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Nuclear Genome Diversity and Relationships among Naturally Occurring Buffalograss Genotypes Determined by Sequence-related Amplified Polymorphism Markers

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***Abstract*. Buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.] has the potential for increased use as a turfgrass species due to its low maintenance and water conservation characteristics. This study was conducted to estimate diversity and relationships among naturally occurring buffalograss genotypes based on the nuclear genome, using sequence-related amplified polymorphism (SRAP) markers. The 56 genotypes studied represented five ploidy levels collected from diverse geographic locations in the North American Great Plains. In addition, blue grama [*Bouteloua gracilis* (*H.B.K.*) Lag. Ex Steud.] and perennial ryegrass (*Lolium perenne* L.) were included as outgroups. Twenty-five combinations of forward and reverse primers were used. Ninety-five intensively amplified markers were scored and used to infer diversity and relationships among the genotypes. All buffalograss genotypes were discriminated from each other with similarity values ranging from 0.70 to 0.95. Principal component analysis (PCA) suggested that the 56 genotypes could be reduced to 50 due to high similarity levels among some of the genotypes. The distance between buffalograsses, blue grama, and perennial ryegrass were consistent with current taxonomical distances. This research indicates that SRAP markers can be used to estimate genetic diversity and relationships among naturally occurring buffalograss genotypes.**

Buffalograss is used for home lawns, road sides, golf course roughs, cemeteries, pastures, and rangelands (Beard, 1973). Recently, considerable attention has been given buffalograss as a turfgrass species, due to its drought resistance and low maintenance requirements (Riordan, 1991). Its aggressive stoloniferous growth habit and dense sod-forming characteristic help prevent wind and water soil erosion (Wenger, 1943). Buffalograss is native and found to the shortgrass prairie of North America, and is found from Mexico to Canada and from the eastern slope of the Rocky Mountains to the Mississippi River Valley. Buffalograss is mostly dioecious, cross-pollinated, and highly heterogeneous with no evidence of self-pollination (Wu and Lin, 1984).

Current knowledge of the genetic basis for buffalograss agronomic traits, and level of diversity and relationships among buffalograss genotypes is limited. Buffalograss is comprised of a morphologically indistinguishable polyploid series, with a base chromosome number of x=10, and diploid, tetraploid, pentaploid, and hexaploid plants have been reported (Johnson et al., 1998; Reeder, 1971). Diploids occur only in central Mexico and southeastern Texas, while hexaploids are found growing throughout the Great Plains region (Huff et al., 1993; Johnson et al., 2001), and plants collected in Kansas, Nebraska, and Colorado were hexaploids and tetraploids (Johnson et al., 2001; Reeder, 1971).

Genome evolution in polyploids was recently reviewed (Wendel, 2000). Genes duplicated in ploidy level may retain their original or similar function, undergo diversification in protein function and regulation, or may become silenced through mutational and epigenetic interactions. These changes may affect DNA structure, which allows greater diversity in higher ploidy levels. Hence, gene diversification in polyploids may lead to increased polymorphism detected by molecular markers such as randomly amplified polymorphic DNA (RAPD) markers, simple sequence repeats (SSRs), and restriction fragment-length polymorphisms (RFLPs). Johnson et al. (2001) found higher adaptability in hexaploid buffalograsses, which could be explained by gene diversification as suggested by Wendel (2000).

Molecular markers for classification of genotypes are abundant, and unlike morphological markers are not affected by environment (Staub and Serquen, 1996). Molecular markers can be used to identify unique genotypes and associated agronomic traits. Considerable RAPD marker diversity was found among diploid buffalograsses at the inter- and intra-population level (Huff et al., 1993). Recently, sequence-related amplified polymorphism (SRAP) markers were used to assess diversity and relationships among selected buffalograss clones and cultivars (Budak, 2004a, 2004b). Although diversity was reported among selected manipulated clones and cultivars, limited information on naturally occurring buffalograss genotypes is available.

SRAP markers were recognized as a new and useful molecular marker system for mapping and gene tagging in *Brassica* (Li and Quiros, 2001). SRAP markers are polymerase chain-reaction (PCR)-based markers that amplify open reading frames (ORFs) and produce a number of codominant markers per amplification. SRAPs use forward and reverse primers, 17 or 18 nucleotides long, and primers consist of a core sequence of 13 or 14 bases, at the 5', CCGG in the forward primer and AATT in the reverse primer, targeting ORFs in genomic sequences. This core sequence is followed by three selective nucleotides at the 3' end of each primer. SRAP markers are more consistent and repeatable than RAPD markers, and are less labor intensive and time consuming to produce than amplified fragment-length polymorphisms (AFLPs) (Budak et al., 2004b; Li and Quiros, 2001; Welsh and McClelland, 1990). Understanding the genetic structure and germplasm characterization data is essential for efficient plant breeding programs. This study was initiated to estimate genetic diversity and relationships among naturally occurring buffalograss genotypes based on SRAP markers.

Materials and Methods

Plant materials. Fifty-six buffalograss genotypes and two outgroups, blue grama and perennial ryegrass were evaluated in this study (Table 1). The 56 buffalograsses included

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53 naturally occurring genotypes, 2 selected clones from each of cultivars ‘Bowie’ and ‘Cody’ populations, and a vegetative cultivar, ‘Density’. The 53 genotypes were diverse in their ploidy level and geographic origin, and were obtained from a plant collection maintained at the University of Nebraska-Lincoln. All genotypes were vegetatively cloned using a single stolon from each population, therefore, each genotype may not represent original population.

Buffalograss genotypes were placed in plant adaptation regions (PARs) developed by Vogel et al. (2005) based on longitudes and latitudes from which the genotypes were sampled (Fig. 1). PARs were developed by overlaying ecoregions (Bailey 1998) and USDA Plant Hardiness Zones (Cathey, 1990). Thermal and moisture zones and subzones define conditions for plant growth in a geographical area characterize ecoregions. Plant Hardiness Zones have been developed to classify plants to hardiness or survival zones, which are latitudinal climatic zones modified by nonlatitudinal geographic features.

Table 1. Buffalograss germplasm studied, ploidy levels, PARs, and number of bands scored for each genotype.

Buffalograsses	Ploidy[z]	PAR	N. bands[x]
Density	Diploid	Unknown	29
PX3 5.1	Triploid[y]	Unknown	30
45B	Tetraploid	315	31
66	Tetraploid	331	31
98	Tetraploid	313	31
46	Tetraploid	315	33
87A	Tetraploid	315	---[w]
119	Tetraploid	315	31
143	Tetraploid	321	---
97	Tetraploid	331	---
132	Tetraploid	321	31
174	Tetraploid	321	34
47	Tetraploid	315	---
Prestige	Tetraploid	Maternal parent from Dallas, Texas	---
378	Pentaploid	Hebron, Neb.	---
49	Pentaploid	315	35
20B	Pentaploid	331	34
68	Pentaploid	331	33
84	Hexaploid	315	33
78C	Hexaploid	315	-
70	Hexaploid	331	33
2	Hexaploid	315	---
4A	Hexaploid	315	---
83	Hexaploid	315	35
188	Hexaploid	331	34
170	Hexaploid	321	31
77	Hexaploid	331	34
209	Hexaploid	315	38
126	Hexaploid	315	---
123	Hexaploid	315	30
193	Hexaploid	321	37
187	Hexaploid	315	35
223A	Hexaploid	255	33
203	Hexaploid	331	32
34	Hexaploid	331	33
184A	Hexaploid	315	33
153B	Hexaploid	315	33
136	Hexaploid	321	35
28	Hexaploid	331	34
189A	Hexaploid	331	---
89	Hexaploid	315	32
178	Hexaploid	315	35
152	Hexaploid	321	31
17	Hexaploid	332	33
234	Hexaploid	321	31
196	Hexaploid	315	34
240	Hexaploid	315	31
7	Hexaploid	311	32
137	Hexaploid	313	32
32B	Hexaploid	331	33
102	Hexaploid	331	33
10B	Hexaploid	315	34
95-55	Hexaploid	Nebraska	33
DP-2F	Hexaploid [y]	Unknown-	37
04-038[v]	Hexaploid	Maternal parents from F. Collins, Colo., and Holdrege, Neb.	33
04-046[u]	Hexaploid	Maternal parents from Ariz., Nebraska and Okla.	32
Perennial ryegrass	Unknown	Unknown	---
Blue grama	Unknown	Aspermont, Texas (PI 477959)	---

[z] Ploidy levels determined by Johnson et al. (2001).

[y] Gulsen et al., unpublished.

[x] Number of bands scored for each genotype.

[w] Genotypes with partially missing data.

[v] A clonal selection from a population of the seeded-type ‘Cody’.

[u] A clonal selection from a population of the seeded-type ‘Bowie’.

DNA extraction. Total DNA was extracted from 40 to 50 mg young frozen leaf tissue of individual genotypes, using a DNA extraction kit, Puregene (Gentra Systems, Minn.). DNA concentration was measured with a fluorometer (Hoefer Scientific Ins., San Francisco) and 5 $\text{ng}\cdot\mu\text{L}^{-1}$ DNA templates were made using TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

PCR parameters and gel analysis. The 25 combinations of 13 forward and 16 reverse SRAP primers previously evaluated in buffalograsses by Budak et al. (2004a) were used in this study. Each 25 µL reaction consisted of 5 $\text{pM}\cdot\mu\text{L}^{-1}$ of each of primer pairs, 200 µM of each of dNTPs, 2.5 µL of 10× PCR buffer, 5 µL of Q Solution, 2 mM of $MgCl_2$ as a final concentration, 6 µL ddH_2O, and 1 unit of Taq polymerase (Qiagen, Valencia, Calif.), 25 ng of template. Perkin Elmer Cetus DNA Thermal Cycler (Shelton, Conn.) was used and cycling parameters included; one cycle of 2 min at 94 °C, 34 cycles of 1 min at 94 °C, 1 min at 47 °C, 1 min at 72 °C, and for extension, one cycle 5 min at 72 °C. PCR products were separated on 2.5% agarose gel at 90 volt for 5 or 6 h.

Scoring gels and data analyses. Each band 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, N.Y.) (Rohlf, 1993). A similarity matrix was constructed based on Dice`s coefficient (Dice, 1945), which considers only one to one matches between two taxa for similarity. The similarity matrix was used to construct a dendogram using the unweighted pair group method arithmetic average (UPGMA) to determine genetic relationships among the germplasm studied. PCA allows easy visualization of the differences among the individuals and determines the optimum number of clusters in a study (Mohammadi and Prasanna, 2003). PCA was performed based on the variance covariance matrix calculated from marker data using PCA. To provide a goodness-of-fit test for the similarity matrix to cluster analysis, first, COPH module was used to transform the tree matrix to a matrix of ultrametric similarities (a matrix of similarities implied by the cluster analysis) and then, MXCOMP module was used to compare this ultrametric similarities to the similarity matrix produced. In addition, correlations between ploidy level and number of markers scored in each sample were calculated by using SAS Version 8.0 (SAS Institute, Cary, N.C.), PROC CORR. The number of bands was detected based on the observed total number of bands in all genotypes.

The partitioning of molecular variance within and among ploidy levels and PARs was calculated by the AMOVA (Excoffier et al., 1992) in ARLEQUIN ver 2.000 software (Univ. of Geneva, Geneva, Switzerland) (Sch-

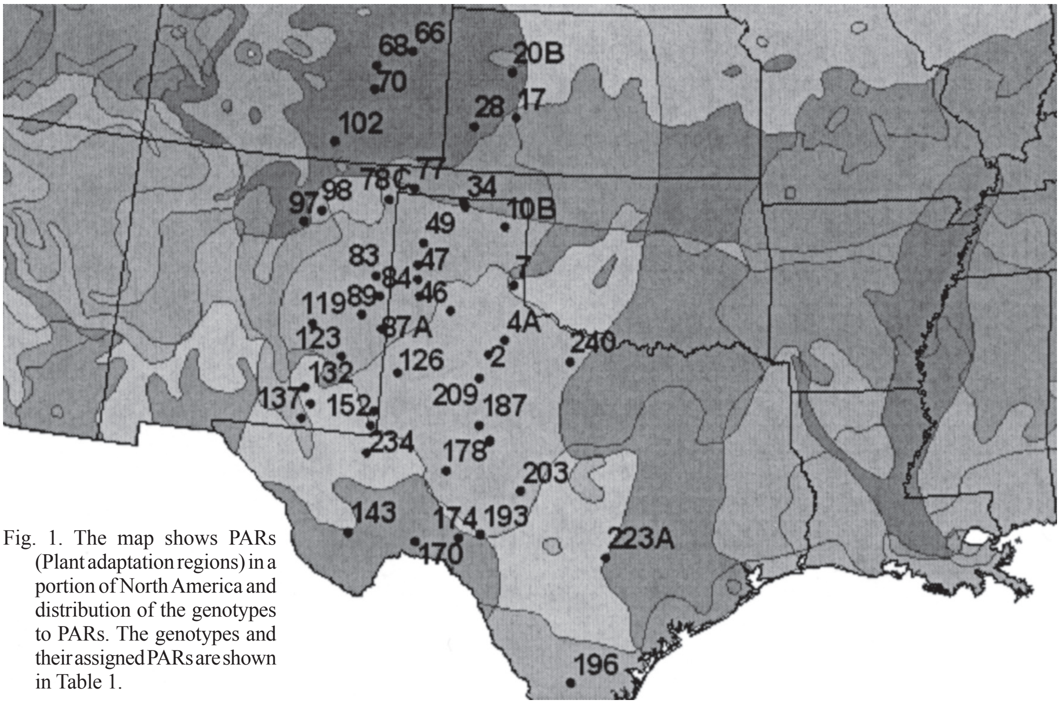

Fig. 1. The map shows PARs (Plant adaptation regions) in a portion of North America and distribution of the genotypes to PARs. The genotypes and their assigned PARs are shown in Table 1.

neider et al., 2001). Euclidean distance matrices (total number of polymorphic bands for pair wise comparisons of individuals plants) were used as data input for AMOVA. The number of permutations for significance testing was set at 10000 for all analyses.

Results and Discussion

In total, 25 combinations of forward and reverse SRAP primers were screened and a total of 95 bands with a high intensity were scored. The number of bands scored per primer ranged from 3 to 17. The scored markers were comprised of fragment sizes ranging from 110 to 1600 base pairs. The number of markers scored per genotype for the 25 combinations of primers ranged from 29 to 38, and were generally greater at the higher ploidy levels (Table 1). Some variation within a ploidy level may be expected in the observed number of bands due to cross-pollination. For example, the number of bands observed in hexaploids ranged from 31 to 38, while in tetraploids, they ranged from 31 to 34. Cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be high ($r = 0.97$, $P < 0.01$), suggesting that the cluster analysis (Fig. 2) strongly represents the similarity matrix.

No two buffalograss genotypes were found to be identical as with other cross-pollinating species (Phan, 2000), which agreed with visual observations of the genotypes (Fig. 2). All buffalograss genotypes studied had a similarity levels ranging between 0.70 and 0.95. This level of similarity was expected. There were no specific ploidy level grouping patterns. For example, tetraploids, pentaploids, and hexaploids occurred in the same group. Two hexaploid genotypes, 203 and 170, and a tetraploid, 45B, were grouped with a high similarity value, 0.97%. In addition, two other hexaploid genotypes, 34 and 89, were grouped with a tetraploid genotype, 98. The grouping responses may be due to autoploidy, resulting in higher ploidy levels being derived from duplication of a single genome. Johnson et al. (1998) also hypothesized that autoployploidy occurred in buffalograss. Blue grama and perennial ryegrass, were distinguished from one another with a considerably lower similarity value of nearly 0.29. As expected, the blue grama genotype used was more closely grouped with buffalograsses than perennial ryegrass, but was still distinguishable from buffalograsses based on SRAP marker determination.

There was a positive correlation between ploidy level and number of bands scored for each genotype ($r = 0.48$, $P < 0.05$) (Table 1). Coupled with broader adaptation zones observed in hexaploids (Johnson et al., 2001), this correlation may infer that extra copies of homologous chromosomes provide new DNA sequences for adaptation to diverse environments as reported by Wendel (2000). This response is the second source of evidence, in addition to ploidy level distribution, demonstrating adaptability of hexaploids throughout the North American Great Plains, especially when compared to diploids, which are more narrowly adapted. Nested AMOVA indicated

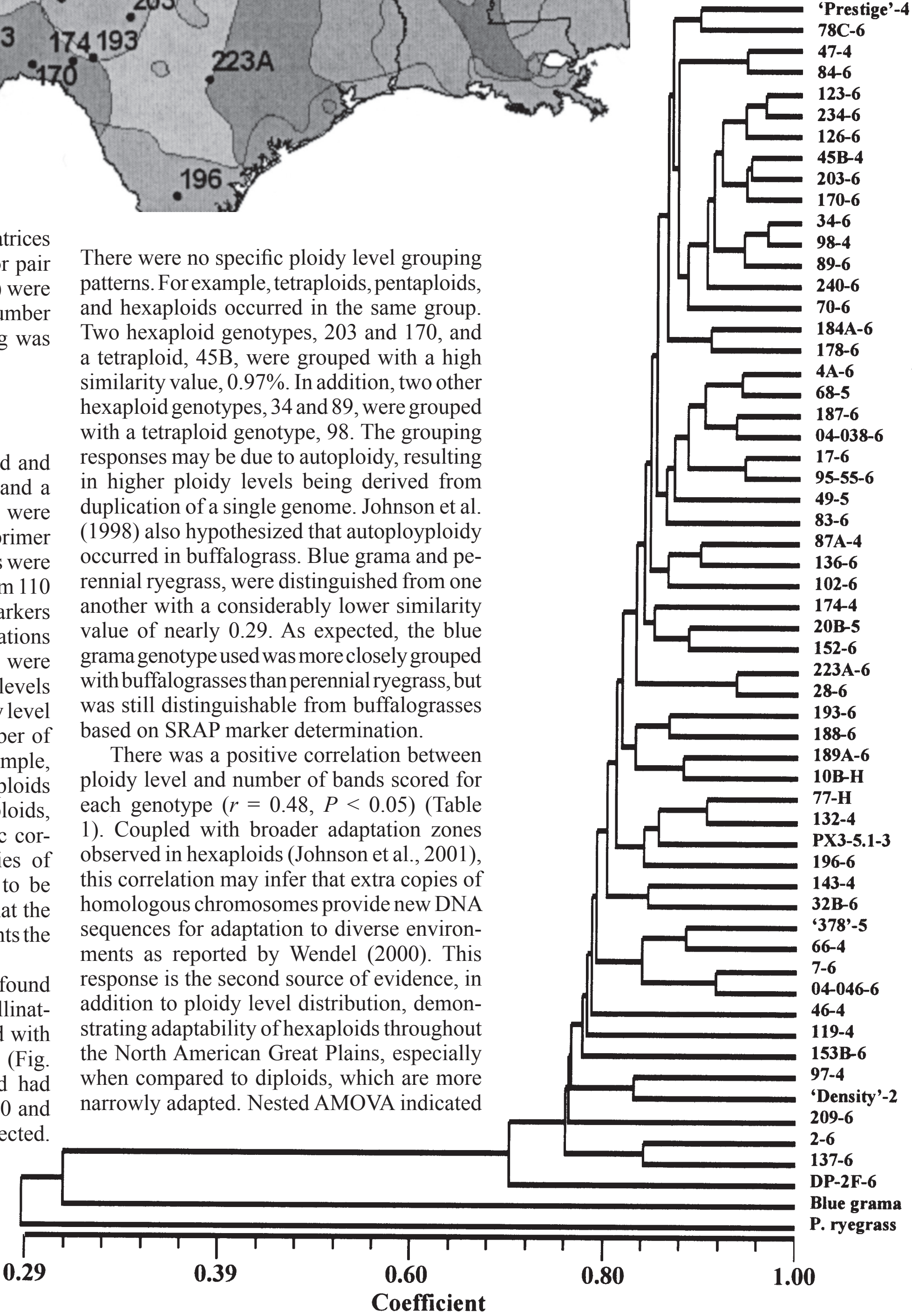

Fig. 2. UPGMA dendogram of 56 buffalograsses and two outgroups based on based on analysis of 95 SRAP markers. The last digits indicate ploidy levels of the genotypes.

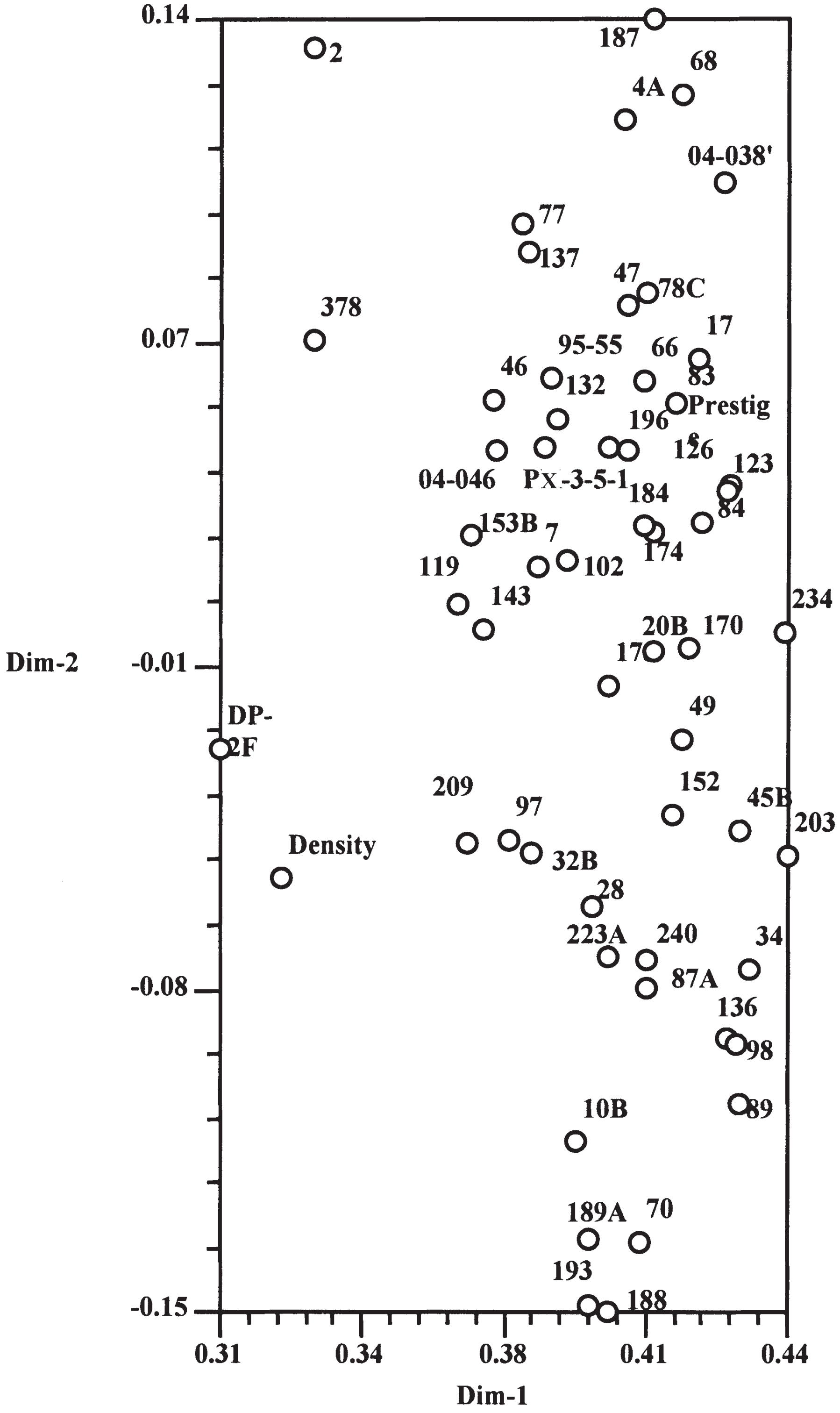

Fig. 3. Relationships among 56 genotypes with different ploidy levels based on principal component analysis (PCA) of SRAP-based genetic similarities.

nonsignificant genetic variation among ploidy levels and PARs (AMOVA not shown). Partitioning of the genetic diversity revealed that almost all of the variation resided within ploidy levels (98.5%) and within PARs (97.5%). Most likely, diversity among buffalograss populations from different ploidy levels and PARs was equally distributed as a result of the cross-pollination coupled with random herbivore seed dispersal, which played a role in early distribution of buffalograsses (Beetle et al., 1950; Quinn et al., 1994).

Principal component analysis was used to define relationships and identify highly similar genotypes (Fig. 3). The PCA gave similar groupings as the UPGMA dendogram. Mohammadi and Prasanna (2003) indicated that PCA becomes very useful for grouping individuals by a scatter plot analysis, when the first two or three eigen vectors explain most of the variation. In this study, the first three eigen vectors comprised 78% of the total observed variation. Based on the results from this study, the genotype number of 56 could be reduced to 50 due to genotype similarity levels based on PCA analysis (Fig. 3). Identifying these similarities will help us efficiently construct a core buffalograss collection and eliminate redundancies.

Based on only one region of cpDNA sequence and one nuclear ribosomal internal transcribed spacer region, Columbus (1999) indicated that some species of *Bouteloua* were more closely related to other genera than to congeners. He speculated that buffalograss should be reclassified to *Bouteloua dactyloides* (Nutt.) J.T. The blue grama genotype in this study was quite different from the buffalograsses, with a low similarity value of 0.31. These results indicate that additional research that includes other related species is needed before taxonomical changes are proposed for buffalograsses.

The SRAP markers efficiently discriminated all naturally occurring buffalograss genotypes in this study. Hence, they may be readily used in establishing germplasm core collections, understanding relationship level, estimating genetic diversity, and integrating markers into genetic linkage maps (Budak et al., 2004a, 2004b). Germplasm organization is of importance for buffalograss breeding programs because of its extensive diversity caused by cross-pollination. Although buffalograsses are known as relatively pest free species, a number of potential pests such as chinch bugs [*Blissus occiduus* (Hemiptera: Lygaeidae)] (Baxendale et al., 1999) and false smut (*Cercospora seminalis* Ellis & Everh.) (Wenger, 1943) have been identified. Initial screening of potential diploid buffalograss parents as convenient ploidy level revealed considerable polymorphism for SRAP markers (Gulsen et al., unpublished). Therefore, the SRAP markers may also have potential in buffalograss breeding programs through marker-assisted selection, which would further enhance buffalograss improvement opportunities.

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