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Organelle DNA Diversity among Buffalograsses from the Great Plains of North America Determined by cpDNA and mtDNA RFLPs

O. Gulsen, R. C. Shearman,* K. P. Vogel, D. J. Lee, and T. Heng-Moss

ABSTRACT

Buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.] is a perennial, warm-season turfgrass that is native to the shortgrass prairie of North America. It offers potential benefits as a turfgrass because of its drought resistance, low incidence of pest problems, and relatively low nutrient requirements. This study was initiated to evaluate the level of organelle DNA diversity among buffalograss genotypes and to determine the mode of inheritance of the chloroplast organelle by cpDNA and mtDNA PCR-RFLPs. The 56 genotypes studied included diploids, triploid, tetraploids, pentaploids, and hexaploids. These genotypes represented 273 buffalograsses collected during 1994 and 1995 from diverse locations in the North American Great Plains. Six cpDNA and three mtDNA noncoding regions were amplified by polymerase chain reaction, using universal chloroplast and mitochondrial primer pairs. Each amplified fragment was digested with two to six different restriction enzymes. On the basis of the use of cpDNA primers, *psbC-trnS*, and restriction enzyme *HaeIII*, cpDNA was determined to be maternally inherited in buffalograss. Of the 225 scored fragments, 189 were polymorphic (84%), which included the outgroups, perennial ryegrass (*Lolium perenne* L.) and blue grama [*Bouteloua gracilis* (H.B.K.) Lag. Ex Steud.]. Similarities among all genotypes ranged from 0.41 to 1.0, with a mean of 0.70. The UPGMA-generated dendrogram clustered all buffalograsses together with the similarity value of 0.95. Out of the 56 genotypes studied, nine (16%) were discriminated from the other buffalograsses. The remaining 47 genotypes did not differ for cpDNA and mtDNA PCR-RFLPs even though they represented diverse geographic origins and five ploidy levels. These results suggest that low levels of organelle DNA PCR-RFLP are present in naturally occurring buffalograss populations.

BUFFALOGRASS is a perennial, warm-season turfgrass that is native to the shortgrass prairie of North America (Wenger, 1943). It is distributed from Canada to Mexico and from the eastern slope of the Rocky Mountains to the Mississippi River Valley. Buffalograss is mostly a dioecious, cross-pollinated species with no evidence of self-pollination (Wu et al., 1984). Therefore, buffalograss is highly heterogeneous. It has received attention for use as a turfgrass species because of its drought resistance and relatively low maintenance requirement (Riordan, 1991). Buffalograss is used in lawns, parks, cemeteries, airfields, athletic fields, roadsides, golf courses, pastures, and rangeland (Beard, 1973). Its aggressive stoloniferous growth habit and dense sod forming capabilities are highly suited to turfgrass use and make it an excellent conservation species,

since it helps prevent soil erosion by wind and water (Wenger, 1943).

The base chromosome number of buffalograss is $x = 10$, with a polyploid series of diploid, tetraploid, pentaploid, and hexaploid plants being reported in the literature (Johnson et al., 2001). The five ploidy levels are morphologically indistinguishable and their genome relationships are unclear. Diploids are reported to occur only in central Mexico and southeastern Texas (Huff et al., 1993; Johnson et al., 1998), while hexaploids are found growing throughout the Great Plains. Buffalograss plants collected in Kansas, Nebraska, and Colorado were hexaploids and tetraploids (Johnson et al., 2001), while in the southwestern parts of the Great Plains the accessions were tetraploids. There is no clear association between ploidy level and adaptation. However, it has been suggested that cold hardiness appears to be associated with higher ploidy levels (Johnson et al., 1998; Kenworthy et al., 1999; Riordan, 1991).

Both cpDNA and mtDNA are well suited for grouping individuals into defined classes and explaining evolutionary relationships because generally they have a lower mutation rate than nuclear DNA (Wolfe et al., 1987), and they are maternally inherited and relatively conserved through evolution (Caetano-Anoles, 1998). In a review of 235 angiosperm species representing 80 different plant families, Corriveau and Coleman (1988) reported 192 species had no plastid DNA in the generative cells of pollen, indicating that maternal inheritance was likely the norm in those angiosperms studied. All 18 grass species in their study were reported to have maternal inheritance of cpDNA. As a consequence, cpDNA provides a simple and reliable tool for tracing evolutionary linkages along maternal lines. For this reason, cpDNA has been widely studied (Clegg et al., 1984; Pillay and Hilu, 1990; Hultquist et al., 1997; Perez de la Rosa et al., 1995; Cipriani et al., 1998; Parducci and Szmidt, 1999; Gulsen and Roose, 2001).

The cpDNA conservation allows the design of pairs of "universal" primers, which can amplify noncoding regions in most plants (Taberlet et al., 1991). Universal primers for amplification of specific cpDNA sequences can be used in PCR-based RFLP analyses of cpDNA and mtDNA. This process involves PCR amplification of cpDNA or mtDNA, digestion of amplified PCR fragments with endonucleases, separation of fragments by electrophoresis, and detection of digested PCR products. Either specifically extracted organelle DNAs or total DNA extractions can be used to amplify cpDNA and mtDNA from a great range of plants (Demesure et al., 1995).

The genetic structure, diversity, and relationships among buffalograsses have not been fully resolved through the use of conventional methods or molecular

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Table 1. Buffalograss genotypes studied and their ploidy levels, sex expression and geographic origins.

Buffalograss genotypes	Ploidy levels†	Sex expression	Geographic origin	
			latitude (N)	longitude
2	hexaploid	F/M	33° 37'	100° 43'
4A	hexaploid	F	33° 54'	100° 20'
83	hexaploid	M	34° 56'	103° 21'
68	pentaploid	F‡	38° 50'	103° 43'
188	hexaploid	F‡	32° 02'	100° 34'
170	hexaploid	M/F	30° 04'	102° 01'
77	hexaploid	F‡	36° 36'	102° 36'
209	hexaploid	M‡	33° 10'	100° 53'
126	hexaploid	F	33° 11'	102° 42'
123	hexaploid	F	33° 25'	103° 56'
193	hexaploid	F	30° 17'	100° 39'
46	diploid	M	34° 56'	102° 24'
187	hexaploid	M	32° 19'	100° 49'
70	tetraploid	F	38° 23'	103° 43'
20B	pentaploid	F	38° 52'	100° 30'
223A	hexaploid	M‡	29° 56'	97° 59'
203	hexaploid	F	31° 07'	99° 50'
34	hexaploid	F	36° 20'	101° 24'
184A	hexaploid	M	34° 24'	101° 36'
153B	hexaploid	F‡	32° 27'	103° 08'
PX3 5.1	triploid§	No flower observed	Unknown	Unknown
136	hexaploid	F‡	32° 28'	104° 32'
28	hexaploid	M	37° 49'	101° 20'
189A	hexaploid	No flower observed	32° 01'	100° 33'
89	hexaploid	No flower observed	34° 12'	103° 34'
47	tetraploid	M‡	35° 11'	102° 24'
178	hexaploid	F	31° 26'	101° 29'
174	tetraploid	F	30° 12'	101° 06'
78C	diploid	F/M	36° 22'	103° 11'
152	hexaploid	F	32° 11'	103° 12'
17	hexaploid	M‡	38° 01'	100° 21'
49	pentaploid	M	35° 37'	102° 18'
143	tetraploid	M	30° 10'	103° 28'
84	diploid	M	34° 34'	103° 12'
234	hexaploid	M	31° 39'	103° 12'
97	tetraploid	F	35° 49'	105° 04'
132	tetraploid	M/F	32° 46'	104° 42'
196	hexaploid	F	27° 35'	98° 38'
240	hexaploid	M	33° 34'	98° 54'
7	hexaploid	M	34° 55'	100° 13'
66	tetraploid	M	39° 08'	102° 53'
98	tetraploid	M	36° 04'	104° 42'
87A	tetraploid	F	33° 57'	103° 08'
119	tetraploid	M	33° 58'	104° 39'
45B	diploid	No flower observed	34° 37'	102° 19'
137	hexaploid	M	32° 12'	104° 43'
32B	hexaploid	M	36° 26'	101° 29'
102	hexaploid	M	37° 20'	104° 34'
10B	hexaploid	F	35° 59'	100° 28'
Cody	hexaploid	M/F	Maternal parents from Arizona, Nebraska, and Oklahoma	
Bowie	hexaploid	M/F	Maternal parents from F. Collins, CO, and Holdrege, NE	
91-118	tetraploid	F	Maternal parent from Dallas, TX	
378	pentaploid	F	Hebron, NE	
95-55	hexaploid	F	Nebraska	
Density	diploid	F	Unknown	
DP-2F	unknown	M/F	Unknown	
Perennial ryegrass	unknown		Unknown	
Blue grama	unknown		Aspermont, Texas (PI 477959)	

† Ploidy levels determined by Johnson et al. (2001).

‡ Flower counts observed were less than 10.

§ Ploidy level determined by Gulsen et al. (unpublished).

markers. Random amplified polymorphic DNA (RAPD) markers (Huff et al., 1993), allozymes, (Peakall et al., 1995), and sequence related amplified polymorphism (SRAP) (Budak et al., 2004) have been used to measure diversity and genetic relationships within and among buffalograss genotypes. These studies reported considerable RAPD, allozyme, and SRAP marker diversity among buffalograss germplasm. Molecular characterization of genetic variation within and among genotypes is

extremely beneficial in evolutionary biology studies and plant breeding programs. However, molecular marker development, identification, and use in buffalograss breeding lags behind other major crops. To develop new cultivars with desirable traits, accumulation of data on the genetic structure and characterization of germplasm is important. With these thoughts in mind, this study was initiated to evaluate the level of organelle DNA diversity among buffalograss genotypes, and to determine the

mode of inheritance of the chloroplast organelle, using cpDNA and mtDNA PCR-RFLPs.

MATERIALS AND METHODS

Fifty-eight buffalograss genotypes were evaluated, including 48 representing populations collected from diverse geographic locations in the Great Plains, four cultivars (Density, 378, Cody, and Bowie), and four other genotypes. Blue grama and perennial ryegrass were included as outgroups for comparison purposes (Table 1). The 48 genotypes were obtained from a collection located at Utah State University, Logan, UT. Two additional genotypes, PX3-5.1 and DP-2F, came from the same source. Cody and Bowie were planted from seed and genotypes were cloned and vegetatively propagated to represent these cultivars. Vegetative materials of NE 91-118, 378, Density, and NE 95-55 were obtained and cloned from the John Seaton Anderson Turfgrass Research Facility located near Mead, NE.

The 56 buffalograss genotypes were planted in 15-cm diam by 15-cm deep pots with a soil mixture of 350 g kg⁻¹ peat, 320 g kg⁻¹ vermiculite, 90 g kg⁻¹ soil, and 240 g kg⁻¹ sand. The greenhouse was maintained at 25 ± 1°C with supplemental light supplied by metal halide lamps on a 15-/9-h, day/night photoperiod. The pots were saturated biweekly with a soluble fertilizer (21N-3.5P-15K) at 200 mg L⁻¹ nitrogen. Sex expression was recorded visually every week for 6 mo from 1 Jan. to 30 July 2003.

The genotype DP-2F was identified as having one unique fragment for cpDNA primer *psbC-trnS* and restriction enzyme *HaeIII* on the basis of preliminary studies. DP-2F was used as the female parent in a half-sib cross with Genotypes 28, 32B, 102, and 234 used as males. The male sources did not carry the unique fragment identified in DP-2F. Crossing was done in the greenhouse by transferring bulked pollen from the male sources to DP-2F. Five seeds from the half-sib cross were germinated and the plants were maintained in the greenhouse as previously described. Progeny were evaluated for the unique banding pattern.

Total DNA was extracted from 40 to 50 mg young, frozen leaf tissue of individual genotypes by means of a DNA extraction kit, Puregene (Gentra Systems, Minneapolis, MN). DNA concentrations were measured with a fluorometer (Hoefer Scientific Ins., San Francisco, USA) and 5 ng µL⁻¹ DNA

templates were made in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

Six chloroplast and three mitochondrial primer pairs anchored at coding regions and tested previously for a range of plant species (Demesure et al., 1995) were used to amplify coding and noncoding regions (Table 2). Each 50-µL reaction consisted of 5 pM µL⁻¹ of each of the primer pairs, 200 µM of each of dNTPs, 5.0 µL of 10× PCR Buffer, 10 µL of Q Solution, 3 to 6 mM of MgCl₂ as a final concentration, 10 µL ddH₂O, and 1 unit of Taq polymerase (Qiagen, Valencia, CA), and 50 ng of template. Cycling parameters included the following: one cycle of 2 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 54°C, 2.5 min at 72°C, and for extension, one cycle 10 min at 72°C. Reannealing temperatures were modified for some primer pairs (Table 2).

Restriction enzymes were initially screened in this study as follows: *DraI*, *EcoRI*, *EcoRV*, *HaeIII*, *MboI*, *MseI*, *MspI*, *NlaIII*, *RsaI*, and *TaqI*. They were selected on the basis of observation of the rice chloroplast genome sequences as the closest relative among available complete sequences by the restriction analysis option of VECTOR NTI Suite 8 software, Invitrogen Corp., Carlsbad, CA. For the restriction digestion, 7.5 to 10 µL of PCR products were used on the basis of expected numbers and sizes of restriction fragments. Restriction fragments were separated on 2.5% (w/v) agarose gels containing ethidium bromide, and were photographed under the Gel Doc 2000 (Bio-Rad, Hercules, CA).

Each restriction fragment was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) version 2.1 software package (Exeter Software, Setauket, NY) (Rohlf, 1993). A similarity matrix was constructed on the basis of Dice's coefficient (Dice, 1945), which considers only one-to-one similarity matches between two taxa. The similarity matrix was used to construct a dendrogram using the unweighted pair group method arithmetic average (UPGMA) to determine genetic relationships among the germplasm studied. To provide a "goodness-of-fit" test for the similarity matrix to cluster analysis, the tree matrix was transformed to a matrix of ultrametric similarities (i.e., a matrix of similarities implied by the cluster analysis) by the CPH module. Then the MXCOMP module was used to compare the ultrametric similarities to the similarity matrix produced.

Table 2. Primer pairs, corresponding regions, and reannealing temperatures used in this study.

Primer pairs	Flanking genes	Reannealing temperature
Chloroplast primers		°C
5'-TAGTTTCTGTTTGTGGTGACAT-3' and 5'-AAGTAGTAGGATGGTTCTCAT-3'†	† <i>rbcl</i> -ATPase beta subunit	54
5'-TGCGGATCGAACATCAATTGCAAC-3' and 5'-GTGGTAGAAAGCAACGTGCGACTT-3'†	30S ribosomal protein S16- <i>rps16</i>	54
5'-ACGGGAATTGAACCCGCGCA-3' and 5'-CCGACTAGTTCGGGTTTCTGA-3'‡	<i>trnH-trnK</i>	56
5'-GGGTGCCCCGGGACTCGAAC-3' and 5'-CAACGGTAGAGTACTCGGCTTTTA-3'‡	<i>trnK</i> exon 1- <i>trnK</i> exon 2	60
5'-CGAGGGTTCGAATCCCTCTC-3' and 5'-AGAGCATCGCATTTGTAATG-3'‡	<i>trnS-trnT</i>	54
5'-GGTCGTGACCAAGAAACCAC-3' and 5'-GGTTCGAATCCCTCTCTCTC-3'‡	<i>psbC-trnS</i>	56
Mitochondria primers		
5'-GCATTACGATCTGCAGCTCA-3' and 5'-GGAGCTCGATTAGTTTCTGC-3'‡	<i>nad1</i> exon B- <i>nad1</i> exon C	54
5'-CAGTGGGTGGTCTGGTATG-3' and 5'-TCATATGGGCTACTGAGGAG-3'‡	<i>nad4</i> exon 1- <i>nad4</i> exon 2	54
5'-CACGGGTGCGCCCTCGCGTTCCG-3' and 5'-GTGTGGAGGATATAGGTGT-3'‡	<i>rps14-cob</i>	52

† Donald J. Lee, per. communication, 2002.

‡ Demesure et al. (1995).

Table 3. Chloroplast and mitochondrial DNA primer pairs, restriction enzymes, and number of restriction fragments scored in each digestion.

Primer pairs	Restriction enzymes									
	<i>MseI</i>	<i>TaqI</i>	<i>HaeIII</i>	<i>RsaI</i>	<i>EcoRI</i>	<i>MboI</i>	<i>MspI</i>	<i>EcoRV</i>	<i>DraI</i>	<i>NlaIII</i>
Chloroplast primers										
<i>rbcL</i> -ATPase beta subunit	6	3		7	6		3			
30S ribosomal protein S16- <i>rps16</i>	11	6		3			6		4	8
<i>trnH</i> - <i>trnK</i>	11	3				8		3	5	
<i>trnK</i> exon 1- <i>trnK</i> exon 2	5			3		3				
<i>trnS</i> - <i>trnT</i>		9		5			5		4	
<i>psbC</i> - <i>trnS</i>	13	5	14	7	4		11			
Mitochondria primers										
<i>nad1</i> exon B- <i>nad1</i> exon C		8	3			11				
<i>nad4</i> exon 1- <i>nad4</i> exon 2			8	3		6				
<i>rpS14</i> -cob		2				3				

RESULTS AND DISCUSSION

The number of scored fragments ranged from 2 (*rpS14*-cob with endonuclease *TaqI*) to 14 (*psbC*-*trnS* with endonuclease *HaeIII*) (Table 3). Of the 225 fragments scored from all genotypes studied, 189 were polymorphic (84%). The use of Vector NTI Software Suite 8 to detect endonucleases with higher number of cut sites greatly increased the number of restriction fragments detected without additional testing of restriction enzymes in the lab. This software enhanced efficiency and decreased costs for conducting this study. Total length of the amplified region was 14 250 bp (base pairs). These results compare similarly with those of *Citrus* and *Quercus* (Table 4) (Demesure et al., 1995; Gulsen and Roose, 2001). On the basis of the primer pairs used, similarities were found for size among amplified regions for the three genera. For example, *Buchloë*, *Citrus*, and *Quercus* had 2600, 2800, and 2580 bp fragments, respectively, on the basis of the chloroplast primer *psbC*-*trnS*.

Genetic similarities among all genotypes ranged from 0.41 to 1.0, with a mean similarity of 0.70 (Fig. 1). Buffalograss similarities with perennial ryegrass and blue grama were found to be considerably lower, 0.41 and 0.64, respectively. These results are consistent with current taxonomical distances; as expected, buffalograss was closer in genetic similarity to blue grama than perennial ryegrass. Since all scorable bands (i.e., polymorphic and non-polymorphic) were taken into account with the similarity calculations, the similarity values suggest true organelle DNA similarity among the accessions studied.

Cophenetic correlation between ultrametric similarities

Table 4. Pairs of cpDNA and mtDNA primers and PCR product sizes used in studies of *Buchloë*, *Citrus*, and *Quercus*.

Primer pairs	PCR product size (bp)		
	<i>Buchloë</i>	<i>Citrus</i> †	<i>Quercus</i> ‡
Chloroplast primers			
<i>rbcL</i> -ATPase beta subunit	900	—	—
30S ribosomal protein S16- <i>rps16</i>	950	—	—
<i>trnH</i> - <i>trnK</i>	1950	2000	1690
<i>trnK</i> exon 1- <i>trnK</i> exon 2	2600	2800	2580
<i>trnS</i> - <i>trnT</i>	1550	1750	1800
<i>psbC</i> - <i>trnS</i>	1600	1400	1500
Mitochondria primers			
<i>nad1</i> exon B- <i>nad1</i> exon C	1450	—	1550
<i>nad4</i> exon 1- <i>nad4</i> exon 2	1850	—	1700
<i>rpS14</i> -cob	1400	—	1640

† Gulsen and Roose, 2001.

‡ Demesure et al. (1995).

ties of the tree and a similarity matrix was high ($r = 0.99$) and suggests that the cluster analysis (Fig. 1) performed for this data strongly represents the similarity matrix. DNA extractions, PCR amplifications, and restriction analyses were repeated for all samples indicating polymorphism. The cpDNA and mtDNA PCR-RFLPs were 100% repeatable for these samples.

The UPGMA-generated dendrogram clustered all buffalograss genotypes together with the similarity value of 0.95 (Fig. 1). Out of 56 buffalograsses studied, nine (16%) were discriminated from the rest. The 47 genotypes representing various ploidy levels from diverse geographic regions did not differ for cpDNA or mtDNA PCR-RFLPs. In this study, the small variation in the maternally inherited genome indicates a narrow base for the maternal origin of buffalograsses, perhaps single or few origins of cpDNA and mtDNA (Fig. 2). However, the paternal origin remains unclear and would require further detailed study involving related species and a combination of nuclear and cytoplasmic genome markers to clarify.

The nine genotypes with polymorphic markers differed by only a few markers out of the 225 scored fragments from the main group. Genotypes 7 and DP-2F had a similarity value of 0.97 and differed with the same markers (i.e., *psbC*-*trnS* primers and endonuclease *HaeIII* combination) from the rest of the accessions (Fig. 2). These two accessions, most likely, have a restriction site for this endonuclease at 380 bp and had two additional restriction fragments. DP-2F was used as maternal parent in a half-sib cross with four, hexaploid male genotypes results of which are described later in this manuscript.

Two diploid genotypes, 45B and 46, collected from nearly the same location (i.e., 34°37'N and 34°56'N) differed by 5 markers. The Genotype 78C, also a diploid, had only one marker differentiating it from the other genotypes (i.e., *trnK* exon 1-*trnK* exon 2 with the endonuclease *MseI*). It had a similarity value of 0.98 to the 47 genotypes, which are referred to as the main group. Another group containing Genotypes 87A, 187, 46, and Bowie had 5 different markers from the main group. This group likely evolved their cytoplasmic genomes independently from the others. Density, a female diploid, had six different restriction fragments with a similarity value to the main group of 0.95. Cytoplasmic compatibility is important in breeding programs to produce

Fig. 1. UPGMA dendrogram of 56 buffalograsses and two outgroups based on analysis of 36 monomorphic and 189 polymorphic fragments from 6 cpDNA and 3 mtDNA sequences.

viable seeds. Results from this study indicate no apparent cpDNA/mtDNA variation among many of the genotypes, which suggests that cytoplasmic incompatibility within a ploidy level cross is unlikely to be a concern in buffalograss. However, further research is needed to support this conclusion.

In a recent study, Columbus (1999) speculated that the genus *Buchloë* was relatively similar to the genus

Bouteloua and suggested that buffalograss should be included in the genus *Bouteloua*. His conclusion was based on one region of cpDNA sequence and a nuclear ribosomal internal transcribed spacer region. Although the similarity ratio between buffalograss and blue grama from Columbus (1999) are consistent with the results from this study, the genetic similarity value 0.64 appears to be too low to support reclassification of buffalograss

Fig. 2. CpDNA restriction fragments of buffalograss accessions and two outgroups, following amplification with primers *psbC-trnS* and digestion with restriction enzyme *HaeIII*.

with blue grama. More comprehensive study is needed before reclassifying buffalograss would be justified.

Of the 56 buffalograss genotypes from this study, 21 exhibited male and 24 female characteristics, seven genotypes were monoecious, and four did not flower (Table 1). The 21 male to 24 female genotypes were consistent with the findings reported by Quinn and Engel (1986). They found an equal ratio of males to females expressed in the natural populations they studied. Sex expression ratio from this study is offered as evidence that the population used was randomly selected. Huff and Wu (1987) reported that dioecious sex forms of buffalograsses were not affected by environmental conditions, and monoecious forms were genetically determined despite some potential modification by environment and cultural practices. Genetic diversity detected in this study had no relationship with genotype gender or ploidy level. However, the lack of apparent relationship needs more study for verification, since the population size and marker numbers used in this study were insufficient to warrant such a conclusion.

The cpDNA was found to be maternally inherited in buffalograsses on the basis of cpDNA primers *psbC-trnS* and restriction enzyme *HaeIII*. As mentioned earlier, DP-2F had a unique fragment from the other genotypes studied (Fig. 3). It apparently gained an additional restriction site for 380-bp fragments. This marker was also present in the five half-sib progenies developed from crossing Genotypes 28, 32b, 102, and 234 with DP-2F. This response provides the first evidence for maternal inheritance of cpDNA in buffalograsses. Coriveau and Coleman (1988) reported maternal inheritance of cpDNA in studies involving 18 grass species. Verification of cpDNA inheritance is important for future studies, such as ones targeting cytoplasmic male sterility and transgenic buffalograsses. Stewart et al. (2003) indicated there were advantages for chloroplast transformation, which included uniparental transmission of the chloroplast genome and possible elimination of unintended gene flow from transgenic plants to other grasses via pollen.

As cytoplasmic genome markers, cpDNA and mtDNA PCR-RFLPs may shed light on the origin of one parent, which is especially true for polyploid plants with uniparental cytoplasmic inheritance (Soltis et al., 1992). This study is the first to demonstrate cpDNA diversity and cpDNA and mtDNA PCR-RFLP diversity among native buffalograss populations. It is also the first to report a low level of polymorphism, which possibly suggests one or very few maternal origins for the genus *Buchloë*. Interpretation of this polymorphism may play a role in understanding buffalograss germplasm origin and diversity. These data also suggest the potential for compatible

Fig. 3. Verification of maternal inheritance of cpDNA in buffalograsses by using cpDNA PCR-RFLPs with primers *psbC-trnS* and *HaeIII*. The genotype names are as follows: (1) representative of male parents 28, 32B, 102, 234; (2) undigested PCR fragment with primers; (3) DP-2F as maternal parent; (4-8) half-sib progenies; and (St) standard DNA marker.

cytoplasmic interactions in most buffalograss crosses within the same ploidy level. However, further research on actual crosses is needed to support this conclusion.

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