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Microsatellite markers for the native Texas perennial grass, *Panicum hallii* (Poaceae)[1]

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- *Premise of the study:* We developed microsatellites for *Panicum hallii* for studies of gene flow, population structure, breeding experiments, and genetic mapping.
- *Methods and Results:* Next-generation (454) genomic sequence data were used to design markers. Eighteen robust markers were discovered, 15 of which were polymorphic across six accessions of *P. hallii* var. *hallii*. Fourteen of the markers cross-amplified in a *P. capillare* accession. For the 15 polymorphic markers, the total number of alleles per locus ranged from two to 26 (mean: 11.0) across six populations (11–19 individuals per population). Observed heterozygosity (mean: 0.031) was 13.7 times lower than the expected heterozygosity (mean: 0.426).
- *Conclusions:* The deficit of heterozygous individuals is consistent with *P. hallii* having a high rate of self-fertilization. These markers will be useful for studies in *P. hallii* and related species.

Key words: microsatellites; *Panicum hallii*; Poaceae; switchgrass.

Panicum hallii Vasey (Hall's panicum) is a perennial and member of the Poaceae family (Waller, 1976). *Panicum hallii* occurs naturally as two varieties: *P. hallii* var. *hallii*, which is typically associated with xeric calcareous soils and *P. hallii* var. *filipes* (Scribn.) F. R. Waller, which most commonly occurs in mesic conditions (Waller, 1976). Interest in *P. hallii* has recently increased due to its close evolutionary relationship to the biofuel crop switchgrass, *P. virgatum* L., which only occurs as a polyploid. As a diploid ($2n = 2x = 18$), *P. hallii* is an emerging model system because of its short generation time (seed to seed in ~8 wk), small physical size, relatively small genome size (~550 Mb), and production of thousands of seeds per plant (Anderson et al., 2011).

We present here 18 new microsatellite markers, developed by taking advantage of our initial *P. hallii* genome sequencing efforts. These markers will be broadly useful for population genetic analysis, screening of potential hybrids, and genetic mapping.

METHODS AND RESULTS

Genomic DNA from *P. hallii* var. *filipes* (FIL; Appendix 1) was sequenced on a 454-Titanium platform (Roche, Pleasanton, California, USA) using standard Roche protocols at the University of Texas (National Center for Biotechnology Information [NCBI] Sequence Read Archive accessions SRX019673, SRX019674, and SRX019675). We searched for reads containing repeats ≥ 40 bp in length, with ≤ 20% deviation from perfect repeat structure, and flanked by nonrepetitive regions ≥ 20 bp in length using RepeatMasker (http://www.repeatmasker.org). The 2261 repetitive sequences identified were assembled using CAP3 (Huang and Madan, 1999) to combine redundant sequences from the same genomic locus. The 1034 assembled sequences included 208 with nearly perfect repeats, representing 38 unique repeat types. We attempted to design primers flanking each target using Primer3 (Rozen and Skaletsky, 2000), producing usable primers for 124 targets that fit our criteria for GC content and target size.

We initially screened 25 primer pairs for amplification using DNA extracted from one *P. hallii* var. *filipes* (FIL) and one *P. hallii* var. *hallii* accession (HAL). We subsequently expanded the samples to include five more *P. hallii* var. *hallii* accessions (BFL, DRB, MCR, PAJ, PIS) to test an additional 55 primer pairs. DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) protocol (Kelly and Willis, 1998). PCR amplification was conducted on a MJ Research DNA Engine thermocycler (MJ Research, Waltham, Massachusetts, USA) in 10 μL reaction volumes that contained 1× buffer, 0.8 μM $MgCl_2$, 0.08 μM dNTPs, 0.1 μM forward and reverse primers, 10 ng genomic DNA, and 0.4 U GoTaq Flexi polymerase (Promega, Madison, Wisconsin, USA). A touchdown amplification was carried out with an initial denaturation of 95°C for 3 min, 20 cycles at 95°C for 45 s, 54°C for 30 s, and 72°C for 1 min, where the annealing temperature was decreased by 0.1°C every cycle. This was followed by 21 additional cycles of 95°C for 45 s, 52°C for 30 s, and 72°C for 1 min. Amplification of microsatellites was assessed on 1% agarose gels stained with SYBR Safe (Life Technologies, Carlsbad, California, USA).

For the markers that successfully amplified the test set of DNAs, we screened for polymorphism with forward primers that were modified with an M13 universal tag TGTAAAACGACGGCCAGT, which annealed with a 6-FAM fluorescently labeled primer during the PCR. Products of PCRs were run on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA) with a 500 ROX size standard. The sizes of PCR amplified fragments were scored using GeneMarker (SoftGenetics, State College, Pennsylvania, USA). Of the 33 M13-labeled microsatellite markers, 18 cleanly and robustly amplified fragments. Fifteen of those markers were polymorphic across *P. hallii* var. *hallii*, while the remaining three were monomorphic (Table 1). The forward primers of the 15 polymorphic markers were modified with 6-FAM and HEX labels for population genetic analysis. A total of 11–19 individuals per population were genotyped across six populations (Appendix 1) using the same PCR conditions and genotyping methods as above. The population genetic summary statistics (Table 2) expected heterozygosity (H_e), observed heterozygosity (H_o), and number of alleles (N_a) were calculated using GenAlEx 6.41 (Peakall and Smouse, 2006). Deviations from Hardy–Weinberg equilibrium (HWE) were assessed with GENEPOP (Raymond and Rousset, 1995). Finally, we assessed cross-amplification of the markers in a single accession (BRF) of a

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Table 1. Characterization of 18 microsatellite markers developed in *Panicum hallii*.

Primers[a]	Primer sequences (5′–3′)	Repeat motif	Size range (bp) var. *hallii*	Size (bp) var. *filipes*[b]	Amplification in *P. capillare*	Label[c]	GenBank accession no.
PhMicro8	F: ACCGTACATCTGTTAGCGGG R: CATTTAGCTCCTCTTTGGCG	$(TTC)_{16}$	315–366	348	No	HEX	JN627839
PhMicro18	F: CTCGCGTGATTATCCCAAGT R:CCACAACACATGCATGAACA	$(TCTA)_{14}$	254–290	304	Yes	HEX	JN627840
PhMicro37	F: TATCTGACTTGCTCTCCCGC R: CGATCGACGTGAATGACAAC	$(CA)_{17}$	208–273	258	Yes	6-FAM	JN627841
PhMicro39	F: TCCTCCTTGTGGATGAAACC R: CGACCACATCGACTCTGCTA	$(TTG)_{17}$	386	386	Yes	6-FAM (M13)	JQ004275
PhMicro46	F: AGCCTAACAGCCCAATCGTA R: GCAAGGGCCTTTCTCAATTA	$(TATATG)_{6}$	261–308	258	Yes	6-FAM	JN627842
PhMicro51	F: GGGCTCAAGACTGAAAGCAC R: GCGAGCCAAGAAAATGAAAG	$(CTCA)_{10}$	192–215	199	Yes	6-FAM	JN627843
PhMicro54	F: TACAAGCAGGCAATTTGCAG R: CTCAGCCATTCCTCCTTCAG	$(TTC)_{6}$	282–364	286	No	HEX	JN627844
PhMicro56	F: TAAAAGGTTGCCATCGGAAG R: CAACATCTGAATCCCAAGCC	$(TTTC)_{7}$	423–445	454	Yes	HEX	JN627845
PhMicro59	F: TGTGTCATCTGTGCAAGCAA R: AGGTAATCCGGCCTGCTACT	$(GGAA)_{8}$	303–314	322	Yes	6-FAM	JN627846
PhMicro60	F: TGCTTGTGAATTGGTTCCAG R: TTCAGGCTGCTTGTGTTGAC	$(GGGAGA)_{5}$	195–218	199	Yes	HEX	JN627847
PhMicro62	F: TGTGCTGGAGAGGAACAACA R: CGCCTCATATCCACATCCTT	$(TTA)_{16}$	311	318	Yes	6-FAM (M13)	JQ004276
PhMicro73	F: TGCTCACGTGTATGCTCTCC R: GACTGTCACACATCCACAACTAAG	$(TTC)_{16}$	338–432	355	No	6-FAM	JN627848
PhMicro77	F: GAAGAACCAGCATCAAGGGA R: TAACAGCCATGGTTAAGGGC	$(CATA)_{10}$	338–360	344	Yes	6-FAM	JN627849
PhMicro78	F: AGCAAGTACGGTTACTGCGG R: CAACTTACGTCCGGTCTCGT	$(CGG)_{8}$	342	342	Yes	6-FAM (M13)	JQ004277
PhMicro84	F: CACTTCCTGCCAACCAAGTT R: CGGCGGCTATGCATATTAGT	$(CA)_{12}$	273–295	315	Yes	HEX	JN627850
PhMicro86	F: GTGTTGGCGAAGGAGTAGGA R: TTCCACTCCAGTCATCCCTC	$(GGA)_{10}$	298–316	307	Yes	HEX	JN627851
PhMicro87	F: GTGGACATCAAGGCGAAGAT R: AAACAGGCGCTATGTGAATG	$(CATA)_{8}$	287–295	295	No	HEX	JN627852
PhMicro88	F: GGGAGCATCTGGAGATTTTG R: GCAAATTAGCTCGCATAGCC	$(CGG)_{3}$	303–306	304	Yes	6-FAM	JN627853

[a] A touchdown PCR with an annealing temperature gradient from 54°C to 52°C was used to amplify all markers.
[b] Allele size for a single accession of *P. hallii* var. *filipes.*
[c] Fluorescent label on the forward primer.

Table 2. Population genetic summary statistics for the 15 polymorphic markers in *Panicum hallii* var. *hallii.*[a]

Marker	BFL (N = 11)			DRB (N = 16)			MCR (N = 19)			PAJ (N = 14)			STW (N = 24)			PIS (N = 11)		
	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e
PhMicro8	2	0.000	0.408	10	0.214	0.847*	4	0.056	0.548*	6	0.000	0.663*	3	0.043	0.538*	4	0.000	0.656*
PhMicro18	6	0.091	0.772*	5	0.000	0.562*	1	0.000	0.000	4	0.154	0.737*	2	0.000	0.083	5	0.000	0.711*
PhMicro37	2	0.000	0.397*	4	0.000	0.704*	4	0.111	0.619*	4	0.167	0.642*	4	0.087	0.643*	4	0.100	0.475*
PhMicro46	5	0.000	0.716*	10	0.143	0.888*	5	0.000	0.401*	5	0.083	0.601*	5	0.000	0.712*	8	0.091	0.831*
PhMicro51	3	0.000	0.570*	3	0.071	0.482*	2	0.000	0.484*	3	0.000	0.620*	3	0.000	0.517*	2	0.000	0.165
PhMicro54	4	0.000	0.667*	4	0.071	0.487*	4	0.222	0.597*	6	0.143	0.587*	5	0.000	0.667*	3	0.000	0.430*
PhMicro56	3	0.000	0.340	4	0.000	0.640*	3	0.000	0.547*	1	0.000	0.000	2	0.000	0.375*	3	0.000	0.314*
PhMicro59	2	0.000	0.397*	2	0.000	0.486*	2	0.000	0.117	2	0.000	0.245	3	0.000	0.512*	2	0.000	0.165
PhMicro60	2	0.000	0.397*	2	0.071	0.497*	2	0.000	0.100	4	0.077	0.636*	3	0.043	0.651*	3	0.000	0.562*
PhMicro73	4	0.125	0.414	5	0.071	0.620*	2	0.067	0.420*	2	0.091	0.235	4	0.050	0.471*	4	0.000	0.667*
PhMicro77	2	0.000	0.397*	4	0.000	0.435*	4	0.000	0.562*	1	0.000	0.000	2	0.000	0.483*	1	0.000	0.000
PhMicro84	1	0.000	0.000	2	0.000	0.142	4	0.000	0.593*	3	0.000	0.292*	5	0.000	0.752*	2	0.000	0.165
PhMicro86	3	0.000	0.340	5	0.000	0.469*	3	0.063	0.635*	2	0.083	0.080	1	0.000	0.000	3	0.000	0.340
PhMicro87	1	0.000	0.000	2	0.000	0.500*	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	2	0.000	0.198
PhMicro88	2	0.091	0.434	3	0.063	0.654*	1	0.000	0.000	2	0.000	0.500*	1	0.000	0.000	2	0.000	0.165

Note: H_e = expected heterozygosity; H_o = observed heterozygosity; N = sample size; N_a = number of alleles.
* Significant Bonferroni-corrected ($P < 0.0033$) departures from Hardy–Weinberg equilibrium.
[a] See Appendix 1 for population abbreviations and location information.

related species, *P. capillare* L., using the same PCR and gel electrophoresis protocols as above.

Across six populations of *P. hallii* var. *hallii*, the mean overall H_e was 0.426 (range: 0.375–0.561), H_o was 0.031 (range: 0.013–0.053) due to a deficit of heterozygous individuals, and the total number of alleles per locus ranged from two to 26 (mean: 11.0). Within populations, the number of alleles per locus ranged from one to 10 (mean: 3.2), per locus H_o ranged from 0.000 to 0.222, and per locus H_e ranged from 0.000 to 0.878. Cross-amplification was successful for 14 out of 18 of the markers in *P. capillare.*

CONCLUSIONS

We have developed and validated 15 novel microsatellite primer pairs for a native Texas grass and bioenergy model system, *P. hallii*. The lack of heterozygous individuals suggests that *P. hallii* has a very high rate of self-fertilization. The markers presented here will be useful for future studies of population structure, genetic variation, and genetic mapping in *P. hallii* as well as potentially in *P. capillare*.

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APPENDIX 1. Information for *Panicum* vouchers used in this study. Vouchers are deposited in The University of Texas at Austin Herbarium (TEX).

Population	State, County	Collection no.	Latitude	Longitude
P. hallii var. *hallii*				
BFL	Texas, Travis	DL0100	30°17′09″N	97°46′53″W
DRB	Texas, Val Verde	DL0102	29°56′28″N	100°58′14″W
HAL	Texas, Travis	DL0104	30°11′06″N	97°52′26″W
MCR	Texas, Jeff Davis	DL0101	30°40′58″N	104°08′05″W
PAJ	Texas, Kimble	DL0103	30°29′07″N	99°44′15″W
STW	Texas, Palo Pinto	DL0105	32°36′33″N	98°29′22″W
PIS	Texas, Cameron	DL0106	25°59′15″N	97°19′36″W
P. hallii var. *filipes*				
FIL	Texas, Nueces	DL0107	27°39′15″N	97°24′19″W
P. capillare				
BRF	Texas, Travis	DL0108	30°17′04″N	97°46′51″W