DEVELOPMENT OF MICROSATELLITE MARKERS FOR
BUFFALOGRASS (Buchloë dactyloides; Poaceae),
A DROUGHT-TOLERANT TURFGRASS ALTERNATIVE

JACOB J. HADLE1,2, LAUREN A. KONRADE2, ROCHELLE R. BEASLEY3, STACEY L. LANCE3,
KENNETH L. JONES4, and JAMES B. BECK2,5

1 Department of Biological Sciences, Wichita State University, 537 Hubbard Hall, Wichita, Kansas 67260 USA; 2 Savannah River Ecology Laboratory, University of Georgia, Aiken, South Carolina 29802 USA; 3 Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, Colorado 80045 USA; and 4 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.) Engl. (buffalograss; Poaceae) is a low-growing, perennial C4 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 in North America. Like many dominant Great Plains grasses, B. dactyloides comprises an autopolyploid 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 Skaltsky, 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.

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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 populations at 14 newly developed microsatellite loci.

**Table 2.** Genetic diversity in six *Buchloë dactyloides* populations at 14 newly developed microsatellite loci.

| Locus    | Primers (5’-3’) | Allele size range (bp) | Repeat motif | Fluorescent label | Multiplex |
|----------|-----------------|------------------------|--------------|-------------------|-----------|
| Buda_1   | F: CCCTATGTGACTCTAAATTTGTCCAAGG | 38 | 260–406 | (ATT) | 6-FAM | 1 (0.5 μM) |
|          | R: GTTTAAACTATATCCACTCAGGTATGTGATCCTC | | | | | |
| Buda_2   | F: GAGTGGCATCTGCTACCAACG | 32 | 165–252 | (TTC) | HEX | 1 (0.05 μM) |
|          | R: GTTTAAAGATGGACTGTGTAATG | | | | | |
| Buda_4   | F: GACATGTCAGACGTATG | N/A | 200–288 | (ACTG) | HEX | 2 (0.4 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_10  | F: CAGGAGGCAGACGC | 11 | 174–224 | (ACTG) | HEX | 3 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_11  | F: CTTTACATGTCTACGCAGAGGCG | 22 | 208–284 | (TTCC) | 6-FAM | 4 (0.4 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_12  | F: AGAAGTCTGAGGAGGGAGG | 21 | 160–225 | (AATT) | NED | 2 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_13  | F: TCACTTCCAGGCTAAGGCG | 10 | 167–195 | (AAAG) | NED | 3 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_14  | F: TTAACCTTCCGCGGCACAG | 20 | 132–179 | (AAAG) | 6-FAM | 3 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_15  | F: GACGGAGGAGAGTAGAGTGGCG | 8 | 115–136 | (AGCC) | 6-FAM | 2 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_18  | F: TAAAGGTGCTGGAGAGCG | 14 | 233–280 | (TTCCG) | 6-FAM | 3 (0.4 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_19  | F: CAGGGTCCAGACGGCG | 18 | 130–192 | (AAAG) | 6-FAM | 4 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_20  | F: CAGGGTCCAGACGGCG | 15 | 133–193 | (AAAG) | 6-FAM | 4 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_21  | F: AAGCAGAACGGCAACGGAC | 19 | 167–263 | (ATCT) | 6-FAM | 2 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_22  | F: AGACAATGGACGAGGAGGCG | 27 | 136–204 | (TTCC) | NED | 4 (0.1 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |
| Buda_23  | F: GCCTTGAGAGCAAGGCCAAG | 16 | 128–175 | (AAAG) | 6-FAM | 1 (0.05 μM) |
|          | R: GTTTAACTTGTGATACCTGATG | | | | | |

**Note:** A = number of alleles.

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

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

*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) 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 (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 = 20x), Consistent with their 4x 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.

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

| Voucher | Localityb | Geographic coordinates | Max allelc | Chromosome countd |
|---------|------------|-------------------------|------------|-------------------|
| 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. | 40.78664°N, 104.63803°W | 5 | — |
| JH 2786 | USA, CO, Weld Co. | 40.79728°N, 104.6399°W | 4 | — |
| JH 2787 | USA, CO, Weld Co. | 40.81338°N, 104.63867°W | 4 | — |
| JH 2788 | USA, CO, Weld Co. | 40.81143°N, 104.64822°W | 3 | tetraploid (2n = 20₀) |
| 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 = 20₀) |
| 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 | — |
| 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. | 45.73296°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. | 36.11866°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 = 20₀) |
| JH 2667 | USA, TX, Dallam Co. | 36.41471°N, 102.79685°W | 4 | — |
| JH 2649 | USA, NM, Harding Co. | 36.08549°N, 104.24011°W | 4 | tetraploid (2n = 20₀) |
| 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. | 36.08184°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, 104.321°W | 4 | — |
| JH 2656 | USA, NM, Harding Co. | 36.07302°N, 104.33675°W | 3 | — |

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

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

*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).

*Maximum number of alleles observed at any one of the 13 loci examined.

*Metiotic count from pollen mother cells.