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## DEVELOPMENT OF MICROSATELLITE MARKERS FOR BUFFALOGRASS (*BUCHLOË DACTYLOIDES*; POACEAE), A DROUGHT-TOLERANT TURFGRASS ALTERNATIVE<sup>1</sup>

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- *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<sub>4</sub> 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 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 (Cao 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.

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TABLE 1. Characteristics of 15 *Buchloë dactyloides* loci.<sup>a</sup>

| Locus   | Primer sequences (5'–3') <sup>b</sup>  | A   | Allele size range (bp) | Repeat motif | Fluorescent label | Multiplex <sup>c</sup> |
|---------|--|-----|------------------------|--------------|-------------------|------------------------|
| Buda_1  | F: CCCTATGTTGACTCTAAATATTTGTGTCAGG<br>R: <b>GTTT</b> AAACTATACTCTCACCAGTTGTTTCATCC | 38  | 260–406                | (ATT)        | 6-FAM             | 1 (0.5 μM)             |
| Buda_2  | F: GTAGCTGATCATCCATGCCCC<br>R: <b>GTTT</b> AAAGAATGCACGTGTCATGG                    | 32  | 165–252                | (TTC)        | HEX               | 1 (0.05 μM)            |
| Buda_4  | F: GACAGTGCCAATCTGTTTGC<br>R: <b>GTTT</b> AACTTGGTAATTTCCGGATGTGC                  | N/A | 200–288                | (ACTG)       | HEX               | 2 (0.4 μM)             |
| Buda_10 | F: CACGAAGTGACGACTGGTGC<br>R: <b>GTTT</b> AGATTCTCGTCCAACGACGC                     | 11  | 174–224                | (ACTG)       | HEX               | 3 (0.1 μM)             |
| Buda_11 | F: CTCAAAGTTGGCACCACCG<br>R: <b>GTTT</b> AGCCTGCTTGTCTTGGTAGG                      | 22  | 208–284                | (TTCC)       | 6-FAM             | 4 (0.4 μM)             |
| Buda_12 | F: AATACAATGACGGTGAAGCG<br>R: <b>GTTT</b> AGCGATCGGCTCAGGATAGG                     | 21  | 160–225                | (AATT)       | NED               | 2 (0.1 μM)             |
| Buda_13 | F: TCTAGTCCACCGATCAAGCG<br>R: <b>GTTT</b> AGCTCGTGAGCCGATTCC                       | 10  | 167–195                | (AAAG)       | NED               | 3 (0.1 μM)             |
| Buda_14 | F: TTAACCTCTCCGGCCACAGC<br>R: <b>GTTT</b> AGGTAGTAGAGGAGCAAGATGGC                  | 20  | 132–179                | (AAAG)       | 6-FAM             | 3 (0.1 μM)             |
| Buda_15 | F: GACGAGGAGGATAGATGGCG<br>R: <b>GTTT</b> ATTCTTTCCACCGAACCG                       | 8   | 115–136                | (AGCC)       | 6-FAM             | 2 (0.1 μM)             |
| Buda_18 | F: TTACGCTGCCGTGAGAGGG<br>R: <b>GTTT</b> CACGGGCTCAGACTCAACG                       | 14  | 233–280                | (TCCG)       | 6-FAM             | 3 (0.4 μM)             |
| Buda_19 | F: CAACTGGGCAACAACAAGC<br>R: <b>GTTT</b> CAGTGGCTCCTTGGGTACG                       | 18  | 130–192                | (AAAG)       | 6-FAM             | 4 (0.1 μM)             |
| Buda_29 | F: CAGGAGTCGGCACAAGACC<br>R: <b>GTTT</b> GACGAACTCAAGCAGGACACC                     | 15  | 133–193                | (AAAG)       | HEX               | 4 (0.1 μM)             |
| Buda_30 | F: GCAGCCAAATATCTACTGACGG<br>R: <b>GTTT</b> GATTGTCTCAACCAATTCGC                   | 19  | 167–263                | (ATCT)       | 6-FAM             | 2 (0.1 μM)             |
| Buda_31 | F: AGCCAGAAGGGCCACACC<br>R: <b>GTTT</b> GCAATTTCGTATCCTTATGGCC                     | 27  | 136–204                | (TTC)        | NED               | 4 (0.1 μM)             |
| Buda_34 | F: TCCCTTGAGACATGAGACGG<br>R: <b>GTTT</b> GGAACCTGCAAGATGTTCC                      | 16  | 128–175                | (AAAG)       | 6-FAM             | 1 (0.05 μM)            |

Note: A = number of alleles.

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

<sup>b</sup>Nucleotides added to create PIG-tail noted in boldface.

<sup>c</sup>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<sub>2</sub>O. 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 (Meirman and Van Tienderen, 2004). All measures

TABLE 2. Genetic diversity in six *Buchloë dactyloides* populations at 14 newly developed microsatellite loci.<sup>a</sup>

| Locus   | Weld Co., CO |                |                | Weston Co., WY |                |                | Perkins Co., SD |                |                | Payne Co., OK |                |                | Dallam Co., TX |                |                | Harding Co., NM |                |                |
|---------|--------------|----------------|----------------|----------------|----------------|----------------|-----------------|----------------|----------------|---------------|----------------|----------------|----------------|----------------|----------------|-----------------|----------------|----------------|
|         | A            | H <sub>o</sub> | H <sub>s</sub> | A              | H <sub>o</sub> | H <sub>s</sub> | A               | H <sub>o</sub> | H <sub>s</sub> | A             | H <sub>o</sub> | H <sub>s</sub> | A              | H <sub>o</sub> | H <sub>s</sub> | A               | H <sub>o</sub> | H <sub>s</sub> |
| 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<sub>o</sub> = observed heterozygosity; H<sub>s</sub> = expected heterozygosity.

<sup>a</sup>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  $4x$  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.<sup>a</sup>

| Voucher | Locality <sup>b</sup> | Geographic coordinates    | Max allele <sup>c</sup> | Chromosome count <sup>d</sup> |
|---------|-----------------------|---------------------------|-------------------------|-------------------------------|
| 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.

<sup>a</sup>Voucher specimens are deposited at the Arthur L. Youngman Herbarium at Wichita State University (WICH).

<sup>b</sup>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).

<sup>c</sup>Maximum number of alleles observed at any one of the 13 loci examined.

<sup>d</sup>Meiotic count from pollen mother cells.