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

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Key words : Triticeae ; *Elymus sibiricus* ; ISSRs ; Population structure ; Diversity

Introduction *Elymus sibiricus* L. (Siberian wildrye) is a perennial , self-pollinating and allotetraploid grass indigenous to Northern Asia , possessing the StH genome (Dewey , 1974) . In the subalpine meadows with less than 4000m altitude in Qinghai-Tibet Plateau , *E. sibiricus* usually serves as an important forage species . Climate warming , loss of habitat by deforestation and excessive grazing at high altitude pastures in the entire Qinghai-Tibet Plateau region now begin to threaten its survival . In the present study , we employed ISSR markers to investigate the genetic structure of natural *E. sibiricus* populations from southeast of Qinghai-Tibet Plateau .

Materials and methods Leaves of 93 individuals of *E. sibiricus* were collected from eight populations in southeast of Qinghai-Tibet Plateau , Sichuan Province , China . Individuals generally 5-10m apart from one another were sampled randomly within each population . Genomic DNA was extracted using the CTAB method . 100 primers (University of British Columbia primer set 9) were first screened for PCR amplification . Eighteen ISSR primers (UBC # 807 , 808 , 811 , 813 , 818 , 825 , 835 , 836 , 840 , 842 , 844 , 845 , 853 , 856 , 857 , 864 , 873 and 880) that generated clear , reproducible banding patterns were selected for further analysis . Polymerase chain reaction (PCR) and electrophoresis were carried out as described in Carvalho et al . (2005) . Unequivocally scorable and consistently reproducible amplified ISSR bands were scored as present (1) and absent (0) , each of which was treated as an independent character regardless of its intensity . The genetic structure of studied populations were calculated by POPGENE , Arlequin and TFGA software .

Results 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 to 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 was found between 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 reported for most of inbreeding Triticeae species reported .

Conclusions The high degree of genetic variation found in present study is probably accounted for the wide distribution of *E. sibiricus* . Owing to the fact that the eight population studied are closed located , the possible explanation for the higher intra-population variation patterns revealed in this study is that these studied populations were collected from near the central or founding population .

Reference

Carvalho , A . , Matos , M . , Lima-Brito , J . , Guedes-Pinto , H . & C . Benito (2005) . DNA fingerprint of F_1 interspecific hybrids from the Triticeae tribe using ISSRs . *Euphytica* , 143 , 93-99 .