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Differentiation of bermudagrass (*Cynodon* spp.) genotypes by AFLP analyses

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Abstract Bermudagrasses (*Cynodon* spp.) are major turfgrasses for home lawns, public parks, golf courses and sport fields, and are widely adapted to tropical and warmer temperate climates. Morphological and physiological characteristics are not sufficient to differentiate some bermudagrass genotypes because the differences between them are often subtle and subject to environmental influence. In this study, a DNA-typing technique, amplified fragment length polymorphism (AFLP), was used to differentiate bermudagrass genotypes and to explore their genetic relationships. Twenty seven bermudagrass cultivars and introductions, mostly from the Coastal Plain Experiment Station in Tifton, Ga., were assayed by the radioactive (^{32}P) and the fluorescence-labeled AFLP methods. The AFLP technique produced enough polymorphism to differentiate all 27 bermudagrass genotypes, even the closely related ones. An average of 48–74 bands in the 30–600-bp size range was detected by the ^{32}P-labeled AFLP method. The results indicated that most of the 14 primer combinations tested in this study could be used to distinguish bermudagrass genotypes, and that some single primer-pairs could differentiate all 27 of them. To test the reliability and reproducibility of the AFLP procedure, three DNA isolations (replications) of the 27 bermudagrass genotypes were assayed using five primer pairs. Only 0.6% of the bands were evaluated differently among the three replications. One replication of one genotype (which was most likely a planting contaminant) was grouped in an unexpected cluster using the Unweighted Pair Group Mean Average (UPGMA) method. A one- or two-band difference in scoring did not change the clustering of genotypes or the replications within genotypes. The 27 genotypes were grouped into three major clusters, many of which were in agreement with known pedigrees. Trees constructed with different primer combinations using ^{32}P- and fluorescence-labelling formed similar major groupings. The semi-automated fluorescence-based AFLP technique offered significant improvements on fragment sizing and data handling. It was also more accurate for detection and more efficient than the radioactive labelling method. This study shows that the AFLP technique is a reliable tool for differentiating bermudagrass genotypes and for determining genetic relationships among them.

Key words AFLP (amplified fragment length polymorphism) · Bermudagrass (*Cynodon* spp.) · DNA fingerprinting · Semi-automated fluorescence-based genotyping

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Introduction

Bermudagrasses (*Cynodon* spp.) are perennial, warm-season grasses. They have been used for turf, grazing

and soil conservation (Taliaferro 1995), and are major turfs for home lawns, public parks, golf courses, and sports fields in many countries. As high-quality turf, bermudagrasses have recuperative potential, good color, high density, and wear-tolerance (Beard 1971).

The genus *Cynodon* comprises nine species with *Cynodon dactylon* (L.) Pers. (common bermudagrass) being the most widespread. Common bermudagrass ($2n = 4x = 36$) and African bermudagrass, *Cynodon transvaalensis* Burtt-Davy ($2n = 2x = 18$), are the species best suited for turf. Improved triploid ($2n = 3x = 27$) bermudagrass hybrids between *C. dactylon* and *C. transvaalensis* produce very high-quality turf, and interspecific hybridization of *C. dactylon* and *C. transvaalensis* has been the principle breeding method used to produce high-quality vegetatively propagated turf cultivars such as Tifgreen and Tifway. Diploid or tetraploid bermudagrass can be propagated by seed, but all of the triploid hybrids are sterile, having essentially no pollen production or seed set, and must be propagated vegetatively (Powell et al. 1974). However, the vegetative distribution of cultivars is a potential source of contamination, which can contribute to the production of off-types. Off-types are a serious problem that reduce both quality and performance in the turfgrass industry.

Differentiating among *Cynodon* genotypes has been difficult because of the lack of stable distinctive morphological and physiological characteristics. Isozyme electrophoretic patterns (Dabo et al. 1990; Vermeulen et al. 1991) have been used to differentiate turf-type *Cynodon* genotypes, and some genotypes that were not easily differentiated by morphological characteristics could be distinguished. However, not all genotypes could be differentiated by this method. Expression of some isoenzymes may be influenced by both environmental and developmental factors (Genkel et al. 1974), which also limit the widespread use of this technique for routine genotypic identification (Vermeulen et al. 1991). DNA typing is a preferred method for the identification or comparison of organisms because it can distinguish between even closely related genotypes (Nybom 1994). Caetano-Anollés et al. (1995, 1997) and Caetano-Anollés (1998) found that DNA-amplification fingerprinting (DAF) was an excellent technique to differentiate bermudagrass (*Cynodon*) species and cultivars, as well as to evaluate genetic relationships and the origin of bermudagrass off-types.

The amplified fragment length polymorphism (AFLP) technique for typing genomic DNA is based on the selective PCR-amplification of restriction fragments from total digests of genomic DNA. The AFLP technique combines the reliability of the RFLP technique with the power and ease of the PCR technique. Therefore, the AFLP technique is a new typing method for DNA of any origin or complexity (Heyndrickx et al. 1996; Janssen et al. 1996; Lin et al. 1996a, b). The AFLP technique does not require prior sequence analysis, primer synthesis, library construction, or the characterization of DNA probes. In addition, newly developed semi-automated fluorescence-based detection of AFLPs has improved both fragment scoring and data handling.

The objectives of the present study were to examine the potential of the AFLP technique to differentiate a number of released and experimental bermudagrass genotypes and to explore the genetic relationships among these genotypes.

Materials and methods

Genetic materials

Twenty seven bermudagrass genotypes (eight cultivars and 17 breeding lines from the Coastal Plain Experiment Station, Tifton, Georgia, plus the cultivars FloraDwarf and Midiron) were included in this study (Table 1). Among these 27 bermudagrass genotypes, four were common bermudagrass types (*C. dactylon*), five were African (*C. transvaalensis*) types, and 18 were triploid hybrids (*C. dactylon* × *C. transvaalensis*). Each accession was collected as a single stolon from turf research plots, divided into three propagules and established in three separate pots in the greenhouse in the summer of 1996.

Bermudagrass DNA samples were isolated from fresh leaf tissue with either of two modified protocols, that of Tai and Tanksley (1990) or of Williams and Ronald (1994). For the Williams and Ronald (1994) protocol, a modified PEX-buffer (10 ml of buffer per g of tissue; 100 mM Tris-HCl pH 7.5, 12.5 mM potassium ethyl xanthogenate, 10 mM EDTA pH 8.0, 700 mM NaCl, and 1.25% SDS; stored at 4°C in a brown bottle) was employed.

AFLP analysis

The AFLP protocol was as developed by Keygene, Inc. (Zabeau and Vos 1993; Vos et al. 1995). There are two commercially available AFLP kits: one for radioactive (^{32}P or ^{33}P) labeling from Life Technologies (Gaithersburg, Md.) and the other from Perkin-Elmer (1996) adapted for use with ABI PRISM™ fluorescent dye-labelling and detection technology. AFLP reactions were carried out according to the manufacturers' instructions except for minor modifications. The reaction volume from both protocols was halved. Kits optimized for a genome size of >500 Mb were used for bermudagrass AFLP fingerprinting. The ^{32}P-labelling system was mainly employed to screen primer combinations (see Table 2) and for reproducibility studies. The three best primer combinations from the ^{32}P-labelling AFLP study were tested with the fluorescent-dye labelling system.

There are three major steps for AFLP analysis, as follows: (1) restriction digestion of the DNA and ligation of the oligonucleotide adapters; (2) amplification of the restriction fragments; (3) gel analysis of the amplified fragments. For amplification reactions where the *Eco*RI primer was fluorescently labelled with FAM, 23 PCR cycles were performed, while 25 and 27 cycles were performed for JOE- and TAMRA-labelled primers, respectively.

The ^{32}P-labelled PCR products were separated by electrophoresis on a 6% polyacrymide gel in 1 × TBE buffer at 50 W for about 1.5 h. The gel was dried and exposed to X-ray film overnight. Before loading fluorescent-labelled samples, 2.0 µl of PCR products from E-ACA/M-CAC (FAM labeled), 2 µl from E-AGG/M-CAA (JOE labeled), and 5.0 µl from E-ACC/M-CAT (TAMRA labeled) were mixed. The mixtures were precipitated and resuspended in 3.0 µl of TE buffer. Samples containing 1.0 µl of the mixture of the selective

Table 1 Cultivars and breeding lines used in this study

No	Cultivar or breeding line	Species	Chromosome number (2n)	Source and reference
1	Tifgreen	*C. dactylon* × *C. transvaalenis*	27	Hein 1961
2	Tifdwarf	*C. dactylon* × *C. transvaalensis*	27	Burton 1966 b
3	Tiflawn	*C. dactylon*	36	Hein 1953
4	Tifway	*C. dactylon* × *C. transvaalensis*	27	Burton 1966 a
5	TifEagle	*C. dactylon* × *C. transvaalensis*	27	A mutant from irradiated Tifway 2 stolons
6	Tift94 (MI40)	*C dactylon* × *C. transvaalensis*	27	A mutant from irradiated Midiron stolons
7	Midiron	*C. dactylon* × *C. transvaalensis*	27	RA Keen, Kansas State University 1971
8	#75	*C. dactylon* × *C. transvaalensis*	27	Cross from T574 or × T90 1994 Tifgreen test
9	FloraDwarf	*C. dactylon* × *C. transvaalensis*	27	Dudeck and Murdoch 1997
10	Tifton 10	*C. dactylon*	54	Hanna et al. 1990
11	#18	*C. dactylon* × *C. transvaalensis*	27	Hybrid from T574 × T90 cross
12	TW262	*C. dactylon* × *C. transvaalensis*	27	Dwarf mutant induced in Tifway with gamma radiation
13	#355	*C. dactylon* × *C. transvaalensis*	27	Hybrid from T574 × T90 cross
14	Tifway 2	*C. dactylon* × *C. transvaalensis*	27	Burton 1985
15	#210	*C. dactylon* × *C. transvaalensis*	27	Hybrid from T572 or T573 × T90 cross
16	72-117	*C. dactylon* × *C. transvaalensis*	27	Mutation induced in Tifway with gamma addition
17	#197	*C. dactylon* × *C. transvaalensis*	27	Cross from T572 × T90
18	T572	*C. transvaalensis*	18	Collected in Lesotho in 1985
19	T574	*C. transvaalensis*	18	Collected in Lesotho in 1985
20	T575	*C. transvaalensis*	18	Collected in Lesotho in 1985
21	T576	*C. transvaalensis*	18	Collected in Lesotho in 1985
22	T577	*C. transvaalensis*	18	Collected in Lesotho in 1985
23	T110	*C. dactylon*	36	PI290886 collected in S. Africa
24	T90	*C. dactylon*	36	PI290885 collected in S. Africa
25	TW23	*C. dactylon* × *C. transvaalensis*	27	Dwarf mutant induced in Tifway 2 with gamma radiation
26	TW263	*C. dactylon* × *C. transvaalensis*	27	Dwarf mutant induced in Tifway 2 with gamma radiation
27	D24	*C. dactylon* × *C. transvaalensis*	27	Hybrid from T572-T576 or T110 cross

amplification products, 0.5 µl of a GeneScan 500 ROX internal lane standard, and 2.5 µl of loading buffer (3 formamide: 1 blue dextran dye) were loaded on a 5% denaturing LongRanger gel in 1 × TBE buffer and run at a constant 2500 V for 4 h at 51°C on an automated DNA sequencer (ABI model 377, Perkin-Elmer Applied Biosystems).

Data analysis for genetic relationships among cultivars

Bands on the gel were scored as present (1) or absent (0) from X-ray films of ^{32}P-labeled AFLP patterns. The data matrix obtained from scoring the presence or absence of bands was analyzed by Excel software (version 6.0, Microsoft) to calculate the shared number of fragments. For the semi-automated fluorescence-labelled protocol, the gel file produced by the ABI 377 DNA sequencer was analyzed with GeneScan analysis software (version 2.1, Perkin-Elmer/ABI). Combined data files, containing sizing data for 27 DNA samples from each primer combination, were created using Genotyper (version 1.1, Perkin-Elmer/ABI).

Pairwise comparisons were made for all genotypes and the number of shared fragments for each comparison was calculated with the aid of Mathematica software (version 2.2; Wolfram Research, Incorporated). Relative genetic dissimilarity was estimated according to Nei and Li (1979) by using Excel. Similarity was calculated as $S_{XY} = 2n_{XY}/(n_X + n_Y)$, where n_X and n_Y are the numbers of fragments in individuals X and Y, respectively, and n_{XY} is the number of the fragments shared between individuals. Conversion to dissimilarity, D, was obtained by the equation $D_{XY} = 1 - S_{XY}$. The positive dissimilarity matrices were analyzed using the UPGMA (Unweighted Pair Group Mean Average) method of Saitou and Nei (1987) in NEIGHBOR of PHYLIP software (the Phylogenetic Inference Package, version 3.55c).

Results and discussion

Comparison of AFLP primer combinations

Both commercial AFLP kits included eight *Eco*RI and eight *Mse*I primers. Therefore, 64 total combinations were available for amplification reactions. Initially, 14 primer combinations were examined by the ^{32}P-labelled AFLP technique (Table 2) with 17 bermudagrass genotypes (1–17 in Table 1). An average of 48–74 bands per primer, ranging in size from 30 to 600 bp, was obtained (Table 2). Primer selection was based on the number of fragments amplified in each accession and the amount of polymorphism exhibited among closely related genotypes. All 14 primer pairs could be used to discriminate between some of the 27 bermudagrass genotypes using the ^{32}P-labelled technique. However, results from the ^{32}P-labelled system showed that some primer combinations were more informative than others. Seven of fourteen primer pairs were applied on all 27 bermudagrass genotypes, and each pair discriminated among all 27 bermudagrass genotypes in this study.

Three pairs of primers (Table 2) were selected for the fluorescent dye-labelling technique and assayed in all 27 bermudagrass DNA samples. An increased number of bands were identified compared to the ^{32}P-labelled technique. For example, an average of 94 bands was

Table 2 Average number of bands obtained from different primer combinations. All 14 primer pairs were used in this study. Adapter and primer sequences were developed by Keygene, Inc (Zabeau and Vos 1993). E = GACTGCGTACCAATTCA and M = GATGAGTCCTGAGTAAC

Primer pair	Average number of bands
E-AAC/M-CCA	74
E-AAC/M-CAG	40[c]
E-AAC/M-CAT	39[c]
E-AAG/M-CTG	66
E-AAG/M-CTT	57
E-ACA/M-CAC[a]	66 (71)[b]
E-ACC/M-CAG	66
E-ACC/M-CAT[a]	62 (79)[b]
E-AGC/M-CAC	54
E-AGC/M-CAT	56
E-AGC/M-CTC	49
E-AGC/M-CTT	53
E-AGG/M-CAA[a]	68 (94)[b]
E-AGG/M-CAG	48

[a] Primer pairs used for semi-automated fluorescence-based AFLP
[b] Number in brackets is the average band number from fluorescence-labelled AFLPs
[c] Data from these primer pairs were not included in the results due to very weak PCR amplification products

detected by the automated fluorescence-labelled technique among the 27 genotypes, while only 68 bands were scored by the ^{32}P-labelled technique using the same primer combination (E-AGG/M-CAA) (Table 2).

Reproducibility and reliability of the ^{32}P-labelled AFLP technique

DNA from the 27 bermudagrass genotypes was extracted separately and amplified with five primer combinations (E-AAC/M-CAA, E-AAG/M-CTG, E-ACC/M-CAG, E-ACC/M-CAT and E-AGG/M-CAA) to test the reproducibility of the AFLP technique using the ^{32}P-labelled method. In 15 of the 27 bermudagrass genotypes, the AFLP band patterns obtained from three separate DNA preparations (replications) were identical. Data were obtained from only two replications of three entries and their AFLP band patterns were identical. No PCR product was obtained from one genotype (D24). There were 1- or 2-band differences among the three replications of seven genotypes for a total of 0.6% of the bands to be evaluated differently between replicates. These differences were caused mainly by varying intensities of the amplified fragments on the films, which could be improved by varying the exposure times. The clustering results showed that 1- or 2- band differences did not change the groupings of these seven genotypes. Therefore, the AFLP technique gave reproducible patterns. Among the 27 accessions, a single replication of two accessions (the 3rd replication of both #210 and Tifgreen) showed a band pattern quite different from the two other replications. We re-checked the original plant of #210 growing in the greenhouse, and found the third replication to be morphologically different and probably the result of a contaminant at planting. However, no morphological differences were observed among any of the replications of Tifgreen. Although the lane of the third replication of Tifgreen had three additional bands, the three replications were still grouped on the same branch of the UPGMA tree.

In order to determine the reliability of the AFLP technique as a potential tool for bermudagrass genotype differentiation and varietal protection, 15 'blind' bermudagrass samples of eight genotypes were tested with one primer combination (E-ACC/M-CAG) by the ^{32}P-labelled AFLP technique and compared with standard genotypes. Thirteen of the fifteen samples were correctly identified, or identified as closely related to the correct genotypes. Two samples could not be identified. However, all 15 unknown samples were identified correctly after evaluating the patterns produced with two additional primer pairs (E-AAG/M-CTG and E-AGG/M-CAG).

The 27 genotypes were analyzed in a single laboratory by the semi-automated fluorescence-labelled AFLP technique and in two different laboratories by the ^{32}P-labelled technique to determine if the methods and primer combinations would influence the AFLP-based grouping of bermudagrass genotypes. The results, using the same samples, showed that the AFLP band patterns varied among the different primer pairs, but that all the major branches on the resultant UPGMA trees (Fig. 1) were identical, regardless of the labelling method or the laboratory. RAPD banding patterns can vary depending on the *Taq* polymerase employed (Wolff et al. 1993), but AFLP banding patterns were identical, at least with respect to the low-molecular-weight fragments, using different *Taq* polymerases and buffers in the PCR reaction (Schondelmaier et al. 1996). Reliability of the AFLP technique is also improved by the stringent reaction conditions that are used during primer annealing (Zabeau and Vos 1993). In the AFLP reactions, mispaired primers are not expected to participate in the amplification process. Results from our study demonstrate that the AFLP technique is reliable and gives reproducible results for the differentiation of bermudagrass genotypes. Caetano-Anollés et al. (1997) obtained similar reproducible results using the DAF technique.

Genetic relationships among bermudagrass cultivars

Genetic dissimilarity coefficients based on the fluorescent-labelled AFLP data ranged from 0.05 to 0.67 for the 27 bermudagrass genotypes (Table 3). The dissimilarity coefficient for Tifdwarf and Tifgreen was 0.08, one

Table 3 Genetic disimilarity coefficient calculated for 27 bermudagrass varieties and breeding lines combining data from three primer pairs (E-ACA/M-CAC, E-ACC/M-CAT, E-AGG/M-CAA) by using the fluorescence-labelled AFLP technique. Nei and Li's similarity coefficient (Nei and Li 1979) is calculated as $S_{xy} = 2n_{xy}/(n_x + n_y)$. The disimilarity coefficient between two lines is: $D_{xy} = 1 - S_{xy}$, which can range from 0, when all bands in the two lines are identical, to 1, when there are no bands in common between the two lines

Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 Tifgreen	0.00																										
2 Tifdwarf	0.08	0.00																									
3 Tiflawn	0.36	0.35	0.00																								
4 Tifway	0.56	0.57	0.52	0.00																							
5 TifEagle	0.28	0.33	0.48	0.48	0.00																						
6 Tift94	0.57	0.58	0.56	0.11	0.48	0.00																					
7 Midiron	0.51	0.52	0.59	0.58	0.52	0.57	0.00																				
8 #75	0.57	0.59	0.57	0.38	0.55	0.38	0.46	0.00																			
9 FloraDwarf	0.27	0.32	0.51	0.55	0.27	0.55	0.42	0.50	0.00																		
10 Tifton 10	0.57	0.57	0.50	0.61	0.54	0.60	0.64	0.60	0.56	0.00																	
11 #18	0.54	0.55	0.55	0.37	0.49	0.35	0.52	0.35	0.49	0.52	0.00																
12 TW262	0.60	0.60	0.60	0.30	0.54	0.28	0.57	0.42	0.54	0.54	0.27	0.00															
13 #355	0.26	0.30	0.48	0.54	0.29	0.53	0.50	0.55	0.22	0.56	0.52	0.57	0.00														
14 Tifway2	0.56	0.57	0.55	0.28	0.54	0.25	0.56	0.37	0.55	0.61	0.37	0.25	0.52	0.00													
15 #210	0.56	0.58	0.57	0.39	0.57	0.38	0.57	0.36	0.56	0.61	0.36	0.36	0.50	0.25	0.00												
16 72-117	0.60	0.61	0.59	0.31	0.56	0.27	0.59	0.45	0.56	0.62	0.40	0.30	0.57	0.29	0.38	0.00											
17 #197	0.28	0.31	0.48	0.56	0.29	0.55	0.50	0.55	0.28	0.53	0.50	0.53	0.27	0.55	0.52	0.48	0.00										
18 T572	0.58	0.59	0.61	0.49	0.56	0.50	0.56	0.46	0.55	0.66	0.48	0.50	0.53	0.48	0.43	0.43	0.47	0.00									
19 T574	0.57	0.58	0.61	0.54	0.54	0.56	0.59	0.54	0.55	0.65	0.51	0.58	0.55	0.56	0.54	0.53	0.52	0.40	0.00								
20 T575	0.57	0.59	0.62	0.54	0.55	0.56	0.61	0.57	0.57	0.64	0.53	0.60	0.54	0.55	0.54	0.54	0.53	0.40	0.23	0.00							
21 T576	0.56	0.58	0.64	0.51	0.54	0.51	0.57	0.51	0.55	0.67	0.50	0.56	0.54	0.50	0.47	0.50	0.52	0.33	0.31	0.29	0.00						
22 T577	0.61	0.61	0.63	0.52	0.59	0.52	0.55	0.43	0.57	0.65	0.49	0.52	0.55	0.48	0.45	0.48	0.53	0.18	0.38	0.43	0.36	0.00					
23 T110	0.62	0.62	0.57	0.38	0.58	0.37	0.61	0.40	0.60	0.60	0.38	0.38	0.60	0.36	0.34	0.38	0.59	0.57	0.58	0.61	0.58	0.53	0.00				
24 T90	0.61	0.60	0.57	0.38	0.57	0.37	0.60	0.41	0.60	0.60	0.38	0.39	0.59	0.35	0.32	0.36	0.57	0.56	0.57	0.60	0.58	0.54	0.05	0.00			
25 TW23	0.61	0.61	0.58	0.28	0.59	0.25	0.58	0.43	0.59	0.62	0.39	0.26	0.58	0.21	0.35	0.29	0.57	0.50	0.54	0.58	0.49	0.48	0.36	0.34	0.00		
26 TW263	0.60	0.59	0.59	0.26	0.55	0.22	0.57	0.42	0.55	0.61	0.37	0.25	0.56	0.21	0.34	0.23	0.54	0.46	0.50	0.54	0.47	0.46	0.33	0.31	0.08	0.00	
27 D24	0.60	0.60	0.60	0.26	0.56	0.22	0.59	0.41	0.56	0.62	0.37	0.25	0.57	0.23	0.33	0.26	0.55	0.48	0.53	0.56	0.49	0.48	0.32	0.30	0.10	0.05	0.00

of the lowest for pairwise comparisons among triploid hybrids. Tifdwarf is presumed to be a mutant of Tifgreen. Caetano-Anollés (1998) used the DAF technique to effectively differentiate Tifgreen and Tifdwarf, and the putative somatic mutants, from these two cultivars. T110 and T90, both common bermudagrass accessions from South Africa, showed a distance of 0.05, the lowest among all genotypes, indicating that they are closely related. T576 (*C. transvaalensis*) and Tifton 10 (*C. dactylon*) are different species, one collected in Africa and the other in China, and showed the highest dissimilarity coefficient (0.67).

The UPGMA tree generated by dissimilarity coefficients grouped the 27 cultivars and breeding lines into three major clusters, A, B and C (Fig. 1). Tifgreen and its putative spontaneous somatic mutants, Tifdwarf and Floradwarf, were grouped in Cluster A with Tiflawn, Midiron, and two new interspecific crosses (#197 and #355). Tifway and most of its mutants (Tifway 2, TW23, TW262, TW263 and 72-117) were grouped in Cluster B with Tift94, T90 and T110, and several new interspecific crosses (#18, #75, #210 and D24). Group C contained only the five accessions of the African-type bermudagrasses (*C. transvaalensis*) collected from Lesotho. Group C was quite distinct from group A and group B, which contained the common *C. dactylon* types (Tifton 10, Tiflawn, T90, and T110) and 18 interspecific hybrids (*C. dactylon* × *C. transvaalensis*).

The *C. dactylon* accessions did not form a single group. Among them, Tifton 10, a hexaploid from China, and Tiflawn, a tetraploid, were clustered in Group A. However, the other two *C. dactylon* accessions from South Africa, T90 and T110, were placed in Group B. This shows there is a wide genetic diversity among genotypes within *C. dactylon.*

Hybridization is one of the most common methods used to create variation in bermudagrass (Burton 1992). Most of the bermudagrass cultivars are interspecific hybrids between *C. dactylon* and *C. transvaalensis.* In this study, we included 18 hybrids, which were divided into two distinct groups on the UPGMA trees. Among these hybrids, two of six new crosses were placed in Group A, but four crosses were placed in Group B. Since bermudagrass is highly cross-pollinated, the progeny of genetically heterozygous plants are expected to be highly variable, but we have no explanation for the existence of two main groups.

Major groupings in the UPGMA trees from single primer pairs or from combined data analysis did not change significantly. When UPGMA trees were constructed with data from additional primer combinations (data not shown) using the two AFLP approaches, the major groups formed were very similar to the data presented. AFLP data obtained from the ^{32}P-labelled and fluorescent-labelled AFLP techniques also gave similar results. Although the major groups were similar, the internal structure of these groups

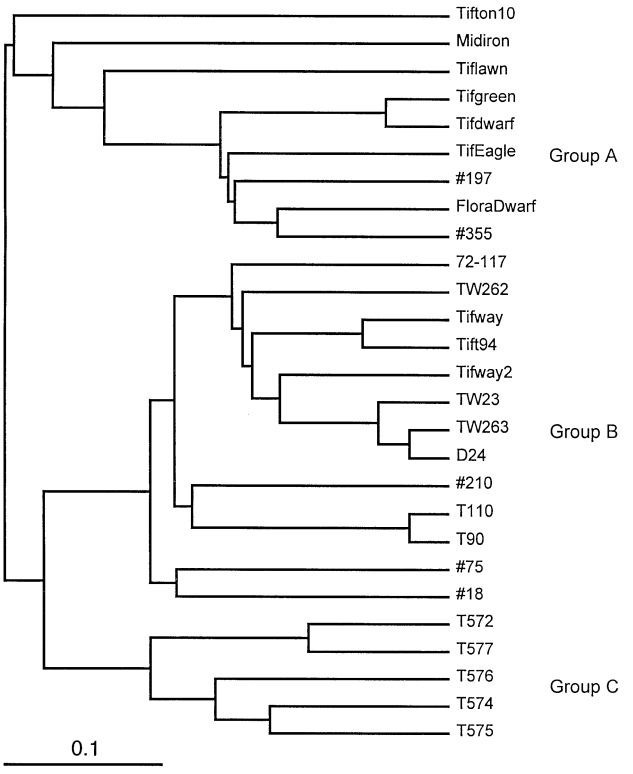

Fig. 1 UPGMA tree of 27 genotypes based on Nei's formula calculated with data from three primer pairs (E-ACA/M-CAC, E-ACC/M-CAT, E-AGG/M-CAA) by using the semi-automated fluorescence-based AFLP technique

varied somewhat with the different data sets. Specifically, the positions of T576, #197, #18 and TW262 changed slightly within the major groups.The AFLP results in this research generally agree with the known breeding history of the bermudagrass cultivars.

Some results in this experiment were unexpected. TifEagle (derived from an irradiated stolon of Tifway 2) and Tifway 2 had a dissimilarity coefficient of 0.54 and were placed in two separate groups on the UPGMA tree. The smaller than expected dissimilarity coefficient (0.11) between Tifway and Tift94 was also surprising, since Tift94 was derived from an irradiated stolon of Midiron. D24, a new *C. transvaalensis* × *C. dactylon* triploid hybrid, showed small dissimilarity coefficients of 0.10 and 0.05, respectively, with TW23 and TW263, mutants of Tifway 2. Tifway 2, a mutant of Tifway, showed a larger than expected dissimilarity coefficient of 0.28 with Tifway. Caetano-Anollés (1998) was not able to separate Tifway and Tifway 2 by the DAF technique. It appears that gamma radiation may cause larger changes in the genome than previously believed, especially since bermudagrass is highly heterozygous (Burton and Hanna 1985) and the use of irradiation is an efficient way to produce new variation in bermudagrass. The triploid bermudagrass hybrids are very susceptible to change by mutation because they have a single chromosome set (nine chromosomes) from

C. transvaalensis, and recessive mutations in this genome would thus be revealed.

The semi-automated fluorescence-labelled AFLP technique

Fluorescent labelling and automated fragment detection and sizing technologies offer significant improvements over radioactive labelling methods.There are two limitations of the radioisotope-labelled AFLP technique – scoring accuracy and typing efficiency. Varying intensities of DNA fragments in PCR reactions may lead to errors in scoring. DAF, like the fluorescence labelled technology, avoids the use of radioactive labelling methods but does not have as effective automated band detection. In the semi-automated process, a much larger range of product sizes can be resolved on each gel compared with the radioactive labelling techniques, because the products pass a detector near the bottom of the gel where the band resolution is greatest. The advantage inherent in direct acquisition of genetic marker data into a computer database management system may ultimately be the most significant benefit of the fluorescence-based approach. Multiplexing of the fluorescence labelling reactions can reduce the cost of the AFLP technique (Mitchell et al. 1997). However, we should note that detection of fluorescence-labelled AFLP products can be complicated by sample overloading, which causes spectral interference between the dye labels during analysis. Overlapping caused by sample overloading could lead to artifacts. The fluorescence-labelled AFLP technique generated some unique fragments for several bermudagrass cultivars. The use of unique fragments might simplify future cultivar identification if they could be cloned and the sequence used to develop SCARS (Paran and Michelmore 1993). However, the sequence difference that gives rise to an AFLP fragment may be as little as a single base change in the restriction site, which could reduce the probability of developing a successful SCAR.

The results from this study showed that the AFLP technique measured sufficient polymorphism for DNA typing, and may be a powerful tool for the genetic dissection of the bermudagrass genome. AFLP analysis of turfgrass will not only provide protection of the proprietary rights, but will also have application as a breeding tool.

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