Shannon's information index (H) at the species level was 0.3154, and the genetic diversity at the population level was 0.1118 (Table 3). The genetic differentiation among the populations was 0.6455 by using the formula $G_{ST} = (H_{sp} - H_{pop})/H_{sp}$, which indicated that 64.55% of the total genetic variation was among populations. A similar level of genetic differentiation among the populations was obtained from AMOVA analysis, and the genetic variation among the populations was 67.57% and 32.43% within populations. The genetic differentiation values of *S. bungeana* populations was highly significant ($P < 0.001$), and Φ_{ST} was 0.6757 (Table 4). The level of gene flow (Nm) was 0.1200 calculated by the formula $Nm = (1-\Phi_{ST})/4\Phi_{ST}$, which indicated that a lower gene exchange among populations existed.

3.3. Correlation of genetic diversity and geographic distance

The correlation between the genetic distances and geographic distances were analyzed using all pairs of the populations (data not shown). Results of the Mantel test indicated that there is a low correlation between the genetic distances and the geographic distances ($r = 0.1126$, $P = 0.204$).

Table 3
Genetic diversity parameters of ten populations of *S. bungeana*.

Population code	NPB	PPB (100%)	na	ne	h	H
P1	112	25.40	1.2540	1.1235	0.0746	0.1146
P2	147	33.33	1.3333	1.1664	0.0967	0.1447
P3	130	29.48	1.2948	1.1347	0.0799	0.1230
P4	75	17.01	1.1701	1.0716	0.0438	0.0361
P5	101	22.90	1.2290	1.1220	0.0722	0.1088
P6	130	29.48	1.2948	1.1604	0.0935	0.1413
P7	129	29.25	1.2925	1.1416	0.0872	0.1028
P8	126	28.57	1.2857	1.1459	0.0882	0.1346
P9	89	20.18	1.2018	1.1182	0.0688	0.0687
P10	131	29.71	1.2971	1.1641	0.0953	0.1437
Mean	117	26.53	1.2653	1.1348	0.0800	0.1118
Species-level	396	89.80	1.8980	1.3121	0.1972	0.3154

Abbreviation: NPB, number of polymorphic band; PPB, percentage of polymorphic band; na, observed number of alleles; ne, effective number of alleles; h, Nei's (1973) gene diversity; H, Shannon's information index.

Table 4
AMOVA of genetic variances within and among *S. bungeana* populations.

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variation (%)	*P* value
Among groups	9	745.690	36.8503	67.57	<0.001
Within groups	190	17.686	17.6855	32.43	<0.001
Total	199	763.376	54.5358	100	

4. Discussion

4.1. Genetic diversity of S. bungeana

A good knowledge of genetic diversity is a necessary prerequisite for the conservation of a specie because it may indicate the status and survival potential of populations (Lande, 1988). Previous studies showed that molecular markers can reveal the dispersal capacity of a species and infraspecific structure (Ouborg et al., 1999). In addition, a loss of genetic diversity usually reduces the ability of populations to adapt the environmental changes (Wang et al., 2003; Hao et al., 2006).

Over grazing and mowing might particularly affect the genetic diversity among populations (Kleijn and Steinger, 2002; Matlaga and Karoly, 2004; Wu et al., 2010). Wu et al. (2010) study indicated that the genetic diversity of *S. grandis* was lower under the condition of mowing. However, in our study, the genetic diversity of *S. bungeana* was relative high under the condition of grazing and mowing than fencing condition for some populations (Table 3). Seed germinating ability of *S. bungeana* was lower, and it mainly depends on clonal reproduction to expand populations. Under fencing condition, the lack of human or animals disturbance is beneficial to increase clonal growth by repeatedly producing tillering ramets from shoot base between genetically closely plants. Thus, we assumed that the low genetic diversity under fencing condition may be related to this specific reproduction pattern.

4.2. Genetic structure of S. bungeana population

Population genetic structure could provide useful information about intraspecific differentiation for conservation and management of populations (Song et al., 2011). In this study, genetic differentiation was consistent with a pattern of strong variation among populations and weak variation (strong homogeneity) within populations. Our genetic differentiation value was higher than other *Stipa* species (Table 5). The genetic differentiation value was also higher than the average coefficients of long-lived perennial species ($\Phi_{ST} = 0.25$, $n = 60$), out-crossing species ($\Phi_{ST} = 0.25$, $n = 73$), mixed breeding species ($\Phi_{ST} = 0.40$, $n = 18$) and the widespread species ($\Phi_{ST} = 0.34$, $n = 32$) (Nybom, 2004). This result indicated that genetic differentiation among *S. bungeana* populations was highly significant ($P < 0.001$). This may be explained by the reproductive system, genetic drift, selection or adaptation, gene flow (dispersal of pollens and seeds), or bottlenecks (Newton et al., 2002; Maki, 2003).

The complex topography, remote distribution and different steppe types of sampling may have decreased the gene flow (dispersal of pollens and seeds) among populations, and promoted the genetic differentiation of *S. bungeana* populations. This conclusion was supported by the result of gene flow in *S. bungeana* (Nm = 0.120). Wright (1951) pointed out that significant genetic differentiation may result from genetic drift when $Nm < 0.5$. Fischer et al. (2000) also suggested that the genetic drift may play an important role in population differentiation if there is no significant correlation between genetic distance and geographical distance. In our study, the genetic distance showed no significant correlation with geographical distance, indicating that genetic drift influenced the genetic structure of *S. bungeana*. Similar results were reported for *S. grandis* and *S. tenacissima* (Zhao et al., 2008; Liu et al., 2009). Therefore, genetic drift may be another important driver factor to increase the larger genetic variation among populations.

4.3. Conservation consideration

As an important dominant species, *S. bungeana* reduced rapidly because of habitat fragmentation, human activities and lower germination rate. Genetic diversity is one primary basis for nature conservation, and the structure of genetic diversity is valuable to proposing conservation strategies (Wu et al., 2010). Considering the relatively low genetic diversity within

Table 5
Comparison of genetic diversity of *S. bungeana* with other *Stipa* plant species.

Species	Genetic diversity values	Markers	References
Stipa grandis	PPBs = 76.06%; Hs = 0.2305; Is = 0.3521; $\Phi_{ST} = 0.2805$	RAPD	Zhao et al. (2006)
Stipa krylovii	PPBs = 97.47%; Is = 0.3820; $\Phi_{ST} = 0.4315$	RAPD	Zhao et al. (2006)
Stipa grandis	PPBs = 100%; Hs = 0.3207; Is = 0.4894; $\Phi_{ST} = 0.2588$	RAPD	Zhao et al. (2008)
Stipa purpurea	PPBs = 96.70%; Is = 0.3517; $\Phi_{ST} = 0.4202$	ISSR	Liu et al. (2009)
Stipa grandis	PPBs = 68.42%; Hs = 0.1952; Is = 0.3035; $\Phi_{ST} = 0.2431$	AFLP	Wu et al. (2010)
Stipa tenacissima	PPBs = 99.07%; Hs = 0.2402; Is = 0.3834; $\Phi_{ST} = 0.3240$	ISSR	Mohamed et al. (2010)

Abbreviation: *PPBs*, percentage of polymorphic loci at species level; Hs, Nei's gene diversity at species level; Is, Shannon's information index at species level; Φ_{ST}, genetic differentiation coefficient among populations.

populations and high genetic differentiation among populations, we should take necessary measures including both in situ and ex situ methods to protect the populations. Taking into account the current grazing and mowing ways and habitat of *S. bungeana*, we suggest that in situ conservation should be considered firstly. For ex situ conservation, to improve germination rate of seed should be carried out as soon as possible, then artificial cultivation may enlarge the vegetation coverage of *S. bungeana*.

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