Genetic variation and geographical differentiation of *Elymus nutans* (Poaceae: Triticeae) from West China

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Introduction

Elymus nutans Griseb. is not only an important alpine forage grass, but also as a crucial gene pool for improving cereal crops. Understanding and maintaining the genetic diversity of the species are essential for both conservation strategy and breeding programs. However, little is known about its genetic and geographical differentiation patterns. *E. nutans* is a perennial, caespitose and allohexaploid (2*n*=6*x*=42) species that contains the St, H and Y genomes. 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 is distributed in the north, northwest and southwest China, particularly in the Qinghai-Tibet Plateau. *E. nutans* is a valuable forage grass in the alpine regions that is resistant to cold, drought and pests, which can be used to improve cereal crops. In addition, it can play an important role in the restoration of disturbed grasslands and the establishment of artificial grasslands, especially at altitudes from 3,000 to 4,500 m (Chen and Jia 2000). During recent decades, its distribution has contracted because of over-exploitation, habitat destruction and fragmentation. Therefore, it is urgent to understand and monitor the genetic and geographical differentiation of wild germplams of *E. nutans*.

Materials and Methods

Plant materials and DNA extraction

A total of 60 accessions of *E. nutans* originating from the areas of Qinghai, Gansu, Tibet, Sichuan and Xinjiang in western China was used in the study Fifteen individuals of each accession were sampled for DNA extraction, and genomic DNA was extracted with the plant genomic DNA kit DP 305 (Tiangen Biotech Co., Ltd, Beijing) according to the manufacturer's directions.

Molecular marker analysis

Four molecular marker systems (SRAP, RAPD, SSR and ISSR) were employed for evaluating the genetic diversity and geographical divergence following the procedures described by Zhou (2005).

Data analysis

The clear bands were scored manually for presence (1) or absence (0) to construct a raw data matrix. Nei and Li's (1979) genetic similarity (GS) coefficients were calculated and used to construct a dendrogram using UPGMA (unweighted pair group method with arithmetic average) cluster analysis performed with the NTSYS pc2.11x. Analysis of molecular variance (AMOVA) was conducted with the program WIN AMOVA 1.55 to study the partition of genetic variation (Excoffier *et al.* 1992). The UPGMA dendrograms were constructed based on the Nei's genetic similarity coefficient for different markers, which showed that clustering was related to geographical origins.

Results

All four molecular marker types were useful in detecting genetic variation in *E. nutans* (Table 1). A high level of genetic diversity was detected with the different markers. Cluster analysis showed some relation to geographical origin. Distinct geographical differentiation among accessions was observed in our study (Tables 2, 3). Using 22 SRAP primer combinations, 20 RAPD primers, 11 pairs of SSR primers and 16 ISSR primers, the rate of polymorphic bands were 85.4%, 91.8%, 99.1% and 91.4%, respectively, and the mean genetic similarity coefficients were 0.745, 0.733, 0.596 and 0.725, respectively.

Table 1. Performances of four molecular markers in the study.

Marker type	Total number of amplified bands	Number of polymorphic bands	Percentages of polymorphic bands	Mean of genetic similarity coefficients
SRAP	495	423	85.4%	0.745
RAPD	443	407	91.8%	0.733
SSR	225	223	99.1%	0.569
ISSR	349	319	91.4%	0.725

Table 2. Pairwise distances among the geographical groups.

Geographical groups	Qinghai	Gansu	Tibet	Sichuan	Northern Xinjiang	Southern Xinjiang
Qinghai	0.0000					
Gansu	0.1418	0.0000				
Tibet	0.1714	0.1569	0.0000			
Sichuan	0.2886	0.2749	0.1736	0.0000		
Northern Xinjiang	0.7527	0.7840	0.6657	0.6866	0.0000	
Southern Xinjiang	0.3686	0.4255	0.3785	0.3930	0.7139	0.0000

Table 3. Analysis of molecular variance (AMOVA) of geographical groups.

Source of variation	df	Sum of squares	Variance component	Percentage of variance	P values
Among regions	1	419.63	16.65	28.83%	<0.001
Within regions	58	2383.51	41.09	71.17%	<0.001
Among groups	5	1163.71	21.87	41.87%	<0.001
Within groups	54	1639.46	30.36	58.13%	<0.001

Conclusion

Substantial genetic diversity and distinct geographical differentiation among accessions were detected in our study. Our results also showed that altitude and landform played a critical role in this differentiation. The Qinghai-Tibet Plateau might be the diversity centre for *E. nutans* and should receive more attention for conservation of *E. nutans* germplasm.

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