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Assessing genetic diversity of *Elymus sibiricus* (Poaceae: Triticeae) populations from Qinghai-Tibet Plateau by ISSR markers

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ABSTRACT

Inter-simple sequence repeats (ISSR) markers were used to assess the genetic diversity and population structure in eight populations of *Elymus sibiricus* L. from the southeast of Qinghai-Tibet Plateau of China. Of the 100 primers screened, 13 produced highly reproducible ISSR bands. Using these primers, 193 discernible DNA fragments were generated with 149 (77.2%) being polymorphic, indicating considerable genetic variation at the species level. In contrast, there were relatively low levels of polymorphism at the population level with the percentage of polymorphic bands (PPB) ranging from 44.04 to 54.92%. The mean gene diversity (H_E) was estimated to be 0.181 within populations (range 0.164–0.200), and 0.274 at the species level. A high level of genetic differentiation among populations was detected based on Nei's genetic diversity analysis (33.1%), Shannon's index analysis (34.5%), Bayesian method (33.2%) and AMOVA analysis (42.5%). No significant statistical differences (analysis of molecular variance [AMOVA], $P = 0.08$) in ISSR variation were found between the sample collection regions. However, among populations (42.5% of the variance) and within populations (57.5% of the variance), there were significant differences ($P < 0.001$). Populations shared high levels of genetic identity. This pattern of genetic variation was different from that for most of inbreeding Triticeae species reported. In addition, a geographical pattern of population differentiation, where the populations from south and north of sampling sites were clearly separated from each other, was revealed by both the cluster and principal coordinates analyses. Generally, the result of this study indicates that *E. sibiricus* contains high molecular variation in its populations. The implications of these results for the conservation of the species are discussed.

1. Introduction

Genetic diversity is an important precursor in a study of any species because its amount and distribution are likely to affect the evolutionary potential of species and/or populations (Futuyma, 1986). Analysis of the genetic structure at intraspecific level is important for future adaptive change or evolution (Schaal et al., 1991), and also for future breeding programs.

Elymus L. is the largest genus in the tribe Triticeae with about 150 species distributed in most temperate regions of the world (Dewey, 1984). As the type species of the genus *Elymus*, *Elymus sibiricus* L. (Siberian wildrye) is a perennial, self-pollinating and allotetraploid grass indigenous to Northern Asia, possessing the St and H genome (Dewey, 1974). Its geographic distribution extends from Sweden to Japan and even to parts of Alaska and Canada (Bowden and Cody, 1961), and

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then extends southerly to Qinghai-Tibet Plateau, which is the highest plateau in the world. *E. sibiricus* usually grows on wet meadows, riverside sands, and among open forest or shrubs. In the subalpine meadows with less than 4000 m altitude in Qinghai-Tibet Plateau, *E. sibiricus* usually serves as an important forage species. Climate warming, loss of habit by deforestation and excessive gazing at high altitude pastures in the entire Qinghai-Tibet Plateau region now begin to threaten its survival. Natural populations of *E. sibiricus* from Qinghai-Tibet Plateau show great geographic and morphological variability with respect to characters (particularly spike length, color of spike and culm, number of spikelets on a spike, height of plants, and the length and width of leaves) (Yan et al., 2006). However, it is not known whether these are attributable to genetic variation, physiological and morphological plasticity, intraspecies hybridization, or a combination of these factors.

ISSRs are amplified from single primer PCR reactions where the primer is designed from di-, tetra-, tri- or penta-nucleotide repeat motifs with a random anchoring sequence of one to three nucleotides (Zietkiewicz et al., 1994). Technically, ISSR fingerprinting is more reproducible than RAPD amplification due to the longer SSR-based primers, thus enables higher stringency DNA amplifications (Wolfe and Liston, 1998). Though ISSR technique has some problems as a dominant marker, it has proved effective tools in addressing problems of systematics and hybridization, as well as population genetics (Reddy et al., 2002).

In the present study, we employed ISSR markers to investigate the extent of genetic variation in *E. sibiricus* populations from southeast of Qinghai-Tibet Plateau. Our objectives are: (1) to determine levels of ISSR variation in *E. sibiricus* populations, (2) to detect levels of genetic variation within and between the populations, and (3) to assess genetic relationship of *E. sibiricus* populations.

2. Materials and methods

2.1. Plant material

Ninety-three individuals of *E. sibiricus* were collected from eight populations in southeast of Qinghai-Tibet Plateau, Sichuan Province, China. Their originations and distributions were shown in Table 1 and Fig. 1. Individual spikes generally 5–10 m apart from one another were sampled randomly to assure they were different individuals. These populations grow in the fringe of alpine forest or shrubs of mixed *Abies*, *Salix*, *Hippophae*, *Caragana*, *Rhododendron*, *Artemisia*, *Deschampsia*, *Bromus*, *Roegneria*, etc. between 3200 and 3700 m a.s.l. Their habitat represents the richly endowed yet rather stable primitive environment in the sampled regions. A single seed from each collected singled spike was planted into an individual pot in the greenhouse, with an approximate temperature 22 °C and 16 h photoperiod. Vouchers of the materials used are kept at the Department of Grassland Science, Sichuan Agricultural University, China.

2.2. DNA extraction and ISSR amplification

Fresh young leaves were powdered in liquid nitrogen and genomic DNA was extracted using the CTAB method described by Doyle (1991). DNA concentration was determined by comparing the sample with known standards of lambda DNA on 1% (w/v) agarose gels. The isolated genomic DNA was diluted to 10 ng/μl and stored at −20 °C for ISSR amplification. In a preliminary study, 100 primers (University of British Columbia primer set 9) were first screened for PCR amplification. Eighteen ISSR primers that generated clear, reproducible banding patterns were selected for further analysis (Table 2).

The effects of Mg^{2+}, dNTP, DNA templates, primers and DNA polymerase on the amplification were tested, and the determined optimal reaction system of ISSR for *E. sibiricus* was as follows: 1× Taq polymerase buffer [10 M Tris–HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 M $MgCl_2$], 1 U Taq DNA polymerase (Tiangen Biotech Co., Ltd., Beijing, China), 30 ng template DNA, 5 pmol primer, 0.2 mM each of dNTP in the total 20 μl reaction volume. PCR amplifications were performed in a PTC-200 thermocycler (Bio-Rad, USA). Initial denaturation was for 2 min at 94 °C, followed by 45 cycles of 1 min at 94 °C, 1 min at 52 °C, 2 min at 72 °C, with a final extension of 10 min at 72 °C. The amplification products were separated via electrophoresis on 2.0% agarose gels (containing 0.1 mg/mL ethidium bromide) with 0.5 ×TBE buffer. The amplified DNA fragments were documented by using Quantity One Software version 4.6 (Bio-Rad, USA). Negative controls, lacking template DNA, were included in each PCR set to test for the possibility of contamination.

Table 1
Populations of *Elymus sibiricus* for ISSR analysis

Population	Voucher	Locality	Sample size	Altitude (m)	Latitude and longitude
WD	Sau-ES 001	Wengda Town, Seda County, Garze Prefecture	10	3344	31°52′N, 100°43′E
XZ	Sau-ES 002	Xuri Town, Seda County, Garze Prefecture	8	3567	31°59′N, 100°36′E
JB	Sau-ES 003	Junba Town, Litang County, Garze Prefecture	11	3673	30°18′N, 100°17′E
GR	Sau-ES 004	Gaoersi Mountain, Yajiang County, Garze Prefecture	18	3525	30°02′N, 101°17′E
CZ	Sau-ES 005	Chuanzhusi Town, Songpan County, Aba Prefecture	9	3214	32°53′N, 103°29′E
CL	Sau-ES 006	Chali Temple, Aba County, Aba Prefecture	10	3324	32°45′N, 102°03′E
NM	Sau-ES 007	Nanmuda Town, Rangtang County, Aba Prefecture	12	3362	32°26′N, 101°02′E
SJ	Sau-ES 008	Shuajingsi Town, Hongyuan County, Aba Prefecture	15	3414	32°05′N, 102°34′E