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Genetic variation and geographical divergence in *Elymus nutans* Griseb. (Poaceae: Triticeae) from West China

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ABSTRACT

Elymus nutans Griseb. as an important forage grass and gene pools for improving cereal crops, widely distributes in west China. However, little is known about its genetic and geographical patterns. Inter-simple sequence repeat (ISSR) markers were applied to assess genetic diversity and geographical divergence among 63 *E. nutans* accessions from west China. The cluster analysis separated the accessions into several groups with geographical origins. The molecular variance analysis (AMOVA) showed that the proportion of variance explained by within- and among-geographical group diversity was 43.2% and 56.8%, respectively. Based on pairwise genetic distances (Φ_{ST}) between geographical groups, the relationships were congruent with the cluster of accessions. The distinct geographical divergence of *E. nutans* was revealed between Qinghai-Tibet Plateau and Xinjiang. The ecogeographical conditions such as climate and mountain ranges and elevation behaved as the crucial factors for genetic divergence. Furthermore, the study also indicated that Qinghai-Tibet Plateau might be the diversity differentiation center of *E. nutans*. The result will facilitate the breeding program and germplasm collection and conservation.

1. Introduction

Genetic diversity as the basis for survival, adaption and evolution of species is ubiquitous in nature (Nevo and Beiles, 1989). However, patterns of genetic variation and the distribution of plant species are not uniform but structured in space and/or time, which are often affected by abiotic ecological factor, biotic agents and species characteristics (Frankel et al., 1995). At a small spatial scale, the microgeographical variation of plant species is usually attributable to species characteristics such as population sizes, breeding system, seed dispersal (Yan et al., 2009a). Conversely, across a wide geographical region, the ecological factor is the crucial factor causing the macrogeographical variation of plant species, which could be divided into different ecogeographical groups or ecotypes (Yahiaoui et al., 2008). Furthermore, the spatial structure of genetic variation of plant species is a prerequisite for effective management of conservation programs (Sun et al., 2001).

Elymus nutans Griseb. is a perennial, caespitose and allohexaploid ($2n = 6x = 42$) species in genus *Elymus* L., which contains the St, H and Y genomes (Lu, 1993). It is native to temperate and tropical Asia, ranging from western and central Asia in the west to China and Mongolia in the east, from Russia in the north to India and the Himalayas areas in the south (Clayton et al., 2006). It grows in grassland, among bushes, along river banks, on mountain slopes, and in swales, at altitudes from 1000 m up to 5000 m (Lu, 1993). In China, it distributes in north, northwest and southwest China, particularly on Qinghai-Tibet Plateau (Kuo, 1987). *E. nutans* is the valuable forage grass in the alpine regions and has the characters of

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resistance for cold, drought and pest that can be used to improve cereal crops, which plays an important role in the restoration of the disturbed grasslands and construction of artificial grasslands, as well as animal production (Chen and Jia, 2002).

Up to the present, there is limited knowledge regarding the genetic and geographical differentiation of *E. nutans*, limiting the utilization and conservation of resources (Yan et al., 2006; Ma et al., 2007). Yan et al. (2009a,b) studied the genetic variation of *E. nutans* and *Elymus burchan-buddae* (Nevski) Tzvelev at a small spatial scale, which revealed that altitude and seed dispersal were the crucial factors affecting the spatial structure of genetic variation. However, effective utilization and conservation of species depends largely on the amount of knowledge of patterns of genetic variation. In west China, there are complicated and diversified environmental conditions, including diverse climate, intricate landform and various soil types. Therefore, it is reasonable to hypothesize that there would be a high level of genetic differentiation among the regions. Here we conducted an analysis of the genetic diversity and spatial variation at a macroscale to test it.

ISSR uses simple sequence repeats anchored at the 5′ or 3′ end by a short arbitrary sequence as PCR primers (Zietkiewicz et al., 1994). ISSR marker is more reproducible than RAPD marker because of the longer SSR-based primers and allowing more stringent annealing temperature (Wolfe and Liston, 1998). Though ISSR technique has some shortcomings as a dominant marker, it has been proved as an effective tool for investigation of genetic variation of species and systematic, as well as population genetics (Reddy et al., 2002). The objectives of the present study were to evaluate genetic diversity of *E. nutans* from west China based on ISSR, and reveal the divergence patterns of this species at the macro-geographic scale.

2. Materials and methods

2.1. Plant materials

A total of 63 *E. nutans* accessions from five provinces representing western regions of China were used in the study (Table 1, Fig. 1). They were obtained from the USDA-ARS, United States; Triticeae Research Institute, Sichuan Agricultural University, China; Sichuan Grassland Science Academy, China. The vouchers of the materials were deposited at the Department of grassland science, Sichuan agricultural university, China. Seeds with a pretreatment at a low temperature were germinated on absorbent filter paper in Petri dishes with a temperature 23 °C. Germinated seeds were transplanted into a sand-peat mixture and the plants maintained in a greenhouse.

Table 1
List of materials used in the study.

Accessions code	Origin	Altitude (m)	Accessions code	Origin	Altitude (m)
PI619586	Dulan, Qinghai	2950	Y2126	Dege, Sichuan	4050
PI499612	Xining, Qinghai	2450	PI619569	Dege, Sichuan	4110
W622069	Xining, Qinghai	2300	Y2155	Ganzi, Sichuan	3340
PI639855	Luqu, Gansu	3060	205097	Hongyuan, Sichuan	4032
PI628698	Tianzhu, Gansu	2860	PI619520	Hongyuan, Sichuan	3300
PI639852	Xiahe, Gansu	2690	PI639862	Kangding, Sichuan	3110
PI499611	Lanzhou, Gansu	2300	W622107	Kangding, Sichuan	3800
Y2171	Changdu, Tibet	3600	Y2081	Kangding, Sichuan	3800
Y2153	Changdu, Tibet	4420	Y2084	Kangding, Sichuan	3350
Y2154	Changdu, Tibet	4420	205106	Litang, Sichuan	3889
Y2186	Changdu, Tibet	4130	205143	Litang, Sichuan	4394
Y2173	Changdu, Tibet	3800	Y2110	Luhuo, Sichuan	3520
Y2160	Gongjue, Tibet	4100	PI619521	Luhuo, Sichuan	3740
PI619522	Gongjue, Tibet	4100	Y2095	Qianning, Sichuan	3310
PI619529	Jiangda, Tibet	3400	Y2097	Qianning, Sichuan	3620
Y2144	Jiangda, Tibet	3400	205218	Rangtang, Sichuan	3824
PI619530	Lasha, Tibet	4020	PI619519	Buerjin, Xinjiang	450
PI619531	Lasha, Tibet	3700	Y1608	Fuyun, Xinjiang	1120
PI619533	Naidong, Tibet	3470	Y1611	Fuyun, Xinjiang	1180
PI619589	Naidong, Tibet	3800	Y1558	Fuyun, Xinjiang	770
PI619532	Yangbajing, Tibet	4150	Y619575	Habahe, Xinjiang	1200
Y2203	Zuogong, Tibet	3800	Y0497	Hejing, Xinjiang	2670
Y2196	Zuogong, Tibet	3640	PI619576	Kashi, Xinjiang	2150
205221	Aba, Sichuan	3534	PI619578	Kashi, Xinjiang	2800
205229	Aba, Sichuan	3324	Y0672	Taxkorgan, Xinjiang	3480
Y2227	Batang, Sichuan	4150	Y0685	Taxkorgan, Xinjiang	2900
205120	Daocheng, Sichuan	3759	Y0605	Yecheng, Xinjiang	1820
205116	Daocheng, Sichuan	3938	Y0627	Yecheng, Xinjiang	3680
205089	Daocheng, Sichuan	4282	Y0639	Yecheng, Xinjiang	3150
205096	Daocheng, Sichuan	4287	Y0655	Yecheng, Xinjiang	2530
Y2120	Dege, Sichuan	4100	Y0618	Yecheng, Xinjiang	3670
Y2122	Dege, Sichuan	4800			