Isolation and Characterization of Polymorphic Microsatellite Loci in *Achnatherum inebrians* and *A. sibiricum*

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Introduction *Achnatherum inebrians* (Hance) Keng and *A . sibiricum* (Linn .) Keng are intoxicating perennial bunchgrasses which mainly grow at grasslands in the North and Northwest of China , where they have caused grassland degradation and environmental deterioration . High infection rates of *Neotyphodium* endophytes have been detected from these two *Achnatherum* species (Li *et al* . 2004 , Wei *et al* ., 2006) ; if animals grazed the endophyte-infected grasses they look as if drunken . In this study , 23 polymorphic microsatellite loci were developed as potential tools to investigate the genetic diversity , genetic structure and gene flow of these species .

Materials and methods Microsatellite loci were isolated using a proprietary magnetic bead-based enrichment procedure that selects for genomic DNA restriction fragments containing [CG]n , [AC]n , and [AG]n repeat motifs , as described in detail in Jones et al . (2000) . Total genomic DNA was extracted from fresh leaves by a modified cetyltrimethyl ammonium bromide (CTAB) method . Primer pairs for amplification of the microsatellite regions were designed on the basis of the sequences flanking the obtained microsatellites using the Primer 5 .0 (Clarke and Gorley , 2001) . Preliminary population genetics analyses were performed using GENEPOP version 3 .4 (Raymond and Rousset ,1995) .

Results Twenty-three polymorphic microsatellite loci were isolated from the *A . inebrians* and *A . sibiricum* . Characterization of 24 individuals form four geographically distant populations (Gansu , Qinghai , Xinjiang and Inner Mongolia provinces) showed moderate to high polymorphism (Table 1) . All 23 loci conformed to Hardy-Weinberg equilibrium ($P > 0.05$) and there was no evidence of significant linkage disequilibrium between pairs of the 23 loci ($P > 0.05$) .

Table 1 *Characteristics of 24 polymorphic microsatellite loci for* A . inebrians *and* A . sibiricum .

Locus	No . alleles	Ho	He	GenBank Accession no .
A . inebrians	3 .0-13 .0	0 .24-0 .50	0 .41-0 .67	EU022691-EU022703
A . sibiricum	4 .0-13 .0	0 .16-0 .56	0 .31-0 .88	EU352687-EU352697

Ho : observed heterozygosity ; H_E , expected heterozygosity .

Conclusions These polymorphic markers increased the number of informative microsatellite markers available for *A . inebrians* and *A . sibiricum* . Moreover , they would provide fertile grounds for analyses of illustrating the phylogenetic relationship and understanding the genetic background of them . Beyond these immediate applications , most of the described loci should prove to be widely applicable to population genetic studies of other grass species .

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