Intra-population genetic diversity of *Buchloe dactyloides* (Nutt.) Engelm (buffalograss) determined using morphological traits and sequence-related amplified polymorphism markers

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Abstract

Genetic variation and diversity are prerequisites for improvement of buffalograss breeding. To assess the within-population genetic diversity of buffalograss, seven morphological traits were evaluated to confirm the variations at the morphological level. The principal component analysis revealed that leaf length, leaf width and stolon branches had a significant contribution to the total variation. The first three principle components showed 72.55% variation. The DNA analysis performed using SRAP primers was used for deducing the diversity at the DNA level. A total of 125 bands were obtained with 8 selected SRAP primer pairs, of which 119 (95.2%) were polymorphic. The polymorphic information content ranged from 0.94 to 0.97 with a mean of 0.96; the marker index ranged from 10.34 to 18.43 with an average value of 14.28. The individuals were successfully assigned to two major groups according to sex in the PCoA and UPGMA dendrogram based on SRAP data, while the individuals could not be grouped based on morphological traits, and the two markers were not significantly correlated ($r=0.0753$, $P=0.8489>0.05$). The molecular data revealed that sex is a critical factor and that female and monoecious plants could be chosen as parents to breed new varieties.

Keywords Buffalograss · Morphological traits · SRAP markers · Genetic variation · Plant sex

Introduction

Buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) is a native dioecious grass of the North American Great Plains. It is a stoloniferous, perennial, open-pollinated, warm season (C4) turfgrass and is highly heterogeneous (Beetle 1950; Budak et al. 2004a). Buffalograss has a series of ploidy levels; diploid, tetraploid, pentaploid, and hexaploidy species have been reported (Reeder 1971). Since buffalograss is a low maintenance species, it has become the most widely

cultivated warm season turfgrass in northern China. Thus, it requires advanced breeding and improvement programs to develop varieties that could be used more widely. Assessment and evaluation of genetic diversity is an essential step for genetic improvement of plants (Grover and Sharma 2014). However, the knowledge of within-population genetic variation of buffalograss is not well understood, which is why it is important for its future improvement.

Genetic variation and diversity are prerequisites for improvement of the breeding and cultivation of plants; understanding its genetic assortment is very important (Verma et al. 2017). It can be obtained from morphological traits or using molecular markers (Mohammadi and Prasanna 2003; Deepa et al. 2018). Morpho-agronomic traits have been the primary variables used for studying genetic diversity (Liu 1997; Bisht et al. 1998). Morphological traits are essential for preliminary evaluation and for assessment of genetic diversity, and it is essential to utilize the germplasm with sufficient genetic variation, which reflects these traits (Ahmed 2017). Variation in morphological traits is limited; environmental and growing conditions also affect these features. At the same time,

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molecular markers offer a complementary estimation of genetic diversity because they are independent of the perplexing effects of environmental factors. The combination of morphological traits and molecular markers is useful and reliable under different environmental conditions (Liu et al. 2016; Taylor et al. 2006; Turchetto et al. 2014).

Many studies have shown that there are significant differences among varieties of buffalograss at the molecular level (Huff et al. 1993; Budak et al. 2004b, 2005; Serba 2009; Rostamiahmadvandi et al. 2013; Jain et al. 2017; Gulsen et al. 2005). Among all molecular markers studied, rapid amplified polymorphic DNA (RAPD) is one of the simplest but provides poor consistency, low reproducibility and limits its effectiveness (Roodt et al. 2002). Although the restriction fragment length polymorphism (AFLP) technique has good reproducibility and reveals high levels of polymorphism, its operation is very elaborate, and the costs are relatively high (Vos et al. 1995). The method based on analysis of SSRs (simple sequence repeats) and ISSR (Inter-simple sequence repeat) requires prior knowledge of the genome sequences to design specific polymerase chain reaction (PCR) primers for amplification (Jones et al. 2009; Pradeep Reddy et al. 2002). In comparison, SRAP (sequence-related amplified polymorphism) markers can overcome most of these limitations (Li and Quiros 2001; Ferriol et al. 2003). SRAPs are co-dominant markers and target open reading frames (ORFs), and are simple markers with a high efficiency that can be used in many aspects of plant molecular research (Ferriol et al. 2003; Li and Quiros 2001). SRAP markers are based on two-primer amplifications, and each primer is 17 or 18 nucleotides long. The primers have a core sequence (13 or 14 bases) at the 5' end, and the first 10 or 11 bases are non-specific; these non-specific nucleotides are followed by in the forward primer by CCGG and in the reverse primer by AATT. The three nucleotides at the 3' end are selective. It has been used in the evaluation of genetic diversity and phylogenetic relationships of *Vicia amoena* (Liu et al. 2013), grasses in the *Festuca-Lolium* complex (Cheng et al. 2016), *Onobrychis sativa* (Sedeh 2017) and ornamental purslane (Jia et al. 2017). Moreover, SRAPs were considered more effective for closely related cultivars of buffalograss and more aligned with the morphological variations (Budak et al. 2004a; Ferriol et al. 2003).

Although previous research has provided rich data regarding genetic diversity among the populations of buffalograss, studies that investigated the levels of variation within buffalograss populations are limited (Liu et al. 2008). In the present study, considering the advantages of morphological and SRAP markers, we utilized these two methods to describe the within-population genetic variability of buffalograss.

Materials and methods

Plant materials

A total of 188 established plants (Cultivar: Texoka) were initially selected as experimental materials. To generate a set of standards for further analyses, the sex expression of the plants was investigated in 2010 and 2013 in the experimental field of China Agricultural University (Hebei province, China). Ultimately, a total of 47 individuals were collected for this study, including 18 female plants (F, F1-F18), 19 male plants (M, M19-M37) and 10 monoecious plants (MO, MO38-MO47). These individuals were chosen because their sexual expression has remained stable over 3 years and they were broadly representative of the morphological and genetic diversity of the buffalograss population.

Morphological analysis

Field data were recorded in the experimental field of China Agricultural University (Hebei Province, China) during the buffalograss growing season (from April to November 2013). Seven morphological traits were scored. Briefly, turf quality (TQ) was scored according to guidelines from the National Turfgrass Evaluation Program (NTEP, <http://www.ntep.org/>). The measured traits were leaf length (LL, cm), leaf width (LW, cm), plant height (PH, cm), the average creeping rate of stolon (ACR, cm·d⁻¹), and a number of stolon branches (SB, integer). The calculated variable was the green period (GP, day), which is determined based on the days between the spring green-up stage and the withering stage (data not shown). The measurements of these traits are provided in Supplemental Table 2. The ACR was calculated based on the stolon length increased during the experimental period (May 15–July 15) and the number of experimental days (N), using the formula $ACR = (L_2 - L_1)/N$, where L_2 is the stolon length measured on July 15 and L_1 is the length measured on May 15. The measurements for all 47 individuals were replicated three times, and each value represents an average of three replications.

Morphological data were analyzed for mean, range, and coefficient of variance. Spearman's correlation of the seven morphological variables was carried out with the IBM Statistical Product and Service Solutions (SPSS) version 22 software package (Allen et al. 2014). Principal component analysis (PCA) was performed, and the results were graphically expressed in 3D plots in R version 3.5.0 (<https://www.r-project.org/>) for analyzing the distribution pattern of the 47 individuals. To delineate clusters

of morphologically similar individuals, unweighted pair group method with arithmetic average (UPGMA) cluster analysis was performed based on a Euclidean distance matrix with MEGA7 (Kumar et al. 2016). The Euclidean distance matrix was obtained from the NTSYS PC version 2.1 software package (Rohlf 2002). Both were used to identify the relationship between individuals and sex.

Molecular analysis

DNA extraction

Young leaf samples were obtained and stored at -20°C until use. The total DNA was extracted from approximately 100 mg of frozen leaf tissue from individual plants using a DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing).

SRAP-PCR

SRAP markers reported in previous studies (Budak et al. 2004b; Zhou et al. 2011) were used in this study. In all, 216 combinations of 12 forward primers and 18 reverse primers (supplied by BioBasic Inc., Shanghai, China) were used (Supplemental Table 1).

SRAP amplifications were performed in 25 μL reaction volumes containing 1 μL of genomic DNA (50 ng/ μL), 12.5 μL of 2 $\times$ Tap PCR mix (0.1 U Taq Polymerase/ μL ; 500 μM each dNTP; 200 mM Tris-HCl, pH 8.3; 100 mM KCl; 3 mM MgCl_2), 2 μL of SRAP primers (1 μL of forward primer and 1 μL of reverse primer) and 9.5 μL of ddH₂O. Amplifications were carried out using a Biometra thermocycler programmed for five cycles of 1 min at 94°C , 1 min at 35°C , and 1 min at 72°C ; 35 cycles of 1 min at 94°C , 1 min at 50°C , and 1 min at 72°C , followed by a final extension at 72°C for 10 min. The PCR products were resolved using electrophoresis on 8.0% polyacrylamide gels using a vertical gel apparatus (Liuyi, China) for 1.5 h or 2 h. Amplified fragments were visualized using silver staining and photographed with a digital camera.

Data scoring and statistical analysis

The SRAP bands were scored visually for their presence (1) or absence (0) to form a binary matrix. Only reproducible and unambiguous SRAP fragments were used for scoring. Primer banding characteristics including the total number of scored bands, number of polymorphic bands (NPB), and percentage of polymorphic bands (PPB) were obtained from the binary matrix. To compare the efficiency of primers, polymorphic information content (PIC), as a marker discrimination power, was computed using the formula $\text{PIC} = 1 - \sum P_i^2$, where P_i is the frequency of the i -th allele at a given locus (Powell et al. 1996). Marker index (MI) was

calculated by applying the formula given by Prevost and Wilkinson (1999).

Principal coordinate analysis (PCoA) was conducted using GenAlEx version 6.5 (Peakall and Smouse 2012). The results were converted to a biplot to reveal the distribution pattern of the individuals in the two-dimensional space. Nei's similarity coefficient and Nei's genetic distance were calculated using the NTSYS PC version 2.1 software package (Rohlf 2002). The unweighted pair-group method with arithmetic average (UPGMA) cluster analysis was performed based on Nei's genetic distance matrix with MEGA7 (Kumar et al. 2016).

Correlation between the distance matrices obtained with both marker types (morphological and SRAP) was estimated by means of the Mantel test in NTSYS pc-2.1 (Mantel 1967).

Results

Morphological variation

Forty-seven individuals were evaluated for morphological variations using seven morphological traits. The LL values ranged from 13.30 to 24.57 cm, the LW values ranged from 0.15 to 0.23 cm, and the maximum PH was 30.46 cm, with a minimum of 14.03 cm. The TQ score ranged from 2.32 to 5.10. The GP ranged from 128 to 150 days. The ACR ranged from 0.00 to 1.08 cm/day, and the number of stolon branches was from 1 to 2. The morphological traits showed a wide variation among the individuals and among the sex groups, as was evident from analysis of variance, range, mean and coefficient of variation for all the morphological traits measured (Table 1). Although there was an obvious difference in the morphological traits among female, male and monoecious plants, we did not find statistically significant differences (data not shown).

In the principal component analysis (PCA), the first three principal components (PCs) explained a total of 72.55% of the morphological variation. PCA showed that the first principal component (PC1) explained 31.26% of the total variance, followed by PC2 (25.55%) and PC3 (15.75%), as shown in Table 2. The principal component analysis also revealed that leaf length, leaf width, and stolon branches had a significant contribution to the total variation. However, the individuals could not be grouped clearly based on any one trait, neither in the PCA 3D plot or in the UPGMA dendrogram. The results showed a combination of all of the traits (Figs. 1, 2).

The Spearman rank correlation coefficients between pairs of variables were calculated; the results are shown in Table 3. Most traits were significantly correlated with each other. Otherwise, LW showed extremely significant correlation with LL ($P < 0.01$) but was not significantly correlated

Table 1 Variability in seven morphological characters among the buffalograss individuals

Traits	Total						Female		Male		Monoecious	
	Min	Max	Mean	SD	SE	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Leaf length	13.30	24.57	17.88	2.23	0.33	12.44	18.59	12.89	17.50	11.12	17.35	11.90
Leaf width	0.15	0.23	0.18	0.02	0.00	8.99	0.19	7.93	0.18	10.50	0.18	5.68
Plant height	14.03	30.46	22.82	3.69	0.54	16.18	21.89	12.60	23.96	15.31	22.33	20.33
Turf quality	2.32	5.10	4.05	0.54	0.08	13.42	4.14	9.88	4.20	10.43	3.62	19.19
Green period	128.00	150.00	138.26	5.06	0.75	3.66	138.78	3.50	137.68	3.87	138.40	3.47
Average creeping rate of stolon	0.00	1.08	0.78	0.19	0.03	24.41	0.78	26.05	0.77	27.89	0.83	11.28
Number of stolon branches	1.00	2.00	1.23	0.42	0.06	34.31	1.06	21.70	1.32	35.33	1.40	34.99

Table 2 Contribution percentages and morphological traits associated with the principal components of 47 individual plants and their Eigen vectors

Principal component	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6	PC-7
Eigen value	2.1880	1.7882	1.1023	0.7297	0.4974	0.4093	0.2851
Explained proportion of variation (%)	31.26%	25.55%	15.75%	10.42%	7.11%	5.85%	4.07%
Cumulative proportion of variation (%)	31.26%	56.80%	72.55%	82.98%	90.08%	95.93%	100.00%
Traits	Eigen vectors						
Leaf length	−0.5705	0.0279	0.2412	0.0109	0.1390	0.5870	−0.5016
Leaf width	−0.3545	−0.2205	0.6066	−0.4599	0.0883	−0.2256	0.4332
Plant height	−0.5152	0.2050	−0.3139	0.3491	−0.1840	0.1960	0.6324
Turf quality	−0.3778	−0.1688	−0.6022	−0.2917	0.4932	−0.3435	−0.1410
Green period	0.3117	−0.5441	−0.0791	0.0508	0.4520	0.5343	0.3289
Average creeping rate of stolon	−0.1837	−0.4990	0.2030	0.7094	0.0449	−0.3866	−0.1459
Number of stolon branches	0.0993	0.5788	0.2556	0.2749	0.6996	−0.1149	0.1075

Fig. 1 Principal component analysis of 47 buffalograss individuals in three-dimensional space based on the morphological data

with other traits. LL and PH showed insignificant correlations with ACR and SB ($P > 0.05$), respectively. Moreover, the relationship between TQ and GP was not significant ($P > 0.05$).

Molecular variation

A total of 125 scored bands were amplified using 8 SRAP primer combinations screened across 47 individuals, out of which 119 (95.2%) were polymorphic (Supplemental Fig. 1). The number of bands amplified by each primer combination ranged from 12 (for combinations ME-1/EM-16 and ME-9/EM-7) to 19 (ME-7/EM-5), with an average of 15.6 bands. The number of polymorphic bands for each primer combination varied from 11 (ME-1/EM-16 and ME-9/EM-7) to 19 (ME-7/EM-5), with an average of 14.9 bands. The percentage of polymorphic bands revealed different levels of polymorphisms ranging from 88.24% (ME-11/EM-4) to 100% (ME-7/EM-5 and ME-8/EM-12). The PIC (polymorphism information content) ranged from 0.94 (ME-9/EM-7) to 0.97 (ME-7/EM-5 and ME-8/EM-13), with an average of 0.96 per primer combination (Table 4). Moreover, the average marker index (MI) was 14.3, indicating that SRAP markers could effectively detect the within-population genetic diversity of buffalograss (Liu et al. 2013).

The genetic similarities among all individuals ranged from 0.45 (F15–MO46) to 0.78 (MO44–MO45), with an average value of 0.62 (Supplemental Table 3). This reflected

Fig. 2 UPGMA dendrogram showing the relationships of 47 buffalograss individuals on the basis of morphological data

the presence of high genetic variability among the individuals under study. The gross structure of the dendrogram and the grouping of individuals into clusters showed a similarity with the sex obtained with that of SRAP data. The

individuals were observed into two major clusters. Cluster A consisted of 39 individuals (female plants, male plants, and 2 monoecious plants) and Cluster B consisted of 8 monoecious plants. Further, the subcluster of female plants and the cluster of monoecious plants were found to span the extremes of the dendrogram (Fig. 3).

The PCoA (principal coordinate analysis) of SRAP data grouped the accession in a two-dimensional space with a clustering pattern synonymous with the dendrogram obtained with SRAP (Fig. 4). Analysis revealed that the first two principal coordinates accounted for approximately 12.16% of the total variance. PCoA divided the cultivars into two groups. Group A included all the female and male plants, and group B consisted of all the monoecious plants.

Morphological–SRAP correlation analysis

The distance matrices from morphological traits and SRAP markers were compared for their correspondence using a Mantel test in NTSYS pc-2.1. Figure 5 shows that the correlation between the coefficient matrices for the morphological and SRAP data was not significant ($r=0.0753$, $P=0.8489 > 0.05$).

Discussion

The present study reveals a high degree of genetic diversity within buffalograss populations using morphological and SRAP markers. The experimental material “Texoka” is a synthetic variety derived from ten selected clones, four female, and six male, which originate from buffalograss in Texas, Oklahoma and Kansas (Voigt et al. 1975). The complex genetic background could be helpful for maintaining high genetic diversity within the population. Complete knowledge and understanding of plant genetic diversity can aid in effective management and genetic improvement (Jia et al. 2017).

Morphological analysis has always been valuable for plant breeding programs (Boczkowska et al. 2014). In the present study, the morphological measurements of 47 individuals revealed a wide within-population variation in morphological characters, which is in accordance with the studies by Huff et al. (1993) and Liu et al. (2008). Moreover, the principal component analysis revealed that leaf length, leaf width, and stolon branches had a significant contribution to the total variation, which will be expected to provided high level of gene availability for transfer during the breeding programs (Aliyu et al. 2000; Jain et al. 2017). The variation of morphological characteristics makes the materials potential donors for breeding.

Even though the morphological traits are generally employed to estimate genetic diversity, the traits could

Table 3 The Spearman rank correlation coefficients among morphological variables

	LL	LW	PH	SB	ACR	TQ	GP
Leaf length (LL)	1						
Leaf width (LW)	0.421**	1					
Plant height (PH)	0.466**	0.004	1				
Number of stolon branches (SB)	−0.024	−0.1	−0.009	1			
Average creeping rate of stolon (ACR)	0.201	0.245	0.071	−0.296*	1		
Turf quality (TQ)	0.294*	0.019	0.467**	−0.330*	0.087	1	
Green period (GP)	−0.338*	−0.124	−0.456**	−0.380**	0.307*	0.057	1

*Correlation is significant at the level of $P < 0.05$ **Correlation is significant at the level of $P < 0.01$ **Table 4** Number of total and polymorphic fragments, polymorphism information content (PIC), and marker index (MI) of all individuals, female plants, male plants, and monoecious plants

Combination	Total number of scored bands	Pol*	% Pol**	PIC	MI
ME-1/EM-16	12	11	91.67	0.95	10.45
ME-3/EM-1	15	15	100.00	0.96	14.40
ME-4/EM-3	17	16	94.12	0.96	15.36
ME-7/EM-5	19	19	100.00	0.97	18.43
ME-8/EM-12	15	15	100.00	0.96	14.40
ME-8/EM-13	18	17	94.44	0.97	16.49
ME-9/EM-7	12	11	91.67	0.94	10.34
ME-11/EM-4	17	15	88.24	0.96	14.40
Total	125	119	95.20	0.96	14.28

*Number of polymorphic fragments

**Percentage of polymorphic fragments

be influenced by the environmental conditions and growing conditions. As a result, the individuals have also been studied using molecular markers. To have a comprehensive idea of the variability within the population of buffalograss, SRAP analysis was carried out. Eight SRAP primer pairs detected a total of 125 scored bands, out of which 119 (95.2%) were consistent with the genotypes of buffalograss (Budak et al. 2004b). Moreover, the PIC value in the present study for SRAP markers ranged from 0.94 to 0.97, with an average of 0.96 (Table 4), and the average marker index (MI) was 14.3. The results indicated that SRAP markers are efficient tools for estimating the genetic variability and the genetic relationships among individuals within buffalograss populations. The most diverse individuals were F15 from female plants and MO46 from monoecious plants, with the minimum genetic similarity value of 0.45. The genetic distance of these two sex groups indicates high diversity in different sex plants prevailing within buffalograss populations, which could provide a reference for parental selection in future breeding programs.

Various clustering methods estimate genetic relatedness of analyzed plants within buffalograss populations.

The PCoA analysis and UPGMA dendrograms based on SRAP markers grouped all individuals into two major groups corresponded to sex which revealed sex is the critical factor to demonstrate the genetic relationship among the individuals. However, the individuals could not be grouped by sex based on morphological traits or any morphological characters. There was less correlation between the divergence of morphological traits and the data obtained using molecular markers (Mantel test). Similar results were obtained earlier in buffalograss using ISSR markers (Liu et al. 2008). The results suggested that the morphological variation may be determined by environmental and genetic factors as reported for *Vitis vinifera* L. (Seyedimoradi et al. 2012), *Phaseolus vulgaris* L. (Kumar et al. 2014) and *Secchium edule* (Jacq.) Swartz (Jain et al. 2017). The preliminary discussion above suggests that the morphological variation may be determined mostly by environmental factors. Moreover, all the experimental materials used in this study were within one population and grown in the same environmental conditions. Morphological traits are associated with a relatively small number of specific gene loci; there may be potential differences in the analysis of large amounts of molecular data (Diederichsen 2009; Jain et al. 2017), suggesting that the genetic diversity can be reflected more accurately by SRAP markers in this study. It also confirms the importance of molecular analyses in addition to morphological data, in future studies, for detecting genetic variation within buffalograss populations.

In conclusion, our results revealed that there was abundant genetic variability within a population of buffalograss and sex is a critical factor for the breeding system. The female and monoecious plants could be chosen as parents to breed new varieties. The findings make it possible to understand the genetic relationship between individuals within a population and will be beneficial for future breeding programs.

Fig. 3 UPGMA dendrogram showing the relationships of 47 buffalograss individuals based on SRAP data

Fig. 4 Principal coordinate analysis of 47 buffalograss individuals in two-dimensional space based on the SRAP data

Fig. 5 Correlation analysis between genetic distance based on morphological and SRAP data

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

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