Development of Microsatellite Markers for Buffalograss (Buchloë dactyloides; Poaceae), a Drought-Tolerant Turfgrass Alternative

Authors: Hadle, Jacob J., Konrade, Lauren A., Beasley, Rochelle R., Lance, Stacey L., Jones, Kenneth L., et al.

Source: Applications in Plant Sciences, 4(8)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1600033

Applications in Plant Sciences 2016 4(8): 1600033

PRIMER NOTE

DEVELOPMENT OF MICROSATELLITE MARKERS FOR BUFFALOGRASS (*BUCHLOË DACTYLOIDES*; POACEAE), A DROUGHT-TOLERANT TURFGRASS ALTERNATIVE[1]

JACOB J. HADLE[2,6], LAUREN A. KONRADE[2], ROCHELLE R. BEASLEY[3], STACEY L. LANCE[3], KENNETH L. JONES[4], AND JAMES B. BECK[2,5]

[2]Department of Biological Sciences, Wichita State University, 537 Hubbard Hall, Wichita, Kansas 67260 USA; [3]Savannah River Ecology Laboratory, University of Georgia, Aiken, South Carolina 29802 USA; [4]Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, Colorado 80045 USA; and [5]Botanical Research Institute of Texas, 1700 University Drive, Fort Worth, Texas 76107 USA

- *Premise of the study: Buchloë dactyloides* (Poaceae) is an important component of Great Plains prairies and a popular drought-tolerant turfgrass alternative in North America. This species comprises an autopolyploid series, and microsatellite primers were developed to understand the distribution of genetic variation among cytotypes and across its large geographic range.
- *Methods and Results:* Fifteen microsatellite loci were designed and successfully amplified in six *B. dactyloides* populations. Within-population genetic diversity was comparatively high, consistent with *B. dactyloides*' life history. Allelic variation at 13 loci was consistent with the cytotype established in chromosome-counted samples.
- *Conclusions:* This variable, interpretable set of loci allows for the determination of multilocus genotype in *B. dactyloides* individuals of varying cytotype. Data such as these from a range-wide sample set can provide important insights for germplasm conservation and crop improvement in this ecologically and economically important species.

Key words: *Buchloë dactyloides*; Great Plains; Illumina sequencing; native turfgrass alternative; Poaceae; polyploidy; simple sequence repeat (SSR) markers.

Buchloë dactyloides (Nutt.) Engelm. (buffalograss; Poaceae) is a low-growing, perennial C_4 grass that is a dominant component of shortgrass prairies of the North American Great Plains (Shearman et al., 2004). Beyond this significant ecosystem role, buffalograss has been widely adopted as a drought-tolerant turfgrass alternative, particularly notable as a native-species option in North America. Like many dominant Great Plains grasses, *B. dactyloides* comprises an autopolypoid series, including diploids ($2n$ = 20), tetraploids, pentaploids, and hexaploids (Johnson et al., 2001). Preserving the full range of buffalograss phenotypic and genotypic diversity and utilizing this diversity for crop improvement will require an understanding of the distribution of genetic variation among cytotypes and across its large geographic range.

Beyond numerous methodological advantages (Guichoux et al., 2011), microsatellites, or simple sequence repeat (SSR) markers, are an attractive genetic tool for studies of wide-ranging polyploid series given their codominant nature and applicability to museum-derived DNAs. Because SSR data are routinely obtainable from DNA extracted from museum tissue (Wandeler et al., 2007), these samples can be used to quickly and economically obtain comparative genotypic data from all portions of a large geographic range. Currently no buffalograss-specific SSR loci are available, as previous studies have relied on a mixture of dominant and codominant loci that were designed for other taxa (Budak et al., 2004). In this study, a set of SSR loci are designed from *B. dactyloides* genomic sequence data. The variability of these loci are then evaluated in six populations from numerous portions of the buffalograss range.

METHODS AND RESULTS

Silica gel–dried tissue was preserved from a *B. dactyloides* individual collected in Kiowa Co., Colorado, USA. A voucher specimen (*Hadle 2228*) has been deposited at the Arthur L. Youngman Herbarium at Wichita State University (WICH). Extraction with a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) yielded 2.4 µg of DNA, and an Illumina paired-end shotgun library was prepared from 1 µg of sheared DNA following the Illumina TruSeq DNA Library Kit protocol (Illumina, San Diego, California, USA) using a multiplex identifier adapter index. The library was sequenced (100-bp paired-end reads) on an Illumina HiSeq 2000. Five million of the resulting reads were screened with PAL_FINDER_v0.02.03 (Castoe et al., 2012) to extract those containing di-, tri-, tetra-, penta-, and hexanucleotide repeats. Such reads were batched to a local installation of Primer3 version 2.0.0 (Rozen and Skaletsky, 1999) for primer design. Single-copy loci were targeted by selecting those for which either primer sequence occurred 1–7 times among the 5 million reads.

[1]Manuscript received 18 March 2016; revision accepted 13 May 2016.

The authors thank the Kiowa, Pawnee, Rita Blanca, and Thunder Basin National Grasslands for permission to sample. This work was supported by the Wichita State University Department of Biological Sciences and by the National Science Foundation (EPS-0903806) with matching support from the Kansas Board of Regents. Manuscript preparation was partially supported by the Department of Energy (DE-FC09-07SR22506) to the University of Georgia Research Foundation. Bioinformatics support came from Biostatistics and Bioinformatics Shared Resource of the University of Colorado Cancer Center (5P30CA046934).

[6]Author for correspondence: jjhadle@wichita.edu

doi:10.3732/apps.1600033

TABLE 1. Characteristics of 15 *Buchloë dactyloides* loci.[a]

Locus	Primer sequences (5′–3′)[b]	*A*	Allele size range (bp)	Repeat motif	Fluorescent label	Multiplex[c]
Buda_1	F: CCCTATGTTGACTCTAAATATTTGTCAGG R: **GTTT**AAACTATACTCTCACCAGTTGTTCATCC	38	260–406	(ATT)	6-FAM	1 (0.5 μM)
Buda_2	F: GTAGCTGATCATCCATGCCC R: **GTTT**AAAGAATGCACGTGTCAATGG	32	165–252	(TTC)	HEX	1 (0.05 μM)
Buda_4	F: GACAGTGCCAATCTGTTTGC R: **GTTT**AACTTGGTAATTTCGGATGTGC	N/A	200–288	(ACTG)	HEX	2 (0.4 μM)
Buda_10	F: CACGAAGTGACGACTGGTGC R: **GTTT**AGATTCTCGTCCAACGACGC	11	174–224	(ACTG)	HEX	3 (0.1 μM)
Buda_11	F: CTCAAAGTTGGCACCACCG R: **GTTT**AGCCTGCTTGCTTTGGTAGG	22	208–284	(TTCC)	6-FAM	4 (0.4 μM)
Buda_12	F: AATACAATGACGGTGGAAGCG R: **GTTT**AGCGATCGGCTCAGGATAGG	21	160–225	(AATT)	NED	2 (0.1 μM)
Buda_13	F: TCTAGTCCACCGATCAAGCG R: **GTTT**AGCTCGTGAGCCGATTCC	10	167–195	(AAAG)	NED	3 (0.1 μM)
Buda_14	F: TTAACTCTCCGGCCACACG R: **GTTT**AGGTAGTAGAGGAGCAAGATGGC	20	132–179	(AAAG)	6-FAM	3 (0.1 μM)
Buda_15	F: GACGAGGAGGATAGATGGCG R: **GTTT**ATTCCTTTCCACCGAACGC	8	115–136	(AGCC)	6-FAM	2 (0.1 μM)
Buda_18	F: TTAACGTGCCGTGAGAGGG R: **GTTT**CACGGGCTCAGACTCAACG	14	233–280	(TCCG)	6-FAM	3 (0.4 μM)
Buda_19	F: CAACTTGGGCAACAACAAGC R: **GTTT**CAGTGGCTCCTTGGGTACG	18	130–192	(AAAG)	6-FAM	4 (0.1 μM)
Buda_29	F: CAGGAGTCGGCACAAGACC R: **GTTT**GACGAACTCAAGCAGGACACC	15	133–193	(AAAG)	HEX	4 (0.1 μM)
Buda_30	F: GCAGCCAAATATCTACTGACGG R: **GTTT**GATTTGTCTCAACCAATTCGC	19	167–263	(ATCT)	6-FAM	2 (0.1 μM)
Buda_31	F: AGCCAGAAGGGCCACACC R: **GTTT**GCAATTCGTATCCTTATGGCG	27	136–204	(TTC)	NED	4 (0.1 μM)
Buda_34	F: TCCCTTGAGACATGAGACGG R: **GTTT**GGAAACCTGCAAGATGTTCG	16	128–175	(AAAG)	6-FAM	1 (0.05 μM)

Note: *A* = number of alleles.

[a] Paired-end sequence data are available from the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.80th2; Hadle et al., 2016).

[b] Nucleotides added to create PIG-tail noted in boldface.

[c] Multiplex set (1–4) and reaction concentration of each primer in each pair. See text for reaction conditions.

Of the 5012 loci that met this criterion, 48 were selected and screened following O'Bryhim et al. (2013). Fifteen polymorphic loci were then evaluated in six *B. dactyloides* populations collected throughout the Great Plains (Appendix 1). Within each population, material from eight individuals separated by >30 m was preserved in silica gel. Voucher specimens for all individuals are archived at WICH. No cross-species amplification was attempted given the monotypic nature of the genus. Floral buds from one individual in five of the six populations (Appendix 1) were fixed in 3 : 1 100% ethanol : glacial acetic acid for 24 h and then transferred to 70% ethanol. Fixed anthers were macerated, stained with 1% acetocarmine, squashed following standard methods, and examined with brightfield microscopy at 1000× magnification. DNA was extracted with the high-throughput protocol outlined in Beck et al. (2012). Amplifications comprised 2 μL (20–25 ng) of genomic DNA, 2.5 μL of QIAGEN Multiplex PCR Master Mix, 0.8 μL of primer mix (see Table 1), and 2.7 μL of H_2O. Loci were either tri- or tetraplexed, with individual primer concentrations optimized to reduce unequal locus amplification (Table 1). Cycling conditions included initial DNA denaturation at 95°C (15 min); 30 cycles of 94°C (30 s), 57°C (90 s), 72°C (60 s); followed by a final extension at 60°C (30 min). All samples were genotyped at the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility (Chicago, Illinois, USA). Alleles were called with GeneMarker 1.91 (SoftGenetics, State College, Pennsylvania, USA), and diversity measures were calculated with GenoDive 2.0 (Meirmans and Van Tienderen, 2004). All measures

TABLE 2. Genetic diversity in six *Buchloë dactyloides* populations at 14 newly developed microsatellite loci.[a]

	Weld Co., CO			Weston Co., WY			Perkins Co., SD			Payne Co., OK			Dallam Co., TX			Harding Co., NM		
Locus	*A*	H_o	H_s	*A*	H_o	H_s	*A*	H_o	H_s	*A*	H_o	H_s	*A*	H_o	H_s	*A*	H_o	H_s
Buda_1	11	0.625	0.889	14	0.875	0.983	12	0.875	0.933	10	0.667	0.945	7	0.625	0.813	10	0.625	0.890
Buda_2	10	0.875	0.895	15	1.000	0.945	15	1.000	0.931	16	0.875	0.935	14	1.000	0.910	11	0.875	0.901
Buda_10	7	0.875	0.862	5	0.875	0.731	8	0.875	0.842	7	1.000	0.797	7	1.000	0.834	7	0.875	0.823
Buda_11	7	0.625	0.858	6	0.500	0.889	10	0.714	0.949	9	0.625	0.837	12	1.000	0.914	9	0.625	0.924
Buda_12	7	0.500	0.872	9	0.750	0.860	9	0.375	0.835	4	0.250	0.533	7	0.375	0.833	10	0.714	0.857
Buda_13	5	0.750	0.781	5	0.500	0.829	4	0.750	0.757	6	1.000	0.801	5	0.750	0.683	7	0.625	0.802
Buda_14	11	1.000	0.909	11	1.000	0.902	9	0.875	0.881	10	1.000	0.873	11	1.000	0.926	11	1.000	0.883
Buda_15	6	0.750	0.830	6	0.875	0.721	6	1.000	0.857	6	1.000	0.775	5	1.000	0.810	6	1.000	0.789
Buda_18	7	0.625	0.857	5	1.000	0.801	6	0.875	0.808	11	0.750	0.928	10	0.714	0.955	6	0.750	0.857
Buda_19	11	1.000	0.901	10	1.000	0.870	8	1.000	0.818	11	1.000	0.883	11	1.000	0.873	9	1.000	0.860
Buda_29	8	0.750	0.843	5	1.000	0.801	9	0.875	0.885	9	1.000	0.864	8	0.875	0.884	8	1.000	0.876
Buda_30	5	1.000	0.767	12	1.000	0.895	9	0.875	0.832	10	0.875	0.858	9	0.875	0.880	9	0.750	0.883
Buda_31	13	1.000	0.952	11	0.714	0.950	8	0.625	0.890	15	1.000	0.950	11	0.875	0.958	14	1.000	0.965
Buda_34	7	1.000	0.848	10	1.000	0.887	7	0.875	0.848	13	0.875	0.949	8	1.000	0.849	8	1.000	0.835
Mean	8.2	0.813	0.862	8.9	0.864	0.862	8.6	0.828	0.862	9.8	0.851	0.852	8.9	0.864	0.866	8.9	0.846	0.868

Note: *A* = number of alleles; H_o = observed heterozygosity; H_s = expected heterozygosity.

[a] All population samples comprised eight individuals; see Appendix 1 for voucher and locality information.

were calculated using observed data only; missing data due to uncertain dosage were not inferred. Note that observed heterozygosity in these polyploid genotypes was calculated as "gametic heterozygosity," the chance that two alleles drawn from an individual are different (Moody et al., 1993).

All but one of the 15 loci were both highly variable and interpretable. One locus (Buda_4) was often difficult to interpret due to excessive stutter and was excluded from further analyses. A total of 271 alleles were identified, with 8–38 (mean 19.4) alleles per locus (Table 1). Mean expected heterozygosity (0.86) (Table 2) was notably higher than the average (0.65) for 71 SSR-based studies of outcrossing plants reviewed by Nybom (2004). This high level of within-population genetic variation is consistent with *B. dactyloides*' predominantly (but not exclusively) dioecious life history (Huff and Wu, 1992). Although variable and interpretable, one locus (Buda_14) consistently exhibited more alleles than expected given the known cytotypes in a set of 79 chromosome-counted specimens analyzed as part of a broader study (Hadle et al., unpublished). All five chromosome-counted specimens were tetraploids ($2n = 20_{II}$). Consistent with their 4*x* cytotype (Appendix 1), each of these five chromosome-counted specimens exhibited a maximum of three or four alleles at the remaining 13 loci (excluding Buda_4 and_14).

CONCLUSIONS

The 14 optimized loci exhibited substantial variability within populations from numerous portions of the buffalograss range, and allelic variation at a set of 13 loci was consistent with the known cytotype in a set of chromosome-counted specimens. Ongoing studies in a set of >550 samples have established that these loci are readily amplifiable in herbarium-extracted DNAs of a wide age range (Hadle et al., unpublished), which will allow for the rapid determination of multilocus genotypes in a large set of samples representing all portions of the buffalograss range. These data have the potential to inform many aspects of buffalograss germplasm conservation and breeding programs, enhancing the conservation, crop, and ecosystem value of this dominant Great Plains grass.

LITERATURE CITED

Beck, J. B., P. J. Alexander, L. Allphin, I. A. Al-Shehbaz, C. Rushworth, C. D. Bailey, and M. D. Windham. 2012. Does hybridization drive the transition to asexuality in diploid *Boechera*? *Evolution* 66: 985–995.

Budak, H., R. C. Shearman, I. Parmaksiz, and I. Dweikat. 2004. Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theoretical and Applied Genetics* 109: 280–288.

Castoe, T. A., A. W. Poole, A. P. J. de Konig, K. L. Jones, D. F. Tomback, S. J. Oyler-McCance, J. A. Fike, et al. 2012. Rapid microsatellite identification from Illumina paired-end genomic sequencing in two birds and a snake. *PLoS ONE* 7: e30953.

Guichoux, E., L. Lagache, S. Wagner, P. Chaumeil, P. Léger, O. Lepais, C. Lepoittevin, et al. 2011. Current trends in microsatellite genotyping. *Molecular Ecology Resources* 11: 591–611.

Hadle, J. J., L. A. Konrade, R. R. Beasley, S. L. Lance, K. L. Jones, and J. B. Beck. 2016. Data from: Development of microsatellite markers for buffalograss (*Buchloë dactyloides*; Poaceae), a drought-tolerant turfgrass alternative. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.80th2.

Huff, D. R., and L. Wu. 1992. Distribution and inheritance of inconstant sex forms in natural populations of dioecious buffalograss (*Buchloë dactyloides*). *American Journal of Botany* 79: 207–215.

Johnson, P. G., K. E. Kenworthy, D. L. Auld, and T. P. Riordan. 2001. Distribution of buffalograss polyploid variation in the southern Great Plains. *Crop Science* 41: 909–913.

Meirmans, P. G., and P. H. Van Tienderen. 2004. GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4: 792–794.

Moody, M. E., L. D. Mueller, and D. E. Soltis. 1993. Genetic variation and random drift in autotetraploid populations. *Genetics* 134: 649–657.

Nybom, H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* 13: 1143–1155.

O'Bryhim, J., C. Somers, S. L. Lance, M. Yau, D. R. Boreham, K. L. Jones, and E. B. Taylor. 2013. Development and characterization of twenty-two novel microsatellite markers for the mountain whitefish, *Prosopium williamsoni* and cross-amplification in the round whitefish, *P. cylindraceum*, using paired-end Illumina shotgun sequencing. *Conservation Genetics Resources* 5: 89–91.

Rozen, S., and H. Skaletsky. 1999. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

Shearman, R. C., T. P. Riordan, and P. G. Johnson. 2004. Buffalograss. *In* L. E. Moser, B. L. Burson, and L. E. Sollenberger [eds.], Warm-season (C_4) grasses, 1003–1026. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA.

Wandeler, P., P. E. Hoeck, and L. F. Keller. 2007. Back to the future: Museum specimens in population genetics. *Trends in Ecology & Evolution* 22: 634–642.

Appendix 1. Collection information for the 48 *Buchloë dactyloides* individuals analyzed in this study.[a]

Voucher	Locality[b]	Geographic coordinates	Max allele[c]	Chromosome count[d]
JH 2782	USA, CO, Weld Co.	40.753266°N, 104.603165°W	4	—
JH 2783	USA, CO, Weld Co.	40.75355°N, 104.635601°W	3	—
JH 2784	USA, CO, Weld Co.	40.77016°N, 104.63829°W	4	—
JH 2785	USA, CO, Weld Co.	40.78644°N, 104.63803°W	5	—
JH 2786	USA, CO, Weld Co.	40.79728°N, 104.6399°W	4	—
JH 2787	USA, CO, Weld Co.	40.81138°N, 104.63867°W	4	—
JH 2788	USA, CO, Weld Co.	40.81143°N, 104.64822°W	3	tetraploid ($2n = 20_{II}$)
JH 2789	USA, CO, Weld Co.	40.8119°N, 104.67879°W	3	—
JH 2804	USA, WY, Weston Co.	43.52456°N, 104.19767°W	4	—
JH 2805	USA, WY, Weston Co.	43.80083°N, 104.4675°W	4	—
JH 2806	USA, WY, Weston Co.	43.79878°N, 104.77735°W	4	—
JH 2807	USA, WY, Weston Co.	43.8001°N, 104.75498°W	4	tetraploid ($2n = 20_{II}$)
JH 2808	USA, WY, Weston Co.	43.80101°N, 104.5977°W	4	—
JH 2809	USA, WY, Weston Co.	43.80121°N, 104.56303°W	4	—
JH 2810	USA, WY, Weston Co.	43.80136°N, 104.47581°W	4	—
JH 2811	USA, WY, Weston Co.	43.80106°N, 104.45981°W	4	—
JH 2819	USA, SD, Perkins Co.	45.73937°N, 102.21776°W	4	—
JH 2820	USA, SD, Perkins Co.	45.72236°N, 102.16819°W	4	tetraploid ($2n = 20_{II}$)
JH 2821	USA, SD, Perkins Co.	45.72587°N, 102.17705°W	3	—
JH 2822	USA, SD, Perkins Co.	45.72926°N, 102.19806°W	5	—
JH 2823	USA, SD, Perkins Co.	45.72287°N, 102.18287°W	4	—
JH 2824	USA, SD, Perkins Co.	42.72815°N, 102.18445°W	4	—
JH 2825	USA, SD, Perkins Co.	45.73206°N, 102.1833°W	4	—
JH 2826	USA, SD, Perkins Co.	45.70429°N, 102.16132°W	6	—
JH 2579	USA, OK, Payne Co.	36.12666°N, 97.21805°W	5	—
JH 2580	USA, OK, Payne Co.	36.12643°N, 97.21787°W	5	—
JH 2581	USA, OK, Payne Co.	36.12586°N, 97.21736°W	5	—
JH 2582	USA, OK, Payne Co.	36.12411°N, 97.21736°W	6	—
JH 2583	USA, OK, Payne Co.	36.12005°N, 97.21874°W	4	—
JH 2584	USA, OK, Payne Co.	36.11686°N, 97.22035°W	6	—
JH 2585	USA, OK, Payne Co.	36.11597°N, 97.22006°W	6	—
JH 2586	USA, OK, Payne Co.	36.12813°N, 97.21822°W	4	—
JH 2660	USA, TX, Dallam Co.	36.41318°N, 102.82968°W	4	—
JH 2661	USA, TX, Dallam Co.	36.41626°N, 102.8273°W	4	—
JH 2662	USA, TX, Dallam Co.	36.4148°N, 102.84119°W	6	—
JH 2663	USA, TX, Dallam Co.	36.41489°N, 102.84902°W	5	—
JH 2664	USA, TX, Dallam Co.	36.41529°N, 102.86018°W	5	—
JH 2665	USA, TX, Dallam Co.	36.41267°N, 102.8642°W	4	—
JH 2666	USA, TX, Dallam Co.	36.41488°N, 102.80423°W	3	tetraploid ($2n = 20_{II}$)
JH 2667	USA, TX, Dallam Co.	36.41471°N, 102.79685°W	4	—
JH 2649	USA, NM, Harding Co.	36.06549°N, 104.24011°W	4	tetraploid ($2n = 20_{II}$)
JH 2650	USA, NM, Harding Co.	36.08134°N, 104.25208°W	4	—
JH 2651	USA, NM, Harding Co.	36.0802°N, 104.26451°W	4	—
JH 2652	USA, NM, Harding Co.	36.08148°N, 104.2731°W	4	—
JH 2653	USA, NM, Harding Co.	36.07953°N, 104.27837°W	3	—
JH 2654	USA, NM, Harding Co.	36.07314°N, 104.29107°W	4	—
JH 2655	USA, NM, Harding Co.	36.07327°N, 104.3221°W	4	—
JH 2656	USA, NM, Harding Co.	36.07302°N, 104.33675°W	3	—

Note: — = not performed; JH = Jacob Hadle.

[a] Voucher specimens are deposited at the Arthur L. Youngman Herbarium at Wichita State University (WICH).

[b] Each set of eight specimens with matching locality information represents a population. Locality is presented as country, state, county (CO = Colorado, NM = New Mexico, OK = Oklahoma, SD = South Dakota, TX = Texas, WY = Wyoming).

[c] Maximum number of alleles observed at any one of the 13 loci examined.

[d] Meiotic count from pollen mother cells.