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Isolation and Characterization of 88 Polymorphic Microsatellite Markers in Kentucky Bluegrass (*Poa pratensis* L.)

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Abstract. **Kentucky bluegrass (*Poa pratensis* L.) is an important facultative apomictic temperate perennial grass species used for both forage and cultivated turf. Through apomixis, this species is able to propagate diverse and odd ploidy levels, resulting in many genetically distinct phenotypes. A wide range of diverse cultivars and accessions of kentucky bluegrass have been previously characterized based on common turf performance or morphological characteristics as well as by random amplified polymorphic DNA (RAPD) markers. Although previous characterization efforts have provided valuable information, the use of both morphological characteristics and RAPD markers for genetic diversity analysis has limitations. In the current report, we developed and characterized 88 novel microsatellite markers for kentucky bluegrass. Polymorphism for each marker was assessed in 265 kentucky bluegrass cultivars, experimental selections, collections, and hybrids. The number of alleles for individual microsatellites ranged from four to 81 with an average of 38.3 alleles per simple sequence repeat. These polymorphic microsatellite markers would be useful tools for investigating genetic diversity, creation of genetic linkage maps, assessment of levels of apomixis in cultivars and experimental varieties, and identification of aberrant progeny in apomictic kentucky bluegrass breeding programs.**

The bluegrasses, also commonly referred to as meadowgrasses, are one the most economically important genera of the *Poaceae* (Huff, 2010; Soreng and Barrie, 1999). Kentucky bluegrass (*Poa pratensis* L.) is the botanical-type species for the genus *Poa* (Soreng and Barrie, 1999) and is recognized as one of the most widely used temperate perennial grass species for both forage and amenity turf in the northern United States and Canada (Huff, 2003, 2010). Kentucky bluegrass is a facultative apomictic species, which has a highly variable chromosome number, creating a series of polyploidy and aneuploidy ranging from 2n = 28 to 154 (Akerberg, 1939; Grazi et al., 1961; Huff, 2003; Love and Love, 1975; Meyer and Funk, 1989; Muntzing, 1933; Nielsen, 1946; Tinney, 1940). Although this complex polyploidy may sometimes present a challenge to breeding efforts in this species, the ability of kentucky bluegrass to propagate diverse and odd ploidy levels through apomixis results in many genetically distinct phenotypes within the species (Huff, 2010). This wide range of diversity of cultivars and accessions of kentucky bluegrass has been previously characterized based on common turf performance or morphological characteristics (Bara et al., 1993; Bonos et al., 2000; Murphy et al., 1997; Shortell et al., 2009) as well as by random amplified polymorphic DNA markers (Curley and Jung, 2004; Huff, 2001; Johnson et al., 2002).

Although currently used in many plant variety protection (PVP) schemes, the use of morphological characteristics to determine genetic diversity and distinctness, uniformity, and stability (DUS) of cultivars has numerous drawbacks, including the maintenance of increasingly large reference collections for comparative analyses, a limited number of descriptors available to distinguish varieties, time-consuming field-based measurement of large numbers of samples and replicates, and the potential for the expression of morphological traits to be influenced by the environment (Giancola et al., 2002; Ibanez et al., 2009; Kwon et al., 2005; Lombard et al., 2000; Roldan-Ruiz et al., 2001). As a result of these drawbacks of using morphological characters for determining genetic diversity or for DUS testing, numerous researchers have proposed using molecular markers for PVP (Bonow et al., 2009; Borchert et al., 2008; Cooke and Reeves, 2003; Giancola et al., 2002; Gunjaca et al., 2008; Heckenberger et al., 2002, 2003, 2005a; Ibanez et al., 2009; Kwon et al., 2005; Roldan-Ruiz et al., 2001; Smith et al., 2009; Smykal et al., 2008; Tommasini et al., 2003; van Eeuwijk and Law, 2004; Vosman et al., 2004). In contrast to morphological characters, molecular markers offer a number of advantages, including a nearly unlimited number of characters, high degree of polymorphism, ease of scoring, and they are unaffected by the environment (Lombard et al., 2001; Smykal et al., 2008; Tommasini et al., 2003). In this report, we describe the development of the first polymorphic microsatellite markers for ongoing molecular genetic research in kentucky bluegrass.

Total genomic DNA was extracted from a single plant of the kentucky bluegrass cultivar Cabernet (Bonos et al., 2004) using the Sigma GenElute Plant Genomic DNA Miniprep Kit (St. Louis, MO) according to the manufacturer's instructions. DNA was sent to Genetic Identification Services Inc. (GIS, Chatsworth, CA) for construction of simple sequence repeat (SSR) libraries enriched for CA, GA, AAT, and CAG SSRs. Methods for DNA library construction and enrichment were developed following Jones et al. (2002). Briefly, genomic DNA was partially digested with a cocktail of seven blunt-end restriction enzymes (*Rsa*I, *Hae*III, *Bsr*B1, *Pvu*II, *Stu*I, *Sca*I, *Eco*RV). Fragments in the size range of 300 to 750 bp were adapted with 20 bp oligonucleotides, which contained a *Hin*dIII site at the 5′ end and subjected to magnetic bead capture (CPG, Inc., Lincoln Park, NJ) using 5′-biotinylated $CA_{(15)}$, $GA_{(15)}$, $AAT_{(12)}$, and $CAG_{(12)}$ as capture molecules according to the manufacturer's instructions. Captured molecules were amplified using a primer complimentary to the adaptor, digested with *Hin*dIII to remove the adaptor sequences, and ligated into the *Hin*dIII site of pUC19. Recombinant plasmids were then electroporated into *Escherichia coli* DH5α. GIS delivered DNA libraries 50% enriched for CA and GA repeats and libraries 15% enriched for the trinucleotides AAT and CAG.

Several 50-μL aliquots of each SSR library were plated out onto LB agar plates containing ampicillin, IPTG, and Bluo-Gal. The plates were incubated at 37 °C overnight. Three thousand individual colonies were chosen from the CA- and GA-enriched libraries and grown in 6 mL LB broth containing ampicillin. Five hundred colonies were chosen from the AAT- and CAG-enriched libraries. DNA was isolated from the cultures using the Qiagen QIAprep Spin Miniprep kit (Valencia, CA). Samples were sequenced using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). A total of 2523 clones were sequenced. Sequence data from clones in the AAT and CAG libraries proved to be complex (long sequences of highly repetitive DNA), and the percentage appropriate for primer design was less than 1%. As a result, only the dinucleotide libraries were used for further analyses. Six hundred seventy-four dinucleotide clones contained no repeat, whereas 546 clones had other problems that precluded primer design (poor sequence, repeat too close to cloning site, etc.). One thousand seventy-one clones contained either CA or GA repeat motifs suitable for primer design.

Sequence data from the clones containing dinucleotide SSRs were analyzed for primer

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Table 1. Primer sequences and characteristics of 88 *Poa pratensis* (L.) microsatellite markers obtained from the cultivar Cabernet tested on 265 *Poa pratensis* cultivars, experimental selections, collections, and hybrids.

Marker ID	GenBank accession no.	Primers (5′-3′)	Repeat motif	N_a	Original cloned allele (bp)	Allele size range (bp)[z]	Range of PIC values for N_a alleles
NJPpGA1	HM136687	F: AAGGCTCGGTTGAGTTCAG R: TTTGGAAGAGGAGGCAGAG	$(CT)_{13}CG(CT)_{10}$	40	252	199–305	0.01–0.48
NJPpGA6	HM136688	F: GGTTGGTCTCGTCTAAGATGG R:GTGACCCTTCATGGCGTATAT	$(GA)_8A(GA)_7$	22	279	181–314	0.01–0.45
NJPpGA9	HM136689	F: GCCGTAAATAGTGGAGAAGAC R: AAAATCCTGACTGTTGGAGAC	$(CT)_{21}$	40	208	142–275	0.01–0.50
NJPpGA107	HM136690	F: TTGGGCTACAGTATTTTATTCC R: AAGTGGAGTCTGTTCATTGATC	$(GA)_{22}$	39	238	187–268	0.01–0.50
NJPpGA108	HM136691	F: GAGTGGAGATCACAACACATG R: CCTTTTCTTTAGGGGACAAG	$(GA)_{20}$	81	213	161–292	0.01–0.44
NJPpGA111	HM136692	F: TGCACGCATGTCTTATGTACC R: GGAGGAGAAGTTGGAGGACTC	$(GA)_{19}$	32	229	179–268	0.01–0.43
NJPpGA115	HM136693	F: CCACCCAGACATTTGACTG R: CTTCGCACTATGACAAGCC	$(GA)_{11}$	24	277	281–328	0.01–0.50
NJPpGA124	HM136694	F: GCCTCTTCTTCCAAGGATAC R: ATGGTTCTGGTGGTGTTTC	$(CT)_9$	33	114	117–173	0.01–0.50
NJPpGA125	HM136695	F: GCAGAACAAAAAGTCATACTGG R: AACCCTATTTAGCCCTCACTC	$(GA)_8GG(GA)_{18}$	64	231	158–439	0.01–0.50
NJPpGA128	HM136696	F: GAAGCCGACGAGGTAGTC R: AGATGGCGAGACGAAGTC	$(CT)_{17}$	30	156	131–199	0.01–0.50
NJPpGA129	HM136697	F: CCAGCACATCTACGAGCAC R: TTCGGAAGAACTTGATTTGG	$(CT)_{13}$	27	296	272–322	0.01–0.48
NJPpGA132	HM136698	F: TTTGCTCCACTTCTCATGC R: AGAGTTGCGGAAGGATGTC	$(CT)_7TT(CT)_2$	4	300	311–321	0.01–0.50
NJPpGA134	HM136699	F: ACACCCCCTTTGTAGATTCG R: CCTTTGCTTGCTTTCCATC	$(CT)_3GT(CT)_{22}$	15	150	129–172	0.02–0.48
NJPpGA274	HM136700	F: GAGCACCAAAACTCGACACT R: CAAACTACGACAACCGGAGT	$(CT)_{19}$	35	298	191–443	0.01–0.50
NJPpGA329	HM136701	F: TGCTTATTTCATTGGAAGCA R: CGAATTGGATAACCTGACATC	$(CT)_{23}$	35	300	292–382	0.01–0.48
NJPpGA379	HM136702	F: AAATTTGTGACCATGTCAACC R: GGCGAAATGGCTTAGAACTAC	$(GA)_{18}$	42	295	286–370	0.01–0.50
NJPpGA393	HM136703	F: CATGAGAGACACGAACAGGA R: AAGTGCATTACCTTTGTCCA	$(CT)_{10}$	43	248	239–335	0.01–0.50
NJPpGA403	HM136704	F: GTCATTTTGTCCCGTGAGTC R: TGCATCCTCTAGGTCTTCTGA	$(GA)_{15}$	18	262	231–297	0.01–0.49
NJPpGA405	HM136705	F: GACGAATTGGATAACCTGACA R: TGCTTATTTCATTGGAAGCA	$(GA)_{21}$	38	300	293–379	0.01–0.48
NJPpGA412	HM136706	F: GCACCGTGGACAAAGTTATT R: AGGGAAGGATGACATCAACA	$(CT)_{17}$	64	260	244–335	0.01–0.41
NJPpGA422	HM136707	F: TGCTGTCCTCCAACATCAAT R: TCCAAGTTCCGACGACTCTA	$(CT)_{24}$	43	296	267–419	0.01–0.50
NJPpGA424	HM136708	F: GGTCAAAAGTTAAGACGATTGG R: TGATTTGCTATTATGCATCTTCA	$(CA)_3(GA)_{14}$	41	284	283–336	0.01–0.48
NJPpGA426	HM136709	F: GGTGACATCCTCCTTGCTAA R: TACATGGTTCTCCCCCTTG	$(GA)_3GT(GA)_{28}$	32	294	242–313	0.01–0.50
NJPpGA434	HM136710	F: GAAGATAACTGACCAATTCAAGAGA R: CGGTTACATGCGATGTTTTT	$(CT)_4CG(CT)_2$ CCC$(CT)_{31}$	41	399	351–425	0.01–0.50
NJPpGA446	HM136711	F: TGGCCTACATAATGGTCAAAA R: AAAATTATGGATACCAGCCTACC	$(CT)_{25}$	79	285	153–409	0.01–0.50
NJPpGA448	HM136712	F: ATCGTCACGGGGAGAATC R: AACTCCTGTCGCTGCGTA	$(CT)_{37}$	36	297	187–317	0.01–0.50
NJPpGA450	HM136713	F: CTCCGTGAGTAGACCGTGTT R: AAACGTGATGCTTCGACAAT	$(CT)_{15}TT(CT)_7$	66	281	216–340	0.01–0.49
NJPpGA470	HM136714	F: TTGAAAGCCCACCACAATTA R: GTGTTGCTACGGGCTCTTTA	$(GA)_{15}$	48	290	269–494	0.01–0.50
NJPpGA480	HM136715	F: ACGGGGATCCTCTAGAGTCG R: TGATGCAGTTGTCGATCTGT	$(CT)_{24}$	52	187	161–242	0.01–0.50
NJPpGA748	HM136716	F: GCTTCTATGTGATCGAGGAAA R: GCTTATTTGTCTACTACAATGTTGC	$(GA)_5GC(GA)_{24}$	37	299	264–339	0.01–0.50
NJPpGA749	HM136717	F: CTGCGAGAGTAGCGAACAAT R: AGGGAATCGATCTTGCCTAT	$(GA)_2TA(GA)_{17}$	35	279	253–337	0.01–0.49
NJPpGA753	HM136718	F: TCACTGAACTGTGACAAGAATTT R: GAATATTTGGATGGCAATGG	$(CT)_{17}$	49	274	255–357	0.01–0.50
NJPpGA771	HM136719	F: TTCAGAGCATACAGAGTAAACA R: CTAATTTGGTGGATCTTGCT	$(GA)_{13}$	36	250	250–317	0.01–0.50
NJPpGA783	HM136720	F: TGCCCAAGGAAAAATTATGA R: GGGCTCACTGAACTCCAAT	$(GA)_{17}AA(GA)_{25}$	77	365	267–398	0.01–0.48
NJPpGA799	HM136721	F: TTGTTGAACTGCTTCTGAAATG R: GAATATTTGGATGGCAATGG	$(CT)_{14}$	29	300	231–335	0.01–0.35
NJPpGA806	HM136722	F: TCCCTCAAGTGACCTACGAA R: TGTAACCACGAGTTAAGTGCAT	$(CT)_{23}$	38	298	249–352	0.01–0.49

(*Continued on next page*)

Table 1. (*Continued*)

Marker ID	GenBank accession no.	Primers (5′-3′)	Repeat motif	N_a	Original cloned allele (bp)	Allele size range (bp)[z]	Range of PIC values for N_a alleles
NJPpGA815	HM136723	F: CACTAAAAGCCAAACCACGA R: AAATGGTAGCAGGAGATGGA	$(GA)_{13}AA(GA)_5$	41	286	179–365	0.01–0.50
NJPpGA832	HM136724	F: TTCAGAGCATACAGAGTAAACA R: CTAATTTGGTGGATCTTGCT	$(GA)_{13}$	45	250	236–338	0.01–0.50
NJPpGA836	HM136725	F: AACATAAAGCCAAACCACGA R: AAATGGTAGCAGGAGATGGA	$(GA)_{19}$	50	286	257–365	0.01–0.50
NJPpGA892	HM136726	F: TGTCCCGTGAGTCTACTTTT R: GCATCCTCTAGGTCTTCTGA	$(GA)_{13}$	20	250	224–291	0.01–0.49
NJPpGA897	HM136727	F: ACTCCAATGACCAAAGTGAG R: CCTCTGGGCATCTTGTTG	$(GA)_{30}GG(GA)_3$	40	300	267–362	0.01–0.50
NJPpGA900	HM136728	F: ATTCCGATTTGGACACGTTA R: GCTAGGAGGTCTTAGATGTAGGG	$(CT)_{22}$	42	299	152–374	0.01–0.49
NJPpGA914	HM136729	F: CCCCAAATCCCTACTCAAAT R: GATATGGACAACCACCATGC	$(GA)_{19}$	60	278	274–353	0.01–0.50
NJPpGA931	HM136730	F: CTTCGTTTGGAGAGGTTGTG R: TCCAACGGCTACCTATTCAG	$(GA)_{25}$	23	299	289–342	0.01–0.50
NJPpGA937	HM136731	F: TACGTCTTCGTTTGGAGAGG R: TCCAACGGCTACCTATTCAG	$(GA)_{22}$	20	298	293–337	0.01–0.50
NJPpGA947	HM136732	F: TGATCAGTTGCTTTGTGCAT R: GGTACGGCAGAGAGACAGAG	$(CT)_4GT(CT)_3$	22	245	183–290	0.01–0.49
NJPpGA954	HM136733	F: ATCTTCAGATCCTGGGTAGC R: TTCCTTGTGTGCAAGAGTTG	$(GT)_7A(GA)_{16}$	12	289	286–419	0.01–0.36
NJPpGA957	HM136734	F: TCCACGGTATTTTTCGGTTA R: GTGAGGCAGTCGACAAGAGT	$(CT)_{17}$	25	286	284–3550	0.01–0.47
NJPpGA963	HM136735	F: TACGGTGACACACAATTTGC R: AACCCGGTTTTGCCTTATAC	$(CT)_{15}$	28	291	289–350	0.01–0.48
NJPpGA964	HM136736	F: ATATTTGACGCGGTGCTCT R: CTGCTTCAGATTCGGACAGT	$(GA)_{14}CA(GA)_{13}$	28	291	264–318	0.01–0.49
NJPpGA986	HM136737	F: GACATCAAGACCTTGACGAAA R: GAGGTGTGGCGACAAGATT	$(GA)_{17}$	25	298	292–346	0.01–0.49
NJPpGA993	HM136738	F: ATTTAAGCAGTGGGCATGAG R: GCGCATTTAAACACAACAAT	$(CT)_{25}$	29	292	274–338	0.01–0.50
NJPpGA1023	HM136739	F: CATGAGAGACACGAACAGGA R: TGAGAATTCTTCTATATGTAA-CCACAA	$(CT)_{19}$	35	296	285–351	0.01–0.48
NJPpGA1033	HM136740	F: GGGGGATACAAACTCGACAT R: CCTAGGCCGGGACTTTTT	$(GA)_{27}$	23	293	270–316	0.01–0.48
NJPpGA1054	HM136741	F: ATACATTGGGGTGCATGGT R: AACAACTGCCTATCCATTACAAA	$(CT)_{23}$	13	286	243–305	0.01–0.50
NJPpGA1071	HM136742	F: CACATTCGGTTTTGGATCAT R: CAGAGAACACGAGCATTGAA	$(GA)_{15}GG(GA)_{23}$	61	334	240–357	0.01–0.50
NJPpGA1092	HM136743	F: CACTGACCGTTAAGAAGGTG R: CACCAGTTTACATATCATCC	$(GA)_{10}TA(GA)_{28}$	38	299	241–317	0.01–0.49
NJPpGA1095	HM136744	F: AACTTTTCAGATCACTAGATC-TTATCC R: CCTAGTCCGATATTTCCACTTC	$(CT)_5GTCTGT$ $(CT)_4C(CT)_{22}$	54	298	238–337	0.01–0.49
NJPpGA1100	HM136745	F: CTAATTTGGTGGATCTTGCT R: TTCAGAGCATACAGAGTAAACA	$(CT)_{14}$	47	250	249–336	0.01–0.50
NJPpGA1101	HM136746	F: GAGACCCAAAAATCGTCCTC R: CGTCTCTTCGTTTGAGATGG	$(CT)_{18}$	35	298	285–342	0.01–0.50
NJPpGA1102	HM136747	F: TTCTCTCTCTCCATCCTTG R: TCCATGTAGGCACAAATA	$(CT)_{29}$	38	300	247–329	0.01–0.48
NJPpGA1110	HM136748	F: TGAGGAGTTGCTCGTCTAGG R: TCTGATGCAGACTTGGAACA	$(GA)_{26}$	39	291	240–365	0.01–0.42
NJPpGA1112	HM136749	F: AAACGTGATGCTTCGACAAT R: CTCCGTGAGTAGACCGTGTT	$(GA)_8AA(GA)_{14}$	55	281	236–328	0.01–0.50
NJPpGA1117	HM136750	F: CTTCGTTTGGAGAGGTTGTG R: TCCAACGGCTACCTATTCAG	$(GA)_{25}$	25	299	253–360	0.01–0.49
NJPpGA1119	HM136751	F: TCCCAGCTGGCATTTCTAT R: CGCTGATGCACAGGTTACTA	$(GA)_{19}$	40	285	257–344	0.01–0.50
NJPpGA1148	HM136752	F: CAAAGGGAATGTAAGAGG R: GCTATGGTCGATAAGAGAA	$(GA)_{14}$	40	250	234–289	0.01–0.47
NJPpGA1152	HM136753	F: AAACGTGATGCTTCGACAAT R: CTCCGTGAGTAGACCGTGTT	$(GA)_8AA(GA)_{13}$	58	281	216–328	0.01–0.50
NJPpGA1153	HM136754	F: TTAAAGACCCTACCAATGCAA R: TTTCATCCTCTCATCGCTTC	$(CT)_{14}$	41	291	276–360	0.01–0.50
NJPpGA9307	HM136755	F: AGCACAAAAGGACACAAAAGA R: CAGGAGCCTTTGATTCTTCA	$(GA)_{20}$	67	283	258–394	0.01–0.44
NJPpGA9314	HM136756	F: GGACAACGTAGTCTTCCCAGT R: TTCTTCGTGGTGATTATTAGC	$(GA)_{19}$	48	290	273–410	0.01–0.44
NJPpGA9317	HM136757	F: GTGCACGGTATGCATGGAG R: CAGAATCTCTTCTTGTTCCGTTA	$(GA)_{33}$	53	294	240–357	0.01–0.45

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Table 1. (*Continued*)

Marker ID	GenBank accession no.	Primers (5′-3′)	Repeat motif	N_a	Original cloned allele (bp)	Allele size range (bp)[z]	Range of PIC values for N_a alleles
NJPpGA9324	HM136758	F: TCATGATGTAGTCGCACAGC R: CGAAGGCTTTAGAGGAACAC	$(CT)_{16}CG(CT)_6$	33	278	245–329	0.01–0.48
NJPpGA9326	HM136759	F: TAGGCGTCCGATTAAGAAAA R: GTAGAGGCGTTCTCGGGTAT	$(GA)_{13}$	38	269	257–341	0.01–0.48
NJPpGA9335	HM136760	F: ATTTTCCCGTTGGCGTTT R: GATGGCTGCTACTTCTAGG	$(CT)_{15}$	31	250	237–298	0.01–0.49
NJPpGT6	HM136761	F: TAGCAATGCCTCCAAAGTAG R: CTCGTGAGTTTGAGAATTTGA	$(CA)_{11}$	40	250	235–309	0.01–0.47
NJPpGT8	HM136762	F: ACAAAATTAAGCGGATGCTATC R: AATCGGATTAGTTTCGATCATG	$(GT)_{12}$	31	223	206–321	0.01–0.48
NJPpGT12	HM136763	F: GAGTTGGTCCGTGGTGTAG R: CGCTATTCTTTTAGTGGTATGC	$(CA)_4CG(CA)_{11}CG(CA)_4$	11	226	227–320	0.01–0.50
NJPpGT102	HM136764	F: GTTCTTGGGTAGTGTGCTGTAT R: CGTGTGAATCATTGCCTAAC	$CAGA(CA)_{13}$	21	178	164–246	0.01–0.46
NJPpGT110	HM136765	F: GTTGCGTGTTTTGTTTCGT R: GCCTCGTCGTATTACTCAGTG	$(GT)_{11}$	24	221	197–254	0.01–0.50
NJPpGT113	HM136766	F: CGGTGGAGAGGATGAGTAGTAG R: CACCCTGTCTATAAGGCTATCG	$(GA)_{10}(GT)_{10}TT(GT)_6$	40	190	151–239	0.01–0.49
NJPpGT123	HM136767	F: GAATGGCCTTTCAATCAGATTC R: ATTTCGTGCTGCTGAATTTACA	$(CA)_{13}$	32	142	128–221	0.01–0.50
NJPpGT134	HM136768	F: CCAAAAAGATTACTCACGGTAC R: TGTGAAGAAACACCAAGTAGAA	$(GT)_{33}$	70	138	95–254	0.01–0.50
NJPpGT135	HM136769	F: GCCGCTCTCTTGTGTCATT R: CGGGTAAGGTTTCTGCTTG	$(GT)_{29}$	57	174	132–241	0.01–0.50
NJPpGT136	HM136770	F: TCTTTGTTGCCCTTACCTG R: ATGACATTGGAGGCTTATCAG	$(GT)_2GC(GT)_7$	17	226	237–253	0.01–0.46
NJPpGT9314	HM136771	F: CTCAGTGACGGAACTGCTTA R: GCTGTCAAGGCACTCTAACC	$(CA)_9CG(CA)_5$	45	295	244–313	0.01–0.48
NJPpGT9318	HM136772	F: CAAGTAGGACAATCGCAACA R: ACCACATCATGGACCTCTGT	$(CA)_{15}$	18	284	248–302	0.01–0.47
NJPpGT9434	HM136773	F: CCCCCTATGCGACACGTA R: GTACCTCGTGCGGCATGT	$(GT)_{15}$	43	249	182–311	0.01–0.50
NJPpGT9446	HM136774	F: TGTTTCTGTGACTGAATCTCAACTG R: TGCACACATGCTCATCTTGA	$(GT)_{10}$	37	248	254–308	0.01–0.48

[z]Includes M13(-21) 18 bp sequence (5′–TGTAAAACGACGGCCAGT-3′) on the forward primer.
N_a = number of alleles; PIC = polymorphism information content.

selection, and polymerase chain reaction (PCR) primers were designed to flank regions surrounding the SSR motif using Primer3 software (Rozen and Skaletsky, 2000). The forward primer in the pair was elongated at the 5′ end using the M13(-21) 18-bp sequence (5′–TGTAAAACGACGGCCAGT-3′) for economic fluorescent labeling (Schuelke, 2000) and synthesized by Integrated DNA Technologies (Coralville, IA). Of the 1071 clones containing dinucleotide repeats, PCR primer pairs were designed for 500 of these sequences, of which 88 (17.6%) produced distinct, repeatable polymorphic bands in a panel of 265 kentucky bluegrass cultivars, experimental selections, collections, and hybrids with texas bluegrass (*Poa arachnifera* Torr.).

For genotyping, PCR reactions were conducted in 13-μL reactions using 50 ng genomic DNA, 1X Ramp-Taq PCR buffer (Denville Scientific, Metuchen, NJ), 2 mM $MgCl_2$, 0.25 mM each dNTP (Denville Scientific), 0.5 U Ramp-Taq DNA polymerase (Denville Scientific), 0.5 pmol forward primer with M13(-21) addition, 1 pmol reverse primer, and 1 pmol forward florescent dye-labeled M13(-21) primer (FAM, NED, PET, or VIC). Thermal-cycling parameters were 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 45 s, 72 °C for 45 s followed by 20 cycles of 94 °C for 30 s, 53 °C for 45 s, 72 °C for 45 s, ending with a final extension of 72 °C for 10 min. PCR reaction products were analyzed on an ABI 3130xl Genetic Analyzer and sized using Genemapper 3.7 software (Applied Biosystems) and LIZ 500(-250) size standard (Applied Biosystems).

Although microsatellites can generate co-dominant data, problems can arise during the identification of polyploid genotypes because it is difficult to determine the number of copies of an allele in heterozygotes (Liao et al., 2008; Markwith et al., 2006; Saltonstall, 2003). Thus, banding patterns observed at particular loci are referred to as ‘‘allele phenotypes’’ (Becher et al., 2000). The individual alleles of the SSR markers used in the current study of 265 polyploid kentucky bluegrasses and hybrids were treated as dominant markers, in which the banding phenotypes were scored as band absence (0) or presence (1). Polymorphism information content (PIC) of each individual SSR allele was calculated according to the formula described by Weir (1990): $PIC = 1 - \sum P_i^2$, where P_i is the frequency of the *i*th allele in the genotypes examined. For dominant markers, this formula can be simplified to $PIC = 2P_iQ_i$ where P_i is the frequency of presence and Q_i is the frequency of absence of a particular band (Tehrani et al., 2008). For dominant markers, the maximum PIC value is 0.50. PIC values for the 88 SSR markers in the current study are given as a range of PIC values for all alleles generated by a particular SSR marker (Table 1).

Characteristics of the SSR markers and their primer sequences are shown in Table 1. All SSR markers produced well-defined discrete alleles. The total number of alleles produced by the 88 SSR markers in the 265 cultivars and accessions was 3373. The number of alleles for individual SSR markers ranged from four to 81 with an average of 38.3 alleles per SSR. The high average number of alleles per SSR is likely the result of the combination of high genetic diversity and high ploidy levels in the genotypes under investigation. The PIC value ranges for the majority of the SSR markers approached the maximum PIC value for dominant markers, indicating that each SSR marker produced alleles that were highly polymorphic (Table 1). PIC values and expected heterozygosity of the 3373 individual alleles are available on request. The high level of polymorphism observed for the described microsatellite markers support their application in genetic studies of kentucky bluegrass. The potential use of these markers includes assessment of genetic diversity, creation of genetic linkage maps, assessment of levels of apomixis in cultivars and experimental varieties, and identification of aberrant progeny in apomictic kentucky bluegrass breeding programs. Additionally, the large number of microsatellite markers described in this report falls within a range (75 to 100 SSRs) that other researchers have suggested to be

appropriate for PVP analyses in other crops (Heckenberger et al., 2005b; Smith et al., 2009).

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