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Genetic Diversity of *Cynodon transvaalensis* Burtt-Davy and Its Relatedness to Hexaploid *C. dactylon* (L.) Pers. as Indicated by AFLP Markers

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ABSTRACT

***Cynodon transvaalensis* Burtt-Davy (African bermudagrass) is valued as turf and for use in interspecific hybridization with *C. dactylon* (L.) Pers. var. *dactylon* to produce turf cultivars. Little information is available regarding the magnitude of genetic variation within the taxon. Accordingly, this study was undertaken to evaluate the genetic diversity among 14 *C. transvaalensis* accessions and to examine the phylogenetic relatedness of *C. transvaalensis*, two hexaploid ($2n = 6x = 54$) *C. dactylon* var. *dactylon* accessions, two *C. transvaalensis* by hexaploid *C. dactylon* var. *dactylon* interspecific tetraploid ($2n = 4x = 36$) F_1 hybrids, and one putative tetraploid *C. dactylon* var. *dactylon* by *C. transvaalensis* triploid ($2n = 3x = 27$) F_1 hybrid. Fluorescence-labeled amplified fragment length polymorphism (AFLP) DNA profiling was used to study the genetic relationships among these accessions. A total of 381 polymorphic AFLP markers were amplified from 13 primer combinations. The 14 *C. transvaalensis* accessions and the putative triploid F_1 hybrid clustered into one group and had genetic dissimilarity coefficients ranging from 0.01 to 0.51. The 14 *C. transvaalensis* accessions had genetic dissimilarity coefficients ranging from 0.01 to 0.34. The *C. dactylon* var. *dactylon* accessions and the two tetraploid F_1 hybrids clustered in the second group, with genetic dissimilarity coefficients ranging from 0.17 to 0.33. The tetraploid F_1 hybrids were more closely related to *C. dactylon* var. *dactylon* than to *C. transvaalensis*, while the opposite was true for the putative triploid F_1 hybrid. The results indicate the presence of genetic diversity in *C. transvaalensis* that could be exploited in intra- and interspecific breeding improvement.**

Cynodon transvaalensis (African bermudagrass) is valued as turf and for use in interspecific hybridization with *C. dactylon* var. *dactylon* to produce turf cultivars. African bermudagrass, a diploid ($2n = 2x = 18$) species, is indigenous to the southwestern Transvaal and the northern part of the central Cape Province of South Africa (Harlan et al., 1970a) where it is found primarily near wet sites (Harlan et al., 1970b). Plants of *C. transvaalensis* are distinctive because of their small size, yellow-green color, erect narrow leaves, and two to four racemes per inflorescence with the spikelets loosely arranged on the racemes (Harlan et al., 1970b). *Cynodon transvaalensis* is adapted to much cooler climates and is more winter hardy than needed in its natural distribution (Harlan et al., 1970a). The rhizomatous and stoloniferous plants spread to form a dense sod because of high shoot density. Because of their dense sod, fine leaf texture, and ability to tolerate relatively low mowing heights, *C. transvaalensis* cultivars such as 'Florida' and 'Uganda' have been used on sporting surfaces such as golf course putting greens, bowling greens, and tennis courts (Juska and Hanson, 1964; Roux, 1969). Characteristics that limit the use of *C. transvaalensis* as turf include relatively high fertility and water requirements, summer decline in turf quality when temperatures are high (≥38°C), and intolerance to sustained very low (≤3.2 mm) mowing heights (Juska and Hanson, 1964).

Information on the magnitude of variation within *C. transvaalensis* is limited. *Cynodon transvaalensis* plants were described by de Wet and Harlan (1971) as being very uniform in appearance. However, substantial variation for morphological and adaptation traits has been observed in segregating populations of *C. transvaalensis* (Taliaferro, 1992). The most important use of *C. transvaalensis* has been in interspecific hybridization with tetraploid *C. dactylon* var. *dactylon* to produce clonally propagated F_1 hybrids. Many turf bermudagrass cultivars, including the industry standards 'Tifgreen' and 'Tifway', were produced by this method (Burton, 1973, 1991; Alderson and Sharp, 1995). However, relatively few *C. transvaalensis* accessions have been used in breeding, genetic studies, or as commercial turf cultivars (Taliaferro, 1992, 1995).

Few hexaploid *Cynodon* plants have been reported, one being *C. dactylon* cv. 'Tifton 10' (Hanna et al., 1990). Tifton 10 originated as a vegetative introduction collected by G.W. Burton in 1974 in Shanghai, China. Its major distinguishing features are coarse-textured foliage with a natural dark bluish-green color, rapid establishment rate, and early green-up in spring (Hanna et al., 1990). The morphological characteristics of Tifton 10 are most consistent with plants classified as *C. dactylon* var. *dactylon* in Harlan et al.'s (1970b) taxonomic classification for the genus *Cynodon*. We have crossed *C. transvaalensis* with Tifton 10 to produce tetraploid F_1 plants.

DNA profiling has been used to estimate genetic relatedness among *Cynodon* plants. DNA amplified fingerprinting (DAF) was used to identify cultivars and study the origin of off-types found in cultivars (Caetano-Anolles, 1998; Caetano-Anolles et al., 1995; Ho et al., 1997; Anderson et al., 2001). Assefa et al. (1999) used DAF to assess genetic relatedness within and among eight *Cynodon* taxa. Roodt et al. (2002) used random amplified polymorphic DNA (RAPD) profiles to determine genetic relatedness of *Cynodon* cultivars in South Africa and to assess genetic variation. Zhang et al. (1999) and

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Abbreviations: AFLP, amplified fragment length polymorphism; DAF, DNA amplification fingerprinting; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

Karaca et al. (2002), respectively, differentiated between 27 and 31 *Cynodon* genotypes using AFLP profiles.

The objectives of this study were to assess the genetic diversity within *C. transvaalensis* accessions, and to quantify the genetic relatedness among *C. transvaalensis* and hexaploid *C. dactylon* var. *dactylon* accessions, and three interspecific hybrids based on AFLP DNA profiling.

MATERIALS AND METHODS

Plant Materials and DNA Isolation

Plant materials consisted of 19 bermudagrass accessions including 14 African bermudagrasses, two hexaploid ($2n = 6x = 54$) *C. dactylon* var. *dactylon* accessions, two tetraploid F_1 hybrid plants from diploid *C. transvaalensis* by hexaploid *C. dactylon* var. *dactylon* cv. Tifton 10 crosses, and triploid PI 290897 (Table 1). All of the accessions were clonally propagated single plants. PI 290897 was included on the basis of its classification as *C. transvaalensis* in the USDA-ARS National Plant Germplasm System (NPGS), Germplasm Resources Information Network (GRIN) database (National Plant Germplasm System, 2003). During the course of our investigation, DNA profiling data showed that PI 290897 is significantly different from other *C. transvaalensis* genotypes. We determined that PI 290897 is not *C. transvaalensis* on the basis of coarser foliage morphology and chromosome number ($2n = 3x = 27$). Chromosome number was determined by the leaf squash procedure of Powell (1968).

The plants were grown in a greenhouse at the Agronomy Research Station, Oklahoma State University, Stillwater, OK, in 15-cm diameter pots containing Metro-Mix 250 growing medium (Scotts-Sierra Horticultural Products Co., Marysville, OH). They were watered daily and fertilized biweekly with M-77 Peat-lite Special water-soluble fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH). All plants were actively growing and healthy at time of sampling. Bermudagrass genomic DNA samples were isolated from fresh leaf tissue with DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA).

AFLP Analysis

AFLP analysis was performed as described by Vos et al. (1995), except for minor modifications as described by Bai et al. (1999). Briefly, 300 ng of genomic DNA was double digested with *Eco*RI and *Mse*I restriction enzymes. AFLP adapters for both enzymes were then ligated to the restriction fragments. The ligated DNA was preamplified with a primer combination based on the sequences of the adapters. The sequences of *Eco*RI and *Mse*I primers were 5-GACTGCG TACCAATTC and 5-GATGAGTCCTGAGTAA, respectively. Pre-amplification started at 94°C for 1 min, then 30 cycles of 30 s at 94°C followed by 1 min each at 65 and 72°C. A 1% (w/v) agarose gel was used to check the products of preamplification. Thirteen pairs of selective AFLP primers (Table 2) with *Eco*RI primers labeled with infrared (IR) fluorescence were used for selective amplification. All PCR reactions were conducted on a MJ PTC-100 thermocycler (MJ Research, Inc., Waltham, MA). A 10-μL PCR mixture consisted of 1.0 μL 10× PCR buffer, 1.0 μL 25 m*M* $MgCl_2$, 0.2 μL 10 m*M* dNTP, 0.4 μL labeled *Eco*RI primer, 0.04 μL Taq polymerase, 0.35 μL *Mse*I primer, 5.01 μL H_2O, and 2.0 μL 1:10 diluted preamplified template DNA. All selective amplifications were conducted using the following touchdown thermal profile: one cycle of 2 min at 94°C; 13 touchdown cycles of 30 s at 94°C, 30 s at 65°C (−0.7°C per cycle), 60 s at 72°C; 23 cycles of 30 s at 94°C, 30 s at 56°C, 60 s at 72°C. One microliter of the selectively amplified PCR products were loaded on a 6.5% (w/v) denaturing Long Ranger gel (BMA, Rockland, ME) and run in 1× TBE buffer at 1500 V for 3.5 h in a LI-COR automated sequencer (LI-COR Inc., Lincoln, NE).

Table 2. Total number of bands and number of polymorphic bands scored for each of 13 AFLP selective primer combinations.

Primer pairs	Total bands	Polymorphic bands
e-AAC/m-CAA†	62	42
e-AAC/m-CAC	44	39
e-AAC/m-CAT	46	20
e-AAC/m-GAC	33	26
e-ACT/m-CAA	60	32
e-ACT/m-CAC	44	30
e-ACT/m-CAG	47	37
e-ACT/m-GAC	54	43
e-AGT/m-CAA	62	12
e-AGT/m-CAC	53	31
e-AGT/m-CAG	66	45
e-AGT/m-CAT	46	12
e-GCTG/m-CAA	54	12
Total	671	381
Average	51.6 ± 9.4	29.3 ± 12.1

† e is the preamplification primer sequence for *Eco*RI site. (5-GACTGCGTACCAATTC) without any selective nucleotides and m is the preamplification primer sequence for *Mse*I site (5-GATGAGTCCT GAGTAA).

Table 1. *Cynodon* accessions used for AFLP analysis.

No.	Species	PI	Other ID	Origin	Chromosome no. (2*n*)
1	*C. transvaalensis*	290894	Sekaapplossfine	South Africa	18
2	*C. transvaalensis*	290897	Harrismith	South Africa	27
3	*C. transvaalensis*	290905	Frankenwald fine	South Africa	18
4	*C. transvaalensis*	291591	Florida	South Africa	18
5	*C. transvaalensis*	289922	Howick	South Africa	18
6	*C. transvaalensis*	290812	41-222	South Africa	18
7	*C. transvaalensis*	290813	42-226	South Africa	18
8	*C. transvaalensis*	290665	35-196	South Africa	18
9	*C. transvaalensis*	–	4048	Oklahoma State University	18
10	*C. transvaalensis*	–	2747	Oklahoma State University	18
11	*C. transvaalensis*	290874	Uganda	Egypt	18
12	*C. transvaalensis*	–	T572†	Lesotho	18
13	*C. transvaalensis*	–	T574†	Lesotho	18
14	*C. transvaalensis*	–	T576†	Lesotho	18
15	*C. transvaalensis*	–	T577†	Lesotho	18
16	*C. dactylon* × *C. transvaalensis*	–	41-8	Oklahoma State University	36
17	*C. dactylon* × *C. transvaalensis*	–	Patriot	Oklahoma State University	36
18	*C. dactylon*	–	Tifton 10	China	54
19	*C. dactylon*	–	A12318	China	54

† Collected by W.W. Hanna, USDA-ARS, Coastal Plains Research Station, Tifton, GA.

DNA size standard (LI-COR Inc., Lincoln, NE) was loaded on first and last lanes of a gel as a molecular weight reference.

DNA samples from eight of the 19 accessions were used to test the reproducibility of the AFLP procedures. Two DNA samples were independently isolated from each of the eight plant accessions, thus constituting two sets of eight samples. The DNA samples in one set were amplified once while those in the second set were amplified three times in each case using the amplification steps outlined above. The amplified DNA products from the two sets were treated as four replicates and run on the same gel to measure reproducibility. The primer combinations e-ACT/m-CAG, e-AAC/m-CAG, e-ACT/m-CAT, and e-AAC/m-CAT, were used for the selective amplification.

Data Analysis

Polymorphic DNA bands were scored as present (1), absent (0) or ambiguous (9) for each accession by visual inspection. To ensure accurate scoring, all markers were scored at least twice. Data were compiled in a binary data matrix. Relative genetic dissimilarity was estimated according to Nei and Li (1979). Similarity was calculated as $S_{xy} = 2n_{xy}/(n_x + n_y)$, where n_x and n_y were the numbers of fragments in individuals X and Y, respectively, and n_{xy} was the number of the fragments shared between individuals. Dissimilarity, D, was calculated by the equation $D_{xy} = 1 - S_{xy}$ using Microsoft Excel. Cluster analysis was performed with the NTSYS-pc (Numerical Taxonomy System) version 2.0 program (Exeter Software, New York, NY) using the unweighted pair-group mean algorithm (UPGMA) within the SAHN module. A goodness-of-fit test of the cophenetic matrix to the similarity matrix was performed using the MXCOMP module in the NTYSY-pc program. A principal component analysis was performed using the DCENTER module of the NTSYS-pc program.

RESULTS AND DISCUSSION

Reproducibility of AFLP Procedures

Reproducibility of the AFLP products was very high. Among the eight plant accessions, each with four selective DNA amplifications, the four primer combinations produced 125 lanes (out of total of 128 lanes) having identical AFLP band patterns. There were three lanes in which DNA band patterns were different from other replicate lane patterns. Forty-nine variant bands were found in these three lanes representing 1.2% of the total number (4054) of bands produced by the four primer combinations (data not shown). These non-reproducible fragments were mainly low intensity bands that showed up in some PCR reactions, but not in others. The results from our study agree with previous reports (Zhang et al., 1999; Rouf Mian et al., 2002) and further confirmed that the AFLP technique is reliable, and generates highly reproducible DNA profiling for bermudagrass.

Genetic Relatedness among Accessions

The 13 primer combinations produced 671 bands ranging in size from 50 to 500 bp. The average bands per primer combination were 51.6 ± 9.4 SD (Table 2). Of the 671 amplification bands scored, 381 (56.8%) were polymorphic as indicated by their absence in at least one of the 19 accessions tested. An AFLP gel with PCR products using primer combination e-ACT/m-CAA is shown in Fig. 1.

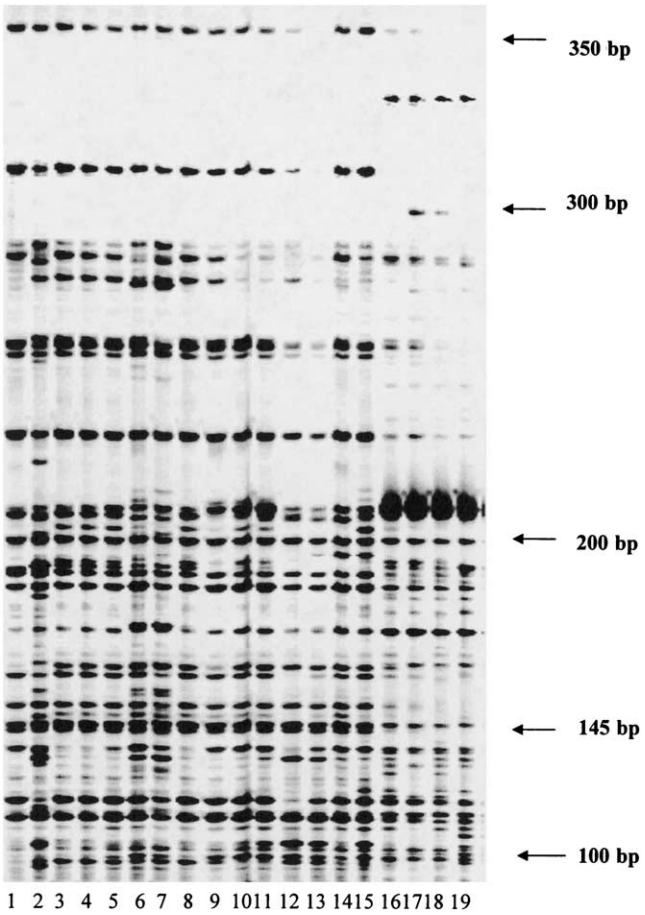

Fig. 1. AFLP fingerprints generated using primer combination e-ACT/m-CAA. Fragment size is indicated on the right. Lanes from 1 to 19 are accessions: 290894, 290897, 290905, 291591, 289922, 290812, 290813, 290665, 4048, 2747, 290874, T572, T574, T576, T577, 41-8, Patriot, Tifton 10, and A12318, respectively.

The 19 accessions clustered into two major groups and subclustered into hierarchy subgroups on the basis of the UPGMA tree of similarity coefficients (Fig. 2). The cophenetic correlation coefficient (r) comparing the relationship of the cophenetic value matrix with the dissimilarity matrix was high, with an r value of 0.96, indicating a very good fit of the dendrogram (Fig. 2) to the dissimilarity coefficients (Table 3) (Mohammadi and Prasanna, 2003). Group A consisted of the 14 *C. transvaalensis* accessions and the triploid accession PI 290897. Group B contained the hexaploid *C. dactylon* var. *dactylon* accessions Tifton 10 and A12318 and the two tetraploid interspecific hybrids 41-8 and 'Patriot'. Genetic dissimilarity coefficients for pair-wise comparisons among the Group A accessions ranged from 0.01 to 0.52, and among Group B accessions from 0.17 to 0.33 (Table 3). The mean dissimilarity coefficients for Groups A and B were respectively 0.24 ± 0.12 and 0.23 ± 0.03. Dissimilarity coefficients among the *C. transvaalensis* accessions ranged from 0.01 to 0.34, with a mean of 0.20 ± 0.07. The 14 *C. transvaalensis* accessions clustered into distinct subgroups as clearly indicated by the UPGMA tree and

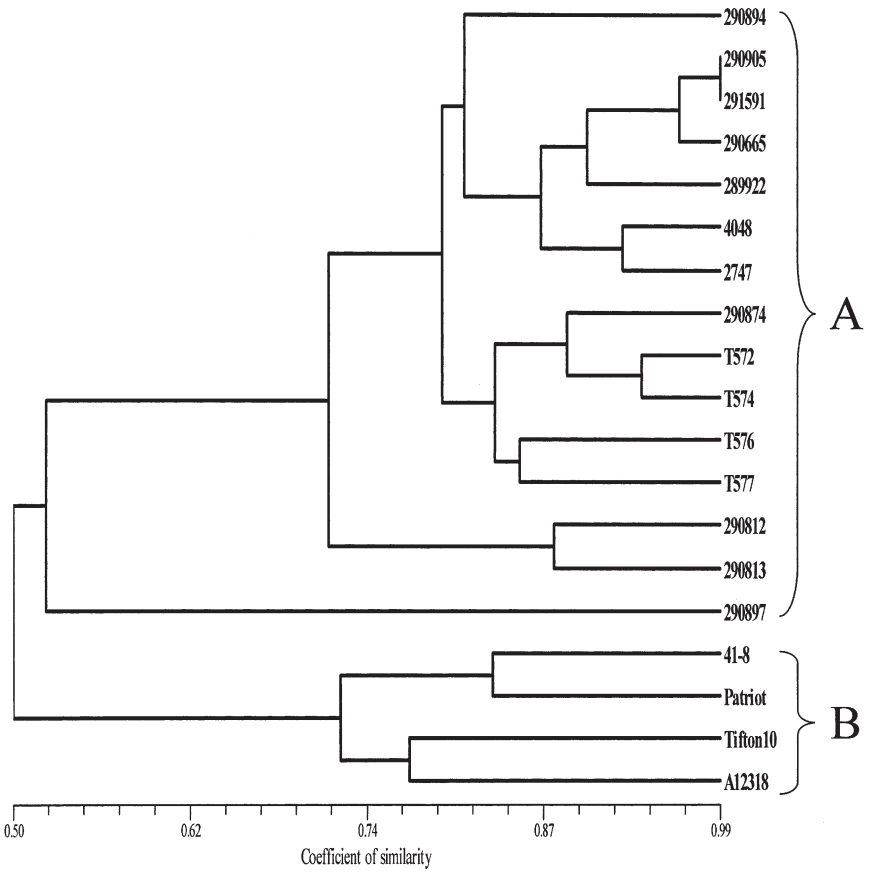

Fig. 2. UPGMA tree of similarity coefficients derived from phenetic analysis of AFLP data.

principal coordinate analysis (Fig. 2 and 3). The *C. transvaalensis* PIs 290665, 289922, 290894, 290905, and 291591 and the OSU breeding lines 4048 and 2747, were included into one subgroup. The grouping of these accessions may reflect their having a common geographic origin in the vicinity of Johannesburg, South Africa, though we were unable to determine the exact origin of all. Records indicate that PIs 290905 and 291591 were originally collected near Johannesburg (Roux, 1969). Field collection notes of W.W. Huffine indicated that PI 290894 was from the Pretoria Horticultural Research Station turf plots and PI 289922 was from the Frankenwald Research Station turf plots. These same notes indicated that PI 290665 was collected as a seedling near the 18th putting green of the Zwartkop Country Club in Pretoria. The PIs in this group were included in a set of 47 *C. transvaalensis* accessions used to synthesize a population from which the two OSU breeding lines were subsequently selected.

The second subgroup was comprised of T572, T574, T576, T577, all from Lesotho, and PI 290874. The same Lesotho *C. transvaalensis* accessions were studied by Zhang et al. (1999), plus another from Lesotho, T575,

Table 3. Genetic dissimilarity coefficients calculated for 19 *Cynodon* accessions encompassing 14 *C. transvaalensis* (Ctr), two hexaploid *C. dactylon* var. *dactylon* (Cdd), and three *C. dactylon* by *C. transvaalensis* F1 hybrids (Hyb) combining data by using AFLP.

290894	Ctr	0.00																		
290897	Hyb	0.48	0.00																	
290905	Ctr	0.16	0.47	0.00																
291591	Ctr	0.15	0.48	0.01	0.00															
289922	Ctr	0.23	0.49	0.09	0.10	0.00														
290812	Ctr	0.29	0.49	0.26	0.27	0.27	0.00													
290813	Ctr	0.33	0.49	0.29	0.30	0.32	0.13	0.00												
290665	Ctr	0.17	0.49	0.04	0.04	0.12	0.28	0.28	0.00											
4048	Ctr	0.21	0.51	0.13	0.14	0.16	0.23	0.27	0.12	0.00										
2747	Ctr	0.21	0.48	0.13	0.13	0.14	0.22	0.28	0.14	0.08	0.00									
290874	Ctr	0.24	0.52	0.18	0.19	0.17	0.25	0.32	0.19	0.15	0.11	0.00								
T572	Ctr	0.26	0.51	0.24	0.25	0.23	0.31	0.34	0.24	0.23	0.22	0.13	0.00							
T574	Ctr	0.22	0.50	0.23	0.23	0.22	0.28	0.30	0.23	0.22	0.19	0.10	0.07	0.00						
T576	Ctr	0.18	0.48	0.15	0.16	0.21	0.24	0.25	0.16	0.18	0.17	0.19	0.21	0.15	0.00					
T577	Ctr	0.24	0.48	0.22	0.22	0.20	0.30	0.32	0.20	0.21	0.19	0.13	0.18	0.14	0.15	0.00				
41-8	Hyb	0.49	0.50	0.47	0.48	0.41	0.44	0.47	0.45	0.42	0.42	0.37	0.38	0.38	0.43	0.35	0.00			
Patriot	Hyb	0.49	0.46	0.47	0.47	0.44	0.46	0.46	0.46	0.41	0.42	0.49	0.46	0.48	0.46	0.47	0.17	0.00		
Tifton10	Cdd	0.60	0.50	0.61	0.61	0.56	0.60	0.59	0.60	0.57	0.58	0.50	0.48	0.50	0.57	0.50	0.25	0.33	0.00	
A12318	Cdd	0.61	0.46	0.60	0.60	0.57	0.56	0.56	0.58	0.54	0.55	0.56	0.55	0.57	0.57	0.56	0.28	0.24	0.23	0.00
Genotype		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

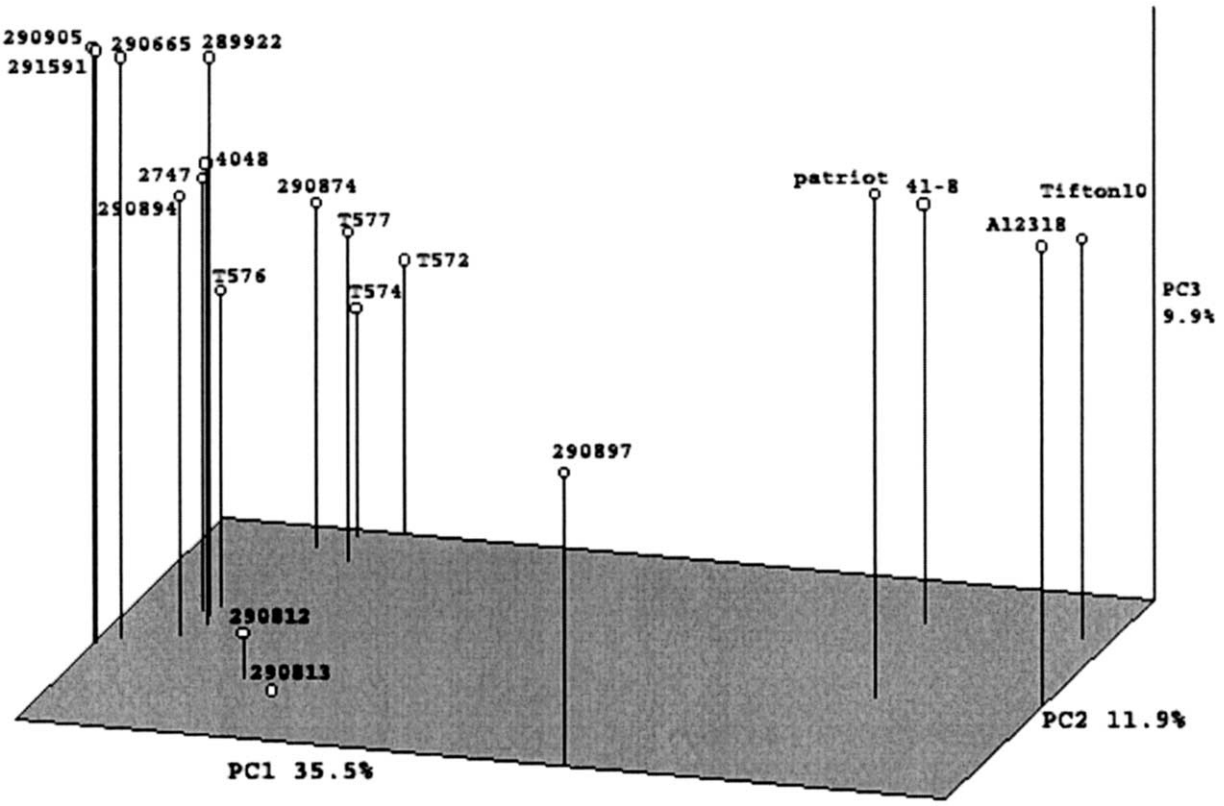

Fig. 3. Principal coordinate analysis of bermudagrass accessions based on AFLP.

and clustered into one distinct group with genetic dissimilarity coefficients ranging from 0.18 to 0.43. Our results compared with those of Zhang et al. (1999) indicated somewhat closer relatedness of the four accessions common to the two studies but were reasonably consistent. The group of 14 *C. transvaalensis* in our study contained accessions more closely related, but none as distantly related as in the group of five *C. transvaalensis* accessions studied by Zhang et al. (1999). Overall, the results of the two studies were reasonably consistent for *C. transvaalensis*. The precise origin of PI 290874 is unclear, though it has been widely distributed as 'Uganda', and was originally introduced to the USA from the Gezira Sporting Club at Cairo, Egypt, as PI 183551 (Juska and Hanson, 1964). Mahdi (1955) indicated that 'Ugandagrass' had been grown in Egypt for 50 yr, but did not provide information on its origin. The name suggests that it made its way from South Africa to Egypt via Uganda.

The PIs 290812 and 290813 clustered into the third subgroup. These accessions were collected in the south central part of South Africa, approximately 200 to 250 km southwest of Johannesburg. Notes made by the collector, W.W. Huffine, indicated that the accessions were found growing in natural settings and not as cultured turf.

The results point to substantial variation within *C. transvaalensis*, but the extent to which the measured variation is representative of the natural range of variation within the species is unknown. The 14 accessions include some that were selected for their attributes as turf cultivars and some that may not have been selected on the basis of evaluations for turf quality. Selected accessions include PIs 289922 (Howick), 290905 (Frankenwald Fine), 290874 (Uganda), 290894 (Sekaappless Fine), and 291591 (Florida). These accessions trace to collections made at various times during the first half of the 1900s and entered into commercial turf use (Juska and Hanson, 1964; Roux, 1969). Many, or perhaps all of these, were included among the *Cynodon* accessions evaluated at the Frankenwald Research Station, Johannesburg, South Africa, beginning in 1930 (Roux, 1969). Available records do not indicate that the other accessions were collected and retained on the basis of their turf value, though it is possible that some were.

Dissimilarity coefficients between PI 290897 (Harrismith) and all other accessions ranged from 0.46 to 0.50, indicating that its genetic relatedness to *C. transvaalensis* accessions and to the *C. dactylon* var. *dactylon* accessions were not different. Morphologically PI 290897 has darker green color foliage and larger leaves and stems than the *C. transvaalensis* accessions. Harrismith was previously introduced to the USA in 1955 as PI 224141 from Pretoria Botanic Gardens and was listed by Juska and Hanson (1964) as *Cynodon* sp. Current NPGS-GRIN records for historical accessions list PI 224141 as *C. dactylon* var. *dactylon* (National Plant Germplasm System, 2003). PI 290897 was introduced in 1963 from the Pretoria Horticulture Research Station. On the basis of chromosome number, morphology, and the AFLP results, PI 290897 as it currently exists in the NPGS *Cynodon* germplasm collection is most likely an interspecific hybrid between a tetraploid *C. dactylon* var. *dactylon* and a diploid *C. transvaalensis* because it is a triploid.

The two hexaploid *C. dactylon* var. *dactylon* accessions Tifton 10 and A12318, and the two Tifton 10 by *C. transvaalensis* F_1 hybrids 41-8 and Patriot clustered into a group, though they respectively formed distinct subgroups (Fig. 2 and 3). Tifton 10 and A12318 are from Shanghai, China. The closer relationship of 41-8 and Patriot to *C. dactylon* var. *dactylon* than to *C. transvaalensis* may result from their having three genomes from the former and one genome from the later. The more intermediate genetic relationship between *C. dactylon* var. *dactylon* and *C. transvaalensis* of the putative triploid hybrid PI 290897 compared with the other two hybrids may also be a function of genomic constitution differences, because the triploid contains two genomes from its tetraploid *C. dactylon* parent and one genome from diploid *C. transvaalensis* parent.

Cynodon L.C. Rich. is a small genus comprised of nine diverse species relative to their geographic distributions, prevalence, and economic importance (Harlan, 1970; Harlan et al., 1970a). Assefa et al. (1999) recognized extensive genetic variation among *Cynodon* species on the basis of DAF markers. They reported the largest within species variation in *C. dactylon* var. *dactylon* (36 accessions) with a mean genetic similarity (GS) coefficient of 0.679. Within species variation was least for *C. arcuatus* J. S. Presl. Ex. C. B. Presl. (5 accessions) and *C. transvaalensis* (four accessions) with mean GS values of 0.94 and 0.948, respectively. The GS coefficients for both *C. arcuatus* and *C. transvaalensis* clustered tightly around the mean. The range in genetic dissimilarity coefficients from our study (0.01–0.61) is consistent with the range for AFLP-based genetic dissimilarity coefficients (0.05–0.67) for the 27 *Cynodon* genotypes in the study by Zhang et al. (1999), both studies encompassing *C. dactylon*, *C. transvaalensis*, and F_1 hybrids from interspecific crossing of the two taxa. These values are also reasonably consistent with the range in GS (0.608–0.977) reported by Karaca et al. (2002) for 31 forage bermudagrass genotypes collected as ecotypes or commercial cultivars from the southeastern USA. The 31 genotypes were reported as *Cynodon* spp., but most would be classified as *C. dactylon*. The GS values from the Karaca et al. (2002) study were based on combined data from AFLP, chloroplast-specific simple sequence repeat length (CpSSRLP), RAPD, and directed amplification of minisatellite-region (DAMD) analyses.

The amount of genetic diversity within *C. transvaalensis* is important relative to the potential for its breeding improvement and its use in interspecific hybridization with *C. dactylon* var. *dactylon* to produce improved turf cultivars. The magnitude of variation for AFLP markers within *C. transvaalensis* suggests probable genetic variation for other traits that could be manipulated by classical breeding. Additionally, the magnitude of variation implies the potential for discovery of *C. transvaalensis* plants with superior combining ability when hybridized with *C. dactylon* var. *dactylon* plants.

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