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SHORT REPORT

Isolation and characterization of 15 polymorphic genomic simple sequence repeat markers in *Poa pratensis* L.

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Abstract

Poa pratensis L. is an important perennial grass used both for forage and turf in temperate regions of the world. In this study, we isolated and characterized 15 novel polymorphic genomic simple sequence repeat (SSR) markers in *P. pratensis*. The polymorphism of each marker was assessed in 14 cultivars. The number of alleles of individual microsatellites ranged from three to six, with an average of 3.8 alleles per SSR. Cross-species amplification tests showed that 13 and seven of the characterized *P. pratensis* markers could be effectively amplified in *P. pratensis* var. *anceps* Gaud and *P. crymophila* Keng, respectively. These polymorphic microsatellite markers should serve as powerful tools for the investigation of genetic diversity and related analyses, and also for molecular-assisted breeding of *P. pratensis*.

Introduction

Poa pratensis L., one of the most economically important perennial grass species, is widely used in temperate areas both for forage and turf. *P. pratensis* exhibits high variability in chromosome number, with frequent cases of polyploidy and aneuploidy arising via facultative apomixis (Grazi *et al.* 1961; Huff 2003). This complex polyploidy is responsible for genetically diverse and distinct genotypes within the species (Huff 2010). Polymorphic simple sequence repeat (SSR) markers are useful tools for investigating genetic diversity and assessing levels of apomixis in cultivars and experimental varieties. At present, 88 polymorphic SSR markers are available for *P. pratensis* (Honig *et al.* 2010). Herein, we report the development of 15 additional polymorphic genomic SSR markers for this species.

Materials and Methods

Microsatellite loci were isolated using the FIASCO (Fast Isolation by Amplified Fragment Length Polymorphism [AFLP] of Sequences Containing Repeats) protocol developed by Zane *et al.* (2002). Total genomic DNA was extracted from leaves of *P. pratensis* 'Qinghai.' Amplified DNA fragments with a size range of 200–1000 bp were enriched for repeats by magnetic bead selection using 5′-biotinylated $(AG)_{16}$, $(AC)_{16}$, $(AAG)_{16}$, $(AAC)_{16}$, $(ACT)_{16}$ and $(ATC)_{16}$ probes. A total of 495 clones with positive inserts were chosen arbitrarily for sequencing. Of these, 246 clones were successfully sequenced, and 163 clones were analyzed and found to contain repeat sequences. Out of the 163 sequences, 114 sequences were unique. A homology Basic Local Alignment Search Tool (BLAST) search against the National Center for Biotechnology Information (NCBI) GenBank database revealed one sequence to be identical to a published SSR sequence (Honig *et al.* 2010). Among the 113 novel sequences, 70 were suitable for primer design. In total, 70 SSR primer pairs were designed using Primer Premier 5.0 software (Premier Biosoft, Palo Alto, CA, USA). Polymorphism of the 70 markers was evaluated by polymerase chain reaction (PCR) amplification in 14 cultivars (Table S1), with the resulting PCR products separated on a non-denaturing 8% polyacrylamide gel.

Because of the heterozygous nature of *P. pratensis*, the individual SSR alleles in the 14 cultivars used in the current study were treated as dominant markers, with the

Table 1 Primer sequences and characteristics of 15 *Poa pratensis* L. simple sequence repeat markers obtained from the cultivar 'Qinghai' and tested in 14 other *P. pratensis* cultivars

Marker	Primers (5′–3′)	Repeat motif	N_a	Allele size range (bp)	Range of PIC values for N_a
poap1†‡	F:GTAAGCCGTCCCAAAGCG R:TAAAATCATGGTACTCTCCCTTCTC	$(CT)_7$	6	227–236	0.12–0.44
poap2†‡	F:TGGCTAGATGCCTGGATA R:GCTACATTGCCTGTAAAACT	$(GA)_{20}$	4	194–205	0.12–0.48
poap3	F:TCCCCTGGTTTGGATAAT R:GGACGTGGTGTAGGTGGT	$(CT)_{16}$	3	161–166	0.39–0.48
Poap4†	F:ATTGCTTCTTAGAATGGAGG R:CGGATAGGGAGATTTTGC	$(GA)_{14}$	3	213–226	0.32–0.50
poap5†	F:GCTTCTTAGAATGGAGGTC R:ATCAGAGGGAGATTTTGC	$(GA)_{22}$	4	224–229	0.12–0.50
poap6†	F:GAGTAAGGTGACGAATCCA R:TGAGTAACAGTGTCAAGGCT	$(CT)_{16}$	3	136–140	0.32–0.48
poap7†‡	F:GAGGAAAACTCTTCTCTCTGTC R:AGAAAGCAACAAGCACCT	$(TC)_8$	3	155–163	0.27–0.48
poap8†	F:TAAAGGAGCCAATGATAGC R:AGTCGTGTCAAGAGGTGC	$(CT)_{17}$	3	205–209	0.27–0.50
poap9†‡	F: ACATAGGCCCCTGCCACC R: CCACCCCTTCTCCAACCC	$(TC)_{30}$	5	154–165	0.12–0.39
poap10†‡	F:TCGCTTGAGTTGCTGTAC R:AAGGTTTCCAAATAGGCT	$(GT)_6$	5	189–201	0.12–0.50
poap11†‡	F:CAAAACGCACAAAACCGAC R:CCCAGCCCGACATCTCAA	$(AAG)_{17}$	5	217–229	0.12–0.44
poap12†	F:CGGATGACTGCGAATGAA R:TGCACCAGGGAGAAACCT	$(TC)_{17}$	3	91–102	0.23–0.48
poap13†‡	F:GAGATTTGCTACATTGCC R:CACCAGGAAGAAACCTATTA	$(CT)_7T(TC)_4$	4	133–142	0.23–0.50
poap14†	F:AGAGTCCAAGGAATCAAATG R:CCTGGTCTTAGAACGTGTG	$(CT)_{21}$	3	167–178	0.32–0.50
poap15	F:TAGAAAACAGTTTGCTGACA R:GTAATGATTTGCGGCTC	$(GA)_5GG(GA)_9$	3	143–148	0.12–0.44

†Primers transferable to *P. pratensis* var. *anceps*. ‡Primers transferable to *P. crymophila*. N_a, number of alleles; PIC, polymorphism information content.

banding phenotypes scored as band absence (0) or presence (1). The polymorphism information content (PIC) of each individual SSR allele was calculated according to the formula described by Tehrani *et al.* (2008): PIC = 2PiQi, where Pi is the frequency of presence and Qi is the frequency of absence.

Result and Discussion

Fifteen of the 70 primer pairs displayed polymorphic banding patterns (Figure S1). Among the 15 SSR markers, 12 were dinucleotide repeats, one was a trinucleotide and two were complex motifs (Table S2). Product sizes ranged from 91 to 236 bp. The number of detected alleles varied from 3 to 6, with an average of 3.8. PIC values of the 15 SSR markers ranged from 0.39 to 0.50, with those of 10 markers approaching the maximum PIC value (0.50) (Table 1).

The high rate of transferability of *Poa* SSR markers across *Poa* spp. were well documented (Kindiger *et al.* 2011, 2013). In this report, cross-species amplification tests were performed on *P. pratensis* var. *anceps* Gaud and *P. crymophila* Keng, two closely related taxa that are important forage and ecological recovery grasses on the Qinghai-Tibetan Plateau of China (Figures S1 and S2). Thirteen of the *P. pratensis* genomic SSR markers could be effectively amplified in *P. pratensis* var. *anceps*, whereas seven were amplifiable in *P. crymophila* (Table 1).

The 15 novel polymorphic SSR markers should be useful tools for investigation of genetic diversity, construction of genetic linkage maps, assessment of levels of apomixis in cultivars and experimental varieties, and even for analysis of plant variety protection in *P. pratensis*. In addition, the developed *P. pratensis* SSR markers can be applied to *P. pratensis* var. *anceps* and *P. crymophila*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Cultivars of *Poa pratensis* used for polymorphism evaluation of the simple sequence repeat (SSR) markers.

Table S2. Simple sequence repeat (SSR) sequences.

Figure S1. Representatives of the simple sequence repeat (SSR) markers showing polymorphic across different *Poa* cultivars.

Figure S2. Representative of the polymorphisms detected with the developed simple sequence repeat (SSR) markers in *Poa pratensis* 'Qinghai' and *P. pratensis* var. *anceps* 'Qinghai.'