Genetic Diversity of *Elymus sibiricus* L. in the Eastern Qinghai-Tibet Plateau of China Detected by SRAP Markers

Jiajun Yan
Sichuan Agricultural University, China

Shiqie Bai
Sichuan Academy of Grassland Science, China

Xiao Ma
Sichuan Agricultural University, China

Xinquan Zhang
Sichuan Agricultural University, China

Changbing Zhang
Sichuan Academy of Grassland Science, China

See next page for additional authors

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Jiajun Yan[1], *Shiqie Bai*[2*], *Xiao Ma*[1], *Xinquan Zhang*[1], *Changbing Zhang*[2], *Minghong You*[2]
([1] *Department of Grassland Science, Sichuan Agricultural University, Ya'an 625014, China*; [2] *Sichuan Grassland Science Academy, Chengdu 611731, China, E-mail: baiturf@yahoo.com.cn*)

Key words: *Elymus sibiricus* L, SRAP, genetic diversity, genetic structure, Qinghai-Tibet Plateau

Introduction Siberian wild ryegrass (*Elymus sibiricus* L.) is one of the most important and widely utilized forage in northeast, northwest and Qinghai-Tibet plateau in China, owing to its good palatability and excellent capability of adaptation to cold and drought conditions. In this study, sequence-related amplified polymorphism (SRAP) molecular markers were used to detect the genetic diversity of 8 natural populations of *E. sibiricus* collected from the eastern part of Qinghai-Tibet Plateau.

Materials and methods Eight natural populations with 20 individuals per population of E. sibiricus were included in the study. A modified version of the CTAB method was used to extract genomic DNA from approximately 0.5g of fresh leaf tissue. Sixteen different primer combinations were employed using eight forward primers and eleven reverse primers. Each 20μL PCR reaction mixture consisted of 600μM of dNTPs, 5mMof $MgCl_2$, 1μM of primer, 2μL of 10×PCR Buffer, 1 unit of Taq polymerase and 40ng of template DNA. PCR amplification was performed as follows: 5 min of denaturing at 94℃, 5 cycles of three steps: 1 min of denaturing at 94℃, 1 min of annealing at 35℃ and 1 min of elongation at 72℃, in the following 35 cycles the annealing temperature was increased to 50℃, with a final elongation step of 10 min at 72℃. PCR products were separated on 6% denaturing polyacrylamide gels at 450V for 90min. Each band was scored as presence (1) or absence (0) and data were analyzed with the POPGENE, AMOVA and NTSYS-pc, version 2.10.

Results The following results were obtained: (1) 384 loci were identified with sixteen primer pairs, out of which 334 loci were polymorphic. The percentage polymorphic loci (PPB%) was 86.98%, Nei's gene diversity (h) was 0.2434 and Shannon's information index (I) was 0.3732 at the species level; while at the level of population, the PPB% was 31.15%, h was 0.1092 and I was 0.1626. (2) AMOVA showed that a higher level of genetic variability (58.64%) resided among populations, whereas 41.36% resided within populations. The Gst values (0.5514) showed the similar result. (3) Nei's unbiased genetic distance matrix compared with a corresponding geographic distance matrix showed the two matrices were significantly correlated (r=0.66681, t=3.1851). Using unweighted pair group method with arithmetic average (UPGMA), 8 populations of E. sibiricus were clustered into three groups, and the populations from the near origin were clustered into one group.

Conclusions The average Nei's gene diversity (h) of monocotyledon was 0.190 (Nybom et al., 2000), and when the percentage polymorphic loci (PPB%) reached to 50%, then the species was thought have an abundant genetic diversity (Ma et al., 2000), so E. sibiricus showed high genetic diversity (h=0.2434, PPB%=86.96%) in this study. A high level of genetic differentiation among populations might be caused by the restricted gene flow (Nm=0.4068) which may result from several factors, such as breeding system of self-pollination, low seed dispersal and geographical isolation of populations.

Reference

Nybom, H., and Bartish, I. V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspect Plant Ecol Evol Syst* 3, 93-114.