

Morphological and RAPD analysis of the dominant species *Stipa krylovii* Roshev. in Inner Mongolia steppe

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ABSTRACT. *Stipa krylovii* Roshev. is an important perennial tussock grass in the Inner Mongolian steppe. It is found over a large area and has considerable ecological and economic importance. In the present study, five natural populations of *S. krylovii* were selected from their typical habitats to study the quantitative trait variation (samples from the natural populations) and RAPD variation. The relationships between quantitative trait variation and RAPD variation, and between either variation and geographic distance and then climatic factors were estimated using Mantel tests. *Stipa krylovii* populations showed differentiation in morphological and RAPD characters, yet no significant relationship existed between genetic variations estimated by morphological and RAPD characters and geographic distance, but both variations were closely associated with the climatic variation. These results indicated that the populational differentiation of *S. krylovii* was not in accord with the model of Isolation-by-distance, but was affected mainly by local climatic factors. Such information would be useful for conservation managers working out an effective strategy to protect this important species and provide the basis for a germplasm collection of it.

Keywords: Climatic factors; Genetic variation; Geographic distance; Mantel test; Quantitative traits; RAPD; *Stipa krylovii* Roshev.

INTRODUCTION

Genetic variation is generally believed to be a prerequisite for long- and short-term survival of a species (Schonewald-Cox et al., 1983; Lande, 1988), and the importance of preserving the genetic diversity of wild and domesticated species is widely acknowledged today. Since the development of bio-techniques in the 1960s, isozyme and DNA molecular markers have been used frequently to get variation estimates for plant species (Chung et al., 1991; Kercher and Conner, 1996; Fahima et al., 1999; Qian et al., 2001). The technique is relatively convenient, yields a large number of useful markers, and often requires very small amount of plant tissue (Fritsch and Rieseberg, 1996). Of these, the most popular marker is RAPD because the technique is quick and reliable and therefore enables a smooth evaluation of the molecular diversity in a species (Black-Samuelsson et al., 1997).

Compared with traditional morphological analysis, which may primarily indicate adaptation in a short as well as long term perspective and can be performed directly in a natural population or by quantitative genetic

studies of progenies under controlled conditions, the molecular markers (isozyme and DNA markers) are generally thought to be useful for detecting the action of non-selective evolutionary forces, such as gene flow and drift (Nei, 1987). Several examples illustrate that data consisting only of selectively neutral markers may fail to reveal adaptively important variation formed through natural selection, and can lead to biologically unsound management strategies (Olfelt et al., 2001). Therefore, reports that quantitative genetic analyses served an important complement in studies of plant species have increased in the past several years. For example, using isozymes and quantitative traits, Knapp and Rice (1998) evaluated the patterns of genetic variation in *Nassella pulchra*; Black-Samuelsson et al. (1997) analysed the patterns of RAPD and morphological traits of the rare plant species *Vicia pisiformis*. Olfelt et al. (2001) used a combination of morphological and molecular genetic markers to study the differentiation of *Sedum integrifolium* in order to apply better conservation priorities and design management strategies. These studies showed that a combination of quantitative traits and molecular markers to analyze the genetic patterns is powerful and comprehensive.

Form. *Stipa krylovii*, consisting of all associations dominated by the species itself, is one of the major

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formations of the moderately-temperate steppe in central Asia. The steppe is principally located in the inland portion of the Eurasian continent. It is not only an important pasture in Inner Mongolia, but also an important green protective defence for the Beijing-Tianjin area, and therefore has considerable economic and ecological importance. Affected by a temperate continental climate, the obvious thermal difference due to latitude and the precipitation discrepancy due to monsoons provides the possibility of genetic variation for *S. krylovii* in different populations. Some populations of this grass might have been genetically differentiated because the fragmented habitats brought about by human activities could have generated geographical isolation and population differentiation driven by genetic drift (Baruch et al., 2004). Zhao et al. (2003) studied the seed morphological traits of eight different geographic populations of *S. krylovii*, and the results showed that some significant differences in awn length, seed length and seed diameter existed among populations. Zhao et al. (2004) then analyzed the genetic differentiation of seven *S. krylovii* populations using RAPD markers. The results demonstrated significant differentiation between populations at the DNA level and that populations in similar habitats had closer genetic distance values and could then be clustered into one subgroup. However, studies that compare quantitative traits and molecular markers of *S. krylovii* have not been reported.

In the present study, five natural populations were selected from the typical region of *S. krylovii* based on their abundance, dominance, and representativeness of the community. In this region, an environmental gradient in aridity formed from the east to the west, and such gradient provided an ideal model system for the study of genetic variation by climatic variation. Accordingly, the purpose of this study was to analyze patterns of morphological and RAPD variations of *S. krylovii* in different locations and climatic conditions, in order to examine whether climatic factors were the main forces in modifying its genetic structure, and whether two levels of population genetic differentiation were affected by the same force. The results will provide a general knowledge about population genetic structure of *S. krylovii*. Such information will be of great significance in explaining the distribution of this important species and in developing conservation strategies for it. The information will also provide the basis for the germplasm collection of this species and for the restoration of northern grassland in China, taking into account that *S. krylovii* communities have been under the pressure of over-grazing and urbanization over the last several decades.

MATERIALS AND METHODS

Plant species

Stipa krylovii is a C_3 , tussock grass forming open steppes that dominate the large semi-arid landscape of the

Inner Mongolian steppe, and the mature plant has very dense tussocks about 20 cm high with long, thin leaves and a very dense matrix of thin roots concentrated in the first 20 cm of the soil layer. It is wind-pollinated, flowering in middle or later July and ripening in late August or early September. A seed consists of a callus, an awn, and a lemma and palea encasing the caryopsis. The sharp callus is covered with backward pointing hairs that readily attach to animals, machinery, and clothing, thus aiding dispersal. Lemmas are firm or even hardened and usually tightly enclose the palea and caryopsis and with a long twisted and twice-bent awn. As fruits dry, the awns become more and more twisted, orienting the seed properly to the soil.

Study site

The plant materials used in this study were taken from the five natural populations mentioned above. These populations were located in the middle and eastern part of the Inner Mongolian steppe in China (Figure 1). They were from three different locations from the east to the west, meadow steppe (one population), typical steppe (two populations) and desert steppe (two populations). The five populations were named Bayanwula, Xilinhot 1, Xilinhot 2, Xinhot, and Mandulatu after their locations from the east to the west (Table 1). The Bayanwula population was from the meadow steppe, where annual precipitation and community species diversity were highest and cumulative temperature in a year was lowest among the five sites. In this community *S. baicalensis* and *Filifolium sibiricum*, found only in meadow steppe, were dominant species, but in relatively high hills, *S. krylovii* became dominant. The Xilinhot 1 and Xilinhot 2 populations were located in the east and west sides of Xilinhot City, respectively. Both study sites were within the typical steppe where annual precipitation was a little lower and cumulative temperature in a year was higher than in meadow steppe, in which *S. krylovii* and *S. grandis* were dominant species and the species diversity was lower than that in Bayanwula. The

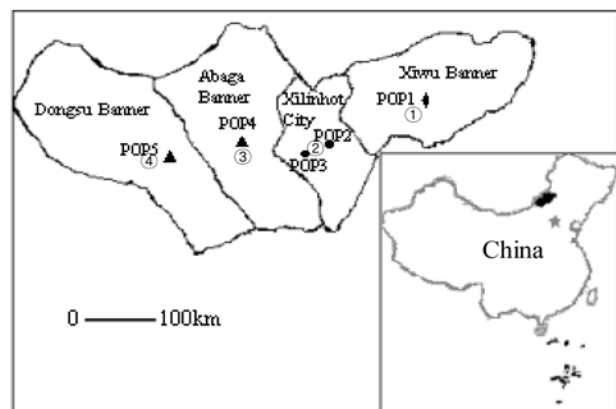


Figure 1. Sampling sites of *S. krylovii* populations (①, ②, ③, ④ indicate the capital city of the corresponding administrative regions, which are Bayanwula, Xilinhot, Xinhot and Mandulatu, respectively).

Table 1. Location, soil type and habitat characters of the five *S. krylovii* populations.

Population	Population code	Administrative area	Vegetation	Soil	Geographic position	Altitude (m)
Bayanwula	POP1	Xiwu Banner	Meadow steppe	Dark Chestnut	44.64 °N, 117.72 °E	1152
Xilinhot 1	POP2	Xilinhot City	Typical steppe	Chestnut	44.14 °N, 116.36 °E	1121
Xilinhot 2	POP3	Xilinhot City	Typical steppe	Chestnut	43.93 °N, 115.74 °E	1088
Xinhot	POP4	Abaga Banner	Desert steppe	Light chestnut	44.12 °N, 114.98 °E	1267
Mandulatu	POP5	Dongsu Banner	Desert steppe	Light chestnut	43.83 °N, 113.82 °E	1157

Xinhot population was from the desert steppe, in which *S. krylovii* was dominant species. The Mandulatu population was from the most western site and within the desert steppe, in which some typical desert species such as *S. gobica* and *Allium polyrhizum* were found. In the latter two sites, annual precipitation was lower than 250 mm, and cumulative temperature in a year was rather higher than at the other three sites.

Data collection for morphological characters

Data were collected for 20 quantitative traits (Table 2). The traits were chosen following previous work on the genus *Stipa* (Zhao et al., 2003). In early September, 2004, 50 vegetative ramets, 50 reproductive ramets and 100 spikes were taken randomly in a quadrat of 50 × 50 m from each population site for morphological character analysis. A distance of 3 m or more was left between individual plants to ensure the independence of the samples. A ramet with spike(s) on it was regarded as being

reproductive while one in a leafy stage was considered vegetative. After a ramet (vegetative or reproductive) was clipped above the ground, its actual length was measured and labeled, and its dry weight was measured after being air-dried at room temperature. A total of 100 spikelets were collected from 100 spikes after being air-dried at room temperature, and a set of measures—including diameter of seed, length of callus, length of lemma, length of the first segment of awn, length of the second segment of awn, length of awn apex, length of the first glume, length of the second glume, length difference of the two glumes, and length of awn—was obtained from an intact spikelet.

RAPD analysis

In each of the five sites, eighteen individuals were randomly collected at an interval of at least 10 m to avoid collecting ramets from the same genet. Leaves were harvested and stored with silica gel in zip-

Table 2. Description of morphological characters of *S. krylovii* plant.

Character	Brief description
Diameter of seed	The diameter of seed (with lemma) measured at the mid-point
Length of callus	Measured from callus tip to base of lemma
Length of lemma	Measured from base of lemma to base of awn
Length of the first segment of awn	Measured from the base of awn to the first geniculate point of awn
Length of the second segment of awn	Measured from the first to the second geniculate point of awn
Length of awn apex	Measured from the second geniculate point to the tip of awn
Length of the first glume	Measured from the base to the tip of the first glume
Length of the second glume	Measured from the base to the tip of the second glume
Length difference of the two glumes	The difference between the first glume length and the second glume length
Length of awn	Measured from the base to the tip of awn
Diameter of the second internode	The diameter of the second internode at its mid-point
Length of the second internode	Measured from node to the tip of the reproductive shoot
Length of flag leaf blade	Measured from the base to the tip of flag leaf blade
Height of reproductive shoot	Measured from top to bottom of the reproductive shoot
Dry matter weight per reproductive shoot	
Height of vegetative shoot	Measured from top to bottom of the vegetative shoot
Leaf blade length per vegetative shoot	Sum of length of leaf blades of all leaves for a vegetative shoot
Dry matter weight per vegetative shoot	
Length of longest leaf of vegetative shoot	
Length of leaf sheath of first leaf	

lock bags for DNA extraction. Genomic DNA was extracted using a modification of the protocol of standard phenol-chloroform (Hillis et al., 1996). DNA concentration and quality were determined with a UV-VIS spectrophotometer (TU-1800) before it was diluted to 30 ng/ μ L and determined again in 0.7% regular agarose (Spain) gels.

Eighty random decamer primers (hit A, I, N, Q, Operon technologies, Inc., Alameda CA, USA) were tested for PCR amplification with two bulked samples. The protocol for RAPD amplification described by Williams (Williams et al., 1990) was optimized for use on a *S. krylovii* template DNA. PCR was carried out in a 25 μ L reaction volume containing about 30 ng template DNA with 2.5 μ L 10 \times reaction buffer, 2.0 mM MgCl₂, 0.2 mM of each dNTP, 1 U *Taq* DNA polymerase and 0.2 mM primer. The PCR reaction was run in a Programmable Thermal Controller-100 (MJ research, Waltham MA, USA), with the following temperature profile: preliminary denaturation of DNA at 94°C for 4 min, followed by 40 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min. After 40 cycles, there was a final step of 10 min at 72°C, followed by soaking at 4°C until recovery. RAPD products were analyzed with electrophoresis in 1.5% regular agarose (Spain) gels containing ethidium bromide (0.5 mg/mL). Molecular marker SD005 (Beijing Dingguo Biotechnology Development Center, China) was used as a size marker. Gels were photographed under UV light to ensure the RAPD reproducibility. The reproducibility and repeatability of the amplification profiles were tested for each primer. Only those bands that were clear and consistently reproduced were considered. RAPD bands were scored as present (1) or absent (0) for each DNA sample, and a matrix of different RAPD phenotypes was established and used for statistical analysis.

Statistical analysis of morphological traits

One-way ANOVA was performed for each of the traits, and Duncan's test was used to test the significance of the differences between populations. A nested ANOVA was also performed to estimate the levels of morphological variation within and among *S. krylovii* populations using the restricted maximum likelihood method of the SAS VARCOMP procedure (SAS, 1989). The proportion of variance accounted for by random factor (population being the only random factor in this analysis) was calculated as the ratio of the variance component to the sum of all variance components (i.e., variance among populations), and the remainder was considered as variance within population or variance among individuals within population (namely error). Euclidean distance coefficients were estimated for each pair of populations after means of each character were normalized using Z-scores in order to avoid effects due to scaling difference. The resulting Euclidean distance matrix was used for cluster analysis

using the unweighted paired group method analysis (UPGMA) (Sneath and Sokal, 1973) in NTSYS-pc (Rohlf, 1994). In order to obtain information on the traits most effective in affecting the cluster, Principal Component Analysis (PCA) was carried out on the mean of the twenty morphological characters. Common components coefficients, eigenvalues, and relative and cumulative proportion of the total variance expressed by single traits were calculated.

Statistical analysis of RAPD markers

The vector of each individual's RAPD marker presence/absence was used to compute a measure of genetic similarity for all pairs of individuals. Jaccard's similarity coefficient (Jaccard, 1908) was used. The Jaccard's distance was calculated by $D_j = 1 - \text{Jaccard}_{xy}$, and the average Jaccard's distances within and between pairwise populations were estimated. UPGMA cluster was generated based on the average Jaccard's distance matrix using NTSYS-pc (Rohlf, 1994). Nei's genetic diversity index and gene differentiation coefficient (G_{ST}) were calculated under the Hardy-Weinberg equilibrium using the POPGENE Version 32 Program (Yeh et al., 1999). At the same time, we used G_{ST} to calculate the average number of immigrants per generation for each locus, namely, $Nm = (1 - G_{ST})/4 G_{ST}$.

Correlation analysis

Data used to calculate indices of climatic differences between populations were obtained from the Xilinhot[Ⓞ]'s Meteorological Station, which was responsible for assembling data from its subordinate meteorological stations located in Bayanwula, Xilinhot (East), Xilinhot (West), Xinhot, and Mandulatu, respectively. Thirty-year average values (1965-1995) for annual precipitation— $\geq 10^\circ\text{C}$ cumulative temperature in a year, annual mean temperature, number of frost-free days, number of windy days, mean temperature in January and July, sunshine hours in April and July, the percentage of sunshine time, mean temperature 10 cm above ground level in May and July—were compiled and transformed into standard units (Table 3). The aridity index is defined as the ratio of cumulative temperature in a year to total precipitation of the year and can be viewed as an indication of the degree of drought of an environment. It is calculated in this article by formula: aridity index = $0.16 * \text{cumulative temperature in a year } (\geq 10^\circ\text{C}) / \text{precipitation in the year}$. The average value, with all variables weighted equally, was used as an index of climatic difference between sites (Knapp and Rice, 1998). Geographic distances among populations were estimated from the Map of the Xilingol League. In order to examine the relationship between genetic variations and geographical distances, and then climatic differences among the sampling sites, the relationships between the Jaccard's distance / Euclidean's distance and

[Ⓞ]Xilinhot is the capital city of the Xilingol League, an administrative division in Inner Mongolia Autonomous Region, which is corresponding to a prefecture.

Table 3. The climatic characters in different populations of *S. krylovii* (mean of 30 years between 1965 and 1995).

Variables	POP1	POP2	POP3	POP4	POP5
Annual precipitation (mm)	340	300	290	230	180
≥10°C cumulative temperature in a year (°C)	2256	2400	2496	2552	2664
Annual mean temperature (°C)	1.5	1.4	1.8	1.2	2.9
Aridity index	1.06	1.28	1.38	1.78	2.37
Number of frost free days (d)	100	106	106	103	120
Number of windy days (d)	69.9	61.1	61.1	84.6	85.5
Mean temperature in January (°C)	-19.4	-19.1	-19.8	-22	-18.9
Mean temperature in July (°C)	19.5	20.7	20.8	20.3	21.4
Sunshine hours in April (h)	261.3	266.5	269	280.4	284.6
Sunshine hours in July (h)	267.7	274.3	276.9	288.4	302.8
The percentage of sunshine time (%)	66	69	70	71	73
Mean temperature 10 cm above ground level in May (°C)	11	11.8	11.9	12.3	15
Mean temperature 10 cm above ground level in July (°C)	20	20.7	21	22.3	24.3

Table 4. Statistical analysis on the morphological characters of *S. krylovii* populations.

Character	POP1	POP2	POP3	POP4	POP5	Mean	SE	CV
Diameter of seed (mm)	0.888 ^a	0.829 ^c	0.814 ^c	0.668 ^d	0.850 ^b	0.810	0.084	0.104
Length of callus (mm)	2.379 ^c	2.496 ^b	2.609 ^a	2.215 ^d	2.420 ^{bc}	2.424	0.146	0.060
Length of lemma (cm)	1.075 ^c	1.102 ^b	1.145 ^a	1.071 ^c	1.062 ^c	1.091	0.033	0.031
Length of the first segment of awn (cm)	3.944 ^a	3.255 ^b	2.995 ^c	2.722 ^d	2.938 ^c	3.171	0.472	0.149
Length of the second segment of awn (cm)	1.305 ^c	1.400 ^b	1.475 ^a	1.377 ^d	1.387 ^a	1.389	0.061	0.044
Length of awn apex (cm)	9.928 ^c	10.354 ^b	11.370 ^a	9.229 ^d	11.660 ^a	10.508	1.008	0.096
Length of the first glume (cm)	2.545 ^c	2.578 ^c	2.874 ^a	2.300 ^d	2.648 ^b	2.589	0.206	0.080
Length of the second glume (cm)	2.402 ^d	2.463 ^c	2.697 ^a	2.179 ^e	2.521 ^b	2.452	0.188	0.077
Length difference of the two glumes (cm)	0.143 ^b	0.115 ^c	0.178 ^a	0.122 ^b	0.127 ^b	0.137	0.025	0.185
Length of awn (cm)	15.177 ^b	15.008 ^b	15.839 ^a	13.327 ^c	15.984 ^a	15.067	1.058	0.070
Diameter of the second internode (mm)	1.070 ^b	1.120 ^b	1.077 ^b	1.208 ^a	1.057 ^b	1.106	0.062	0.056
Length of the second internode (cm)	8.752 ^{bc}	10.056 ^a	9.346 ^b	8.352 ^c	6.584 ^d	8.618	1.306	0.152
Length of flag leaf blade (cm)	11.038 ^a	9.200 ^{bc}	9.581 ^{bc}		8.553 ^c	9.728	0.960	0.099
Height of reproductive shoot (cm)	47.240 ^a	43.980 ^b	46.480 ^a	37.580 ^c	34.454 ^d	41.947	5.655	0.135
Dry matter weight per reproductive shoot (g)	0.305 ^c	0.358 ^{ab}	0.398 ^a	0.332 ^{bc}	0.253 ^d	0.329	0.055	0.166
Height of vegetative shoot (cm)	23.178 ^a	20.820 ^b	24.348 ^a	18.896 ^c	16.564 ^d	20.761	3.155	0.152
Leaf blade length per vegetative shoot (cm)	50.466 ^{ab}	48.468 ^b	53.812 ^a	35.582 ^c	35.026 ^c	44.671	8.763	0.196
Dry matter weight per vegetative shoot (g)	0.041 ^b	0.043 ^{ab}	0.050 ^a	0.029 ^c	0.032 ^c	0.039	0.009	0.220
Length of longest leaf of vegetative shoot (cm)	21.512 ^a	18.260 ^b	22.432 ^a	17.340 ^b	15.168 ^c	18.942	3.002	0.159
Length of leaf sheath of first leaf (cm)	1.666 ^{bc}	2.560 ^a	1.916 ^b	1.556 ^c	1.396 ^c	1.819	0.456	0.250

climatic distance / geographic distance were analyzed using a Mantel test (1967) in NTSYS-pc with significance of the autocorrelation coefficients tested by resampling (3000 auto permutations).

RESULTS

Morphological variations

Statistically significant differences ($P < 0.05$) were

found among populations for the 20 morphological traits studied (Table 4). There was no obvious trend taking all morphological traits into account, but if we only considered the traits related to growth (the last ten characters), an obvious trend did emerge, and that was that except for the length of the flag leaf blade, plants from POP4 and POP5 were smaller than those from other populations. This result suggested that the characters related to growth were significantly affected by local microhabitats and might be indicative of local

environments. Euclidean's dissimilarity / distance coefficients were calculated for all populations based on the morphological data in Table 4. Pairwise Euclidean distances ranged from 0.246 to 0.633 (Table 5). Cluster analysis placed these populations into three main subgroups (Figure 2): POP1, POP2 and POP3 were within the first subgroup, and there were relatively small distance coefficients between them; POP4 and POP5 were in two different subgroups, and both populations had relatively large distance coefficients from any other populations.

The information on variance patterns in the characters of *S. krylovii* is summarized in Table 6. The means of coefficients of variation (CV) estimated for trait varied from 0.058 (length of lemma) to 0.619 (length difference of the two glumes), with the mean being 0.192. Except for the length difference of the two glumes, the CVs of other characters obtained from spike (the first ten characters) were consistent between populations and were smaller than those of characters related to growth (the last ten characters). This result suggested that characters related to growth had higher phenotypic plasticity, and that characters obtained from spike were relatively conservative and had no indication for the local environment. The means of CV estimated for all traits within population ranged from 0.185 (POP4) to 0.200

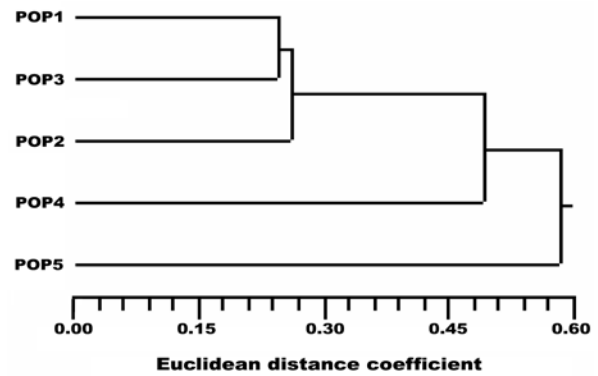


Figure 2. Dendrogram generated with UPGMA based on Euclidean's distances of *S. krylovii* populations.

Table 5. Euclidean's distances between populations of *S. krylovii* calculated from the morphological data.

Population code	POP1	POP2	POP3	POP4	POP5
POP1	-				
POP2	0.262	-			
POP3	0.246	0.260	-		
POP4	0.404	0.520	0.560	-	
POP5	0.605	0.586	0.633	0.509	-

Table 6. Coefficient of variation of morphological characters in five *S. krylovii* populations and the variation distribution within and among populations.

Character	Coefficient of variation						Source of variation	
	POP1	POP2	POP3	POP4	POP5	Mean	Population	Error
Diameter of seed	0.079	0.097	0.098	0.110	0.081	0.093	55.85	44.15
Length of callus	0.126	0.130	0.143	0.130	0.134	0.132	16.30	83.70
Length of lemma	0.044	0.058	0.056	0.055	0.078	0.058	20.52	79.48
Length of the first segment of awn	0.101	0.155	0.140	0.129	0.158	0.137	54.43	45.57
Length of the second segment of awn	0.105	0.136	0.110	0.121	0.146	0.124	10.06	89.94
Length of awn apex	0.125	0.130	0.122	0.098	0.092	0.113	40.87	59.14
Length of the first glume	0.077	0.090	0.089	0.064	0.072	0.078	49.39	50.62
Length of the second glume	0.073	0.089	0.091	0.072	0.068	0.079	47.49	52.52
Length difference of the two glumes	0.598	0.725	0.581	0.537	0.655	0.619	7.26	92.74
Length of awn	0.085	0.097	0.098	0.076	0.082	0.088	38.17	61.83
Diameter of the second internode	0.168	0.141	0.176	0.142	0.144	0.154	9.93	90.07
Length of the second internode	0.133	0.205	0.147	0.161	0.231	0.176	41.64	58.36
Length of flag leaf blade	0.266	0.334	0.339	0.242	0.303	0.297	8.42	91.58
Height of reproductive shoot	0.174	0.151	0.138	0.118	0.133	0.143	44.60	55.40
Dry matter weight per reproductive shoot	0.402	0.293	0.346	0.216	0.307	0.313	19.81	80.19
Height of vegetative shoot	0.138	0.167	0.165	0.162	0.158	0.158	47.05	52.95
Leaf blade length per vegetative shoot	0.165	0.208	0.200	0.231	0.180	0.197	48.84	51.16
Dry matter weight per vegetative shoot	0.271	0.345	0.365	0.458	0.271	0.342	27.40	72.60
Length of longest leaf of vegetative shoot	0.133	0.170	0.160	0.169	0.154	0.157	49.71	50.29
Length of leaf sheath of first leaf	0.448	0.284	0.394	0.404	0.426	0.391	29.16	70.84
Mean	0.186	0.200	0.198	0.185	0.194	0.192	33.34	66.66
SD	0.144	0.149	0.135	0.134	0.144	0.137	16.96	16.96

(POP2), and the divergence was small; furthermore, the difference between populations was not significant ($P>0.05$). The results of the univariate analysis of variance for the characters showed that the mean value of variation among populations was 33.34%, ranging from 7.26% to 55.85%.

A principal components analysis of the twenty quantitative variables yielded three statistically significant components, which accounted for 92.08% of the total variance (50.05% the first, 25.48% the second and 16.55% the third). The coefficient for each variable and significant components were shown in Table 7. The first factor was mostly affected by length of callus, length of lemma, length of the first and the second glume, length difference of the two glumes, height of reproductive and vegetative shoot, leaf blade length per vegetative shoot, dry matter weight per vegetative shoot, and length of longest leaf of vegetative shoot. The second factor was affected by length of awn apex, length of awn and length of flag leaf blade. The third factor was affected by length of the first segment and the second segment of awn.

RAPD variations

After screening eighty 10-base oligonucleotide primers (Operon Technologies, Inc., Alameda CA, USA) against two bulked samples, thirteen primers that showed intense

and reproducible bands were selected for further survey. All these primers generated 237 amplified bands in total, and their length ranged from 300 to 2,000 bp. The Jaccard's similarities between individual plants ranged from 0.221 to 0.938. The average Jaccard's distances between pairs of plants belonging to the same or to different populations were summarized in Table 8, and a UPGMA dendrogram based on average Jaccard's distance

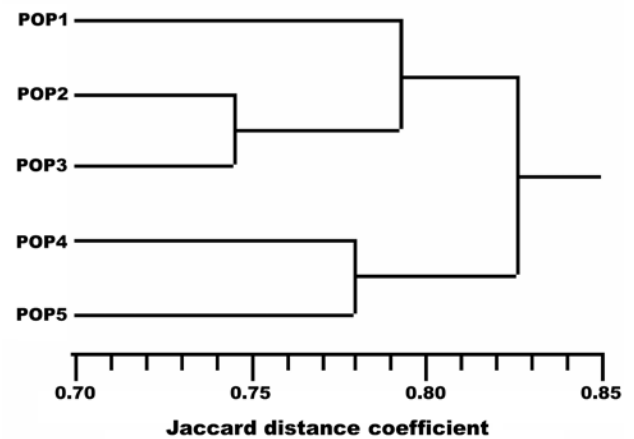


Figure 3. Dendrogram generated by UPGMA based on average Jaccard's distances of *S. krylovii* populations.

Table 7. Coefficients for each morphological variable for the three significant principal components.

Variable	Component		
	First	Second	Third
Diameter of seed	0.547	-0.389	-0.689
Length of callus	0.914	-0.341	0.149
Length of lemma	0.869	0.133	0.466
Length of the first segment of awn	0.347	0.293	-0.869
Length of the second segment of awn	0.498	-0.277	0.813
Length of awn apex	0.482	-0.872	0.076
Length of the first glume	0.861	-0.494	0.063
Length of the second glume	0.841	-0.536	0.066
Length difference of the two glumes	0.758	-0.039	0.024
Length of awn	0.642	-0.716	-0.269
Diameter of the second internode	-0.581	0.617	0.530
Length of the second internode	0.579	0.690	0.226
Length of flag leaf blade	-0.014	0.808	-0.466
Height of reproductive shoot	0.797	0.542	-0.263
Dry matter weight per reproductive shoot	0.641	0.525	0.559
Height of vegetative shoot	0.822	0.519	-0.131
Leaf blade length per vegetative shoot	0.921	0.347	-0.161
Dry matter weight per vegetative shoot	0.985	0.133	0.018
Length of longest leaf of vegetative shoot	0.788	0.495	-0.182
Length of leaf sheath of first leaf	0.499	0.330	0.288
Percentage of Variance	50.05	25.48	16.55

Table 8. Average Jaccard's genetic distances between pairs of individuals belonging to the same (boxed) or different populations based on 237 RAPD markers for five populations of *S. krylovii* (from the minimum to maximum value of paired Jaccard's genetic distances).

	POP1	POP2	POP3	POP4	POP5
POP1	0.549 (0.267-0.729)				
POP2	0.812(0.700-0.923)	0.544 (0.313-0.762)			
POP3	0.774(0.643-0.880)	0.745(0.613-0.854)	0.531 (0.221-0.700)		
POP4	0.875(0.787-0.938)	0.81(0.758-0.913)	0.825(0.742-0.919)	0.567 (0.356-0.705)	
POP5	0.843(0.742-0.913)	0.812(0.710-0.927)	0.791(0.696-0.901)	0.78(0.714-0.874)	0.578 (0.240-0.786)

Table 9. Patterns of genetic diversity of *S. krylovii* populations.

Population code	Sample size	<i>na</i>	<i>ne</i>	<i>h</i>	<i>PPB</i> (%)	<i>G_{ST}</i>	<i>Nm</i>
POP1	18	1.4135	1.2117	0.1279	41.35		
POP2	18	1.5316	1.2428	0.1505	53.16		
POP3	18	1.5316	1.2646	0.1620	53.16		
POP4	18	1.5527	1.2955	0.1756	55.27		
POP5	18	1.6118	1.3272	0.1952	61.18		
Average		1.5282	1.2684	0.1622	52.82		
All populations	90			0.2392 (0.0112-0.5000)	97.47	0.3218	0.5239

Na, Observed number of alleles; *ne*, Effective number of alleles; *h*, Nei's gene diversity; *PPB*, Percentage of polymorphic bands; *Nm*, Estimate of gene flow from *G_{ST}*.

between different populations was shown in Figure 3. The average distances within the populations (boxed in Table 8) were, in all cases, smaller than the distances between plants from different populations. This provided evidence that individuals from different populations diverged more, on average, than individuals within the same populations. The range between the minimum and the maximum of the Jaccard's genetic distances was summarized in brackets in Table 8, and it appeared that the range within populations was larger than that among populations.

A summary of the genetic diversity and genetic differentiation of the five populations of *S. krylovii* was given in Table 9. The Nei's genetic diversity for individual populations (*h*) increased from 0.1279 (POP1) to 0.1952 (POP5). Percentage of polymorphic bands (*PPB*) increased from 41.35% (POP1) to 61.18% (POP5). Observed number of alleles (*na*) increased from 1.4135 (POP1) to 1.6118 (POP5), and effective number of alleles (*ne*) increased from 1.2117 (POP1) to 1.3272 (POP5). All these parameters showed the same pattern as diversity indexes, i.e., the parameters increased with the increase of aridity of the study sites. Total Nei's genetic diversity varied from 0.0112 to 0.5000, the gene differentiation (*G_{ST}*) based on Nei's genetic diversity (1973) was 0.3218, and an estimate for *Nm* was 0.5239, indicating that 32.18% of the genetic diversity was allocated among populations.

Correlation analysis

The relationship between two distance matrixes based

Table 10. Geographic distances (km) (above diagonal) and climatic factor variation coefficients (below diagonal) between the locations of different *S. krylovii* populations.

	POP1	POP2	POP3	POP4	POP5
POP1	-	121.90	176.60	226.54	324.47
POP2	0.0828	-	54.71	110.42	206.10
POP3	0.1110	0.0289	-	64.29	154.00
POP4	0.1950	0.1170	0.0932	-	98.13
POP5	0.2690	0.1900	0.1650	0.0772	-

on morphological traits (Table 5) and RAPD markers (Table 8) was estimated by Mantel's test ($r = 0.3604$, $P = 0.182 > 0.05$, $n = 3000$ permutations), suggesting no significant correlation between the two types of population differentiation. Relationships between either of the two distance coefficient matrixes (Euclidean's or Jaccard's distances) of *S. krylovii* and geographic distance matrix (Table 10) were tested by Mantel test, and the results were not significant. The relationship was significant between Euclidean's distance and climatic distance (Table 10) ($r = 0.5746$, $P = 0.0463$, $n = 3000$ permutations), as well as between Jaccard's distance and climatic distance ($r = 0.7027$, $P = 0.0427$, $n = 3000$ permutations) by Mantel test, suggesting that selection associated with climatic variation played an important role in shaping patterns of morphological and RAPD variations in *S. krylovii*.

DISCUSSION

Consistency of morphological and RAPD markers

In the present study, we used not only selective markers (morphological traits for phenotypic genetic variations) but also selectively neutral markers (RAPD markers for purely DNA genetic variations) to study the intra-specific differentiation of *S. krylovii* and the relationships between differentiation of *S. krylovii* and geographic distances and climatic variations. With these approaches, we arrived at the following conclusions.

First, there was significant differentiation among *S. krylovii* populations both in phenotypic traits and in RAPD markers (Tables 4, 6, 8 and 9), which consistent with former studies on *S. krylovii* by Zhao et al. (2003, 2004).

Second, there was a small proportion of variance among populations and a larger proportion of variance within populations both in morphological traits (33.34% vs 66.656%) and in RAPD characters (32.18% vs 67.82%). Bussell (1999) reviewed RAPD studies on population genetics of 38 plant species and indicated that the average genetic variance component among populations of the 30 out-breeding species was 14.4%. RAPD analysis on out-crossing perennial grasses showed 5-15% of the genetic variation partitioned among populations (Huff et al., 1993, 1998; Huff, 1997). Allozyme analysis of out-crossing species tended to have 17% of the total genetic variation residing among populations (Hamrick and Godt, 1997) while out-crossing grasses exhibited 11% of the variation among populations (Godt and Hamrick, 1998). Because *S. krylovii* is a wind-pollinated perennial grass, about 33% of the variation among populations is significantly higher than other out-crossing perennial grasses in population differentiation. The high genetic differentiation among *S. krylovii* populations was also evidenced by Han et al. (2003). To date, it is well established that the main factors that determine the population genetic structure of plants include the mating and reproductive system, selection or others (Hamrick and Godt, 1989). In our case, there was evidence that habitat destruction and degradation from decades of over-grazing and urbanization throughout the geographic range of *S. krylovii* had significantly decreased its populations in northern China both in scale and in size (Li, 1997) although it remained difficult to rank these according to their significance. A rational explanation of the present results was perhaps fragmentation of habitats and small populations (small in size or decreased in sexual reproduction by over-grazing) resulting from human activities. Similar observations were reported about other wind-pollinated grasses (Qian et al., 2001).

Third, neither phenotypic variation and geographic distance nor molecular variation and geographic distance showed any significant correlation, suggesting that the differentiation of *S. krylovii* estimated by morphological traits or by RAPD markers was not consistent with the Isolation-by-distance model (Wright, 1946). However,

there was significant correlation between phenotypic variation and climatic variation and between genetic variation and climatic variation, which is consistent with the hypothesis that selection associated with climatic variation plays an important role in shaping patterns of morphological and RAPD variations in *S. krylovii*. Shmida et al. (1986) suggested that a decrease in size and organ dimensions is a general rule for plants distributed along a climatic gradient towards the desert. In our study, the habitat climates of POP1 ~ POP3 were relatively humid while POP4 and POP5 were located in the arid desert steppe. In Table 4, the last ten characters related to growth (except for the length difference of the two glumes) were smaller in POP4 and POP5 than in other populations, that is to say, the last ten characters were smaller in the desert steppe. This might be an adaptation to aridity, presumably for reasons of reducing water loss through reduction of the area exposed to radiation (Shmida et al., 1986). From a functional standpoint, it has been argued that smaller leaves may be favored in drier environments, because smaller leaves provide less surface area for transpirational water loss (Givnish, 1979; Nobel, 1991; Dudley, 1996). In addition, smaller organ size and smaller plant size can reduce developmental time (Guerrant, 1988). Similar phenotypic gradients could also be found in other plant and animal groups (Endler, 1977; Nevo, 1988). Directional change in morphological characters along climatic gradient is natural selection, rather than random processes, and plays a dominant role in shaping these characters (Endler, 1977; Davis and Gilmartin, 1985).

Discrepancy of morphological and RAPD markers

Patterns of genetic variation based on morphological traits and RAPD markers were not consistent with each other, and two aspects of the discrepancy should be emphasized.

First, the pattern of the UPGMA dendrogram based on morphological traits and the one based on RAPD markers did not match each other. Populations from the same habitats were clustered into one subgroup based on RAPD markers while the clustering based on morphological traits showed that POP1 (from the meadow steppe) and POP3 (from the typical steppe) were clustered together first. Moreover, the Mantel's test indicted no significant correlation between Euclidean's distance (morphological traits) and Jaccard's distance (RAPD markers). Some authors found that the genetic-phenetic variations were positively and closely correlated (Houle, 1989; Briscoe et al., 1992; Soulè and Zegers, 1996; Waldmann and Andersson, 1998). However, widely differing opinions about the magnitude of the relationship have been expressed, and the extent of the correlation remains controversial. Lewontin (1984) and Lynch (1996) pointed out that genetic-phenotypic correlations were likely to be low. Patterson et al. (1993) found the relationship between molecular and morphological phylogenies to be

weak. Reed and Frankham (2001) analyzed the correlation coefficients of 71 datasets by a meta-analysis, and noted that the mean of correlation coefficients between molecular and quantitative measures of genetic variation was weak ($r = 0.217$). Nevertheless, it is reasonable to assume that the direction or magnitude of selective force acting on the majority of RAPD variation differs from that acting on many morphological traits. From this discrepancy in the present study, we could infer that local selection rather than migration or genetic drift played a more important role in the divergence of *S. krylovii* because if genetic drift was the dominant evolutionary force leading to divergence among populations, selectively neutral markers and selective quantitative trait variations should have shown a stronger relationship (Knapp and Rice, 1998). Further evidence was available in which isozyme and morphological markers were used to study genetic variations among populations (Bryant, 1984; Lagercrantz and Ryman, 1990). Our recent field investigation showed that the current geographic populations have been gradually reduced in size because of increased disturbance from human activities. It is well known that the measure of phenotypic variations is environmentally dependent while molecular markers are rather independent. Thus, phenotypic variations were more affected than molecular variations during the period of intensive human activities, e.g., as grazing intensity increased, the height of plant decreased (Wang et al., 2000). An alternative way to deal with the poor correlation between genetic and morphological distances was proposed by Roldán-Ruiz et al. (2001), who selected only molecular markers linked to phenotypic traits in DUS (Distinctness, Uniformity and Stability) testing.

Second, in the present study, both coefficients of variation of morphological traits within populations and Nei's genetic diversity index within populations indicated a high diversity of *S. krylovii* individuals within populations. Nei's genetic diversity increased from the east to the west populations, but coefficients of variation for morphological traits within populations varied without regularity from the east to the west. A possible explanation for this discrepancy was given in the following: i) Higher genetic diversity within populations was associated with higher capability in adapting to the changing conditions (Sun, 1996; Rajora et al., 1998). In the present study, the environmental conditions turned harsh gradually moving from east to west. Higher genetic diversity was reserved under adverse environmental conditions in evolutionary history. ii) Although RAPD and morphological variations were shaped by climatic selection, the influence of microhabitat may be the major factor that affected population divergence within a population. Selectively morphological traits were environmentally dependent, and higher CVs within populations might represent larger microhabitat selection forces. When disturbance increased with human activities and/or changing climates, the selectively morphological trait variation would have happened due to phenotypic plasticity, but variation at

DNA level would not have happened in such a short term. This result supported a view that *S. krylovii* is more responsible to local selection pressures and explained why this species has a broad ecological amplitude.

Advantages of combination of morphological and RAPD markers

Despite the obvious advantages in analyzing population genetic structure that have been summarized by many authors, disadvantages of the method (selectively neutral markers such as RAPD and selectively quantitative traits) have been noticed by more and more workers involved in methodology development. RAPD markers or other forms of neutral or nearly neutral molecular markers, are unlikely to accurately predict patterns of variation in quantitative traits when selection, rather than drift, is the primary force acting (e.g., local adaptation, speciation) (Reed and Frankham, 2001). In some cases, plant groups with very low levels of molecular differentiation among populations show significant levels of morphological genetic differentiation (Furnier et al., 1991; Karhu et al., 1996). Quantitative trait variation has several disadvantages. Obtaining accurate data is time-consuming and limited by growing season (Camlin and Gilliland, 1994), and the expression of quantitative traits is generally rather plastic with respect to environmental effects. (If the common garden experiments were selected to analyze the quantitative trait variation in order to reduce the influence of environmental factors, it would take more time.) Smith and Smith (1989) suggested that the use of morphological traits was not always the best way to evaluate genetic distance since the degree of divergence between genotypes at the phenotypic level is not necessarily correlated with a similar degree of genetic difference (Hamrick and Godt, 1989). Traditional morphological observation alone cannot determine the roles of phenotypic plasticity and genetic differentiation on population variation (Wen and Hsiao, 1999). Considering the weak correlation between molecular differentiation and quantitative genetic variation, a combination analysis of both quantitative traits and molecular markers might be most desirable because it would allow examination of the relative roles of selection, drift, and gene flow in structuring genetic variation species (Felsenstein, 1986; Rogers, 1986; Knapp and Rice, 1998). The combination has been used more and more in the past several years and has proven to be powerful (Black-Samuelsson et al., 1997; Szczepaniak et al., 2002).

In summary, the five *S. krylovii* populations showed significant differentiation both in quantitative traits and RAPD markers. It was selection, not genetic drift, that was the major factor in this differentiation. As for the biological characters of *S. krylovii*, larger genetic differentiation suggested that genetic diversity had been affected by the declining of population size and fragmentation of the habitat by human activities. Unfortunately, without historical genetic data, we do not know how to compare the current levels of genetic diversity with those prior to habitat reduction and

fragmentation. However, the information obtained in the present study reminds us that it would be prudent to work out some measures to protect *S. krylovii* that would prevent it from larger differentiation in genetic variation and in morphological variation. It is generally believed that mutation and genetic drift due to finite population size, and natural selection will lead to genetic diversification of local populations and that the movement of gametes and individuals (i.e. gene flow) will counter that diversification. We should protect the favorite ecological environments of *S. krylovii* and avoid habitat fragmentation and degradation due to over-grazing, in order to maximize the movement of gamet and individuals. In conserving germplasm resources of *S. krylovii*, we should protect populations with significant differentiation either in genetic variation or in quantitative trait variation. In a word, the combined analysis of both morphological and RAPD markers has undoubtedly provided important information on *S. krylovii*'s genetic diversity and will provide information useful in formulating effective conservation decisions.

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內蒙古草原優勢植物克氏針茅的形態和 RAPD 分析

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克氏針茅 (*Stipa krylovii* Roshev.) 是內蒙古草原一種重要的多年生叢生禾草。它分佈面積廣，具有重要的經濟和生態價值。本文以其分佈區內五個典型樣地的自然種群為研究對象，分析了克氏針茅種群的數量性狀變異和 RAPD 變異，並利用 Mantel 檢驗分析了形態變異與 RAPD 變異，以及這兩種變異分別與種群間的地理距離以及氣候分異之間的相關關係。五個種群的形態數量性狀和 RAPD 分子標記結果都表明克氏針茅種群間分化明顯；兩種方法所得克氏針茅種群間的分化均與地理距離之間不存在顯著的相關關係，但與氣候分異之間相關顯著。這些結果表明克氏針茅種群間的分化不符合“地理—隔離模型”，而主要受當地氣候因子分異的影響。這不僅為決策者制定北方草原保護措施提供有用的信息，同時也為克氏針茅這一重要物種的種質資源保護提供基礎資料。

關鍵詞：氣候因子；遺傳變異；地理距離；Mantel 檢驗；數量性狀；RAPD；克氏針茅。