AFLP analysis of *Cynodon dactylon* (L.) Pers. var. *dactylon* genetic variation

Y.Q. Wu, C.M. Taliaferro, G.H. Bai, and M.P. Anderson

Abstract: *Cynodon dactylon* (L.) Pers. var. *dactylon* (common bermudagrass) is geographically widely distributed between about lat 45°N and lat 45°S, penetrating to about lat 53°N in Europe. The extensive variation of morphological and adaptive characteristics of the taxon is substantially documented, but information is lacking on DNA molecular variation in geographically disparate forms. Accordingly, this study was conducted to assess molecular genetic variation and genetic relatedness among 28 *C. dactylon* var. *dactylon* accessions originating from 11 countries on 4 continents (Africa, Asia, Australia, and Europe). A fluorescence-labeled amplified fragment length polymorphism (AFLP) DNA profiling method was used to detect the genetic diversity and relatedness. On the basis of 443 polymorphic AFLP fragments from 8 primer combinations, the accessions were grouped into clusters and subclusters associating with their geographic origins. Genetic similarity coefficients (SC) for the 28 accessions ranged from 0.53 to 0.98. Accessions originating from Africa, Australia, Asia, and Europe formed major groupings as indicated by cluster and principal coordinate analysis. Accessions from Australia and Asia, though separately clustered, were relatively closely related and most distantly related to accessions of European origin. African accessions formed two distant clusters and had the greatest variation in genetic relatedness relative to accessions from other geographic regions. Sampling the full extent of genetic variation in *C. dactylon* var. *dactylon* would require extensive germplasm collection in the major geographic regions of its distributional range.

Key words: common bermudagrass, AFLP marker, genetic relatedness.

Résumé : Le *Cynodon dactylon* (L.) Pers. var. *dactylon* (chiendent pied-de-poule) est largement distribué entre le lat 45°N et le lat 45°S et atteint même le lat 53°N en Europe. La grande variation observée au niveau des caractères morphologiques et adaptatifs au sein de ce taxon est fort bien documentée, mais il manque d'information sur la variation génétique moléculaire (au niveau de l'ADN) entre les diverses formes. Conséquemment, cette étude a été réalisée afin de mesurer la variation et la parenté génétiques parmi 28 accessions du *C. dactylon* var. *dactylon* provenant de 11 pays situés sur quatre continents (Afrique, Asie, Australie et Europe). Des empreintes génétiques produites à l'aide du polymophisme de longueur des fragments amplifiés (AFLP), révélé par fluorescence, ont été employées pour détecter la diversité et la parenté génétiques. Sur la base de 443 fragments AFLP polymorphes obtenus à l'aide de 8 combinaisons d'amorces, les accessions ont été groupées en groupes et sous-groupes correspondant à leur origine géographique. Les coefficients de similarité génétique (SC) pour les 28 accessions variaient de 0,53 à 0,98. Les accessions provenant d'Afrique, d'Australie, d'Asie et d'Europe formaient les groupements principaux définis à la suite d'analyses de groupement et des coordonnées principales. Les accessions de l'Australie et de l'Asie, bien que formant des groupes distincts, étaient relativement proches et s'avéraient les plus distantes par rapport aux accessions européennes. Les accessions africaines formaient deux groupes assez distants et montraient la plus grande variation quant à leur parenté par rapport aux accessions provenant des autres régions. Un échantillonnage de la gamme complète de variation génétique chez le *C. dactylon* var. *dactylon* nécessiterait un important travail de collection de ressources génétiques au sein des principales régions de sa distribution géographique.

Mots clés : chiendent pied-de-poule, marqueur AFLP, parenté génétique.

[Traduit par la Rédaction]

Introduction

Cynodon dactylon (L.) Pers. var. *dactylon* (common bermudagrass) is the most important member of the genus *Cynodon* because of its widespread distribution in warmer parts of the world and its use as livestock herbage and turf (Harlan 1970). Harlan and de Wet (1969) describe the taxon as the ubiquitous, cosmopolitan weed of the world, contain-

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Table 1. *Cynodon dactylon* var. *dactylon* accessions analyzed for AFLP.

	Identification				
No.	Oklahoma	PI	Other	Chromosome No.	Origin: country (continent)
1	A12374			36	Zimbabwe (Africa)
2	A12375			36	Zimbabwe (Africa)
3		290882		36	South Africa (Africa)
4		291582	Skaaplaas	36	Zimbabwe (Africa)
5		291584	Maadi River	36	Zimbabwe (Africa)
6		291583	Australian Evergreen	36	Australia (Australia)
7	A12376			36	Australia (Australia)
8	A12377			36	Australia (Australia)
9	A12378			36	Australia (Australia)
10			Cn-1	36	Australia (Australia)
11		295339		36	Germany (Europe)
12		251809		36	Italy (Europe)
13			Nr 24*	36	Bulgaria (Europe)
14			Nr 28*	36	Spain (Europe)
15			Nr 34*	36	Italy (Europe)
16			Nr 47*	36	France (Europe)
17			JT-1†	36	Japan (Asia)
18			JT-2†	36	Dubai, UAE (Asia)
19	A12262			36	China (Asia)
20	A12281			36	China (Asia)
21	A12315			36	China (Asia)
22	A12349			36	China (Asia)
23	A12361			36	China (Asia)
24	A12317			54	China (Asia)
25	A12318			54	China (Asia)
26	A12356			54	China (Asia)
27	A12358			54	China (Asia)
28			'Tifton 10'	54	China (Asia)

*Kindly provided by Koos de Bruijn, Barenbrug Tourneur Recherches, Mas Grenier, France.
†Kindly provided by Khorshid Razmjoo, Japan Turfgrass Inc., Chiba, Japan.

Table 2. Primer combinations for pre- and selective-amplification, and total and polymorphic bands scored in *Cynodon dactylon* var. *dactylon* AFLP profiling.

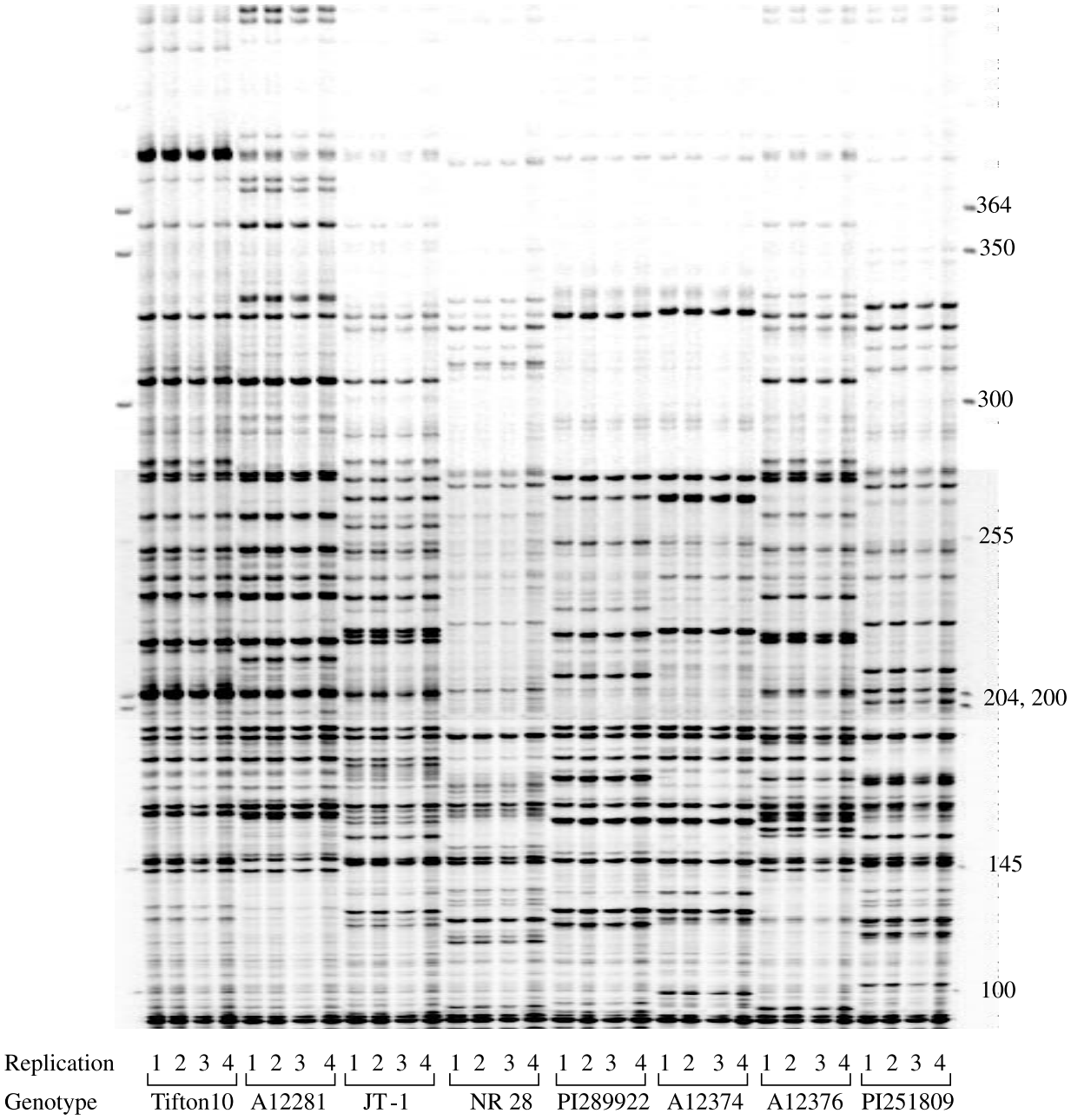
Primer combinations*	Total bands	Polymorphic bands
e-ACT–m-CAG	86	65
e-AAC–m-CAG	52	46
e-AGT–m-CAG	126	81
e-GCTG–m-CAG	76	60
e-ACT–m-CAT	86	74
e-AAC–m-CAT	66	42
e-ACT–m-CAC	50	40
e-AAC–m-CAC	48	36
Total	590	443
Average	74±26	56±17

*e, pre-amplification primer of *Eco*RI (GACTGCGTACCAATTC); m, pre-amplification primer of *Mse*I (GATGAGTCCTGAGTAA).

ing enormous variation ranging from small, fine turfgrasses used as golf course putting green turf to robust types grown for pasture or hay. They indicated that the taxon occurs across all continents and islands between about lat 45°N and lat 45°S, and penetrates to approximately lat 53°N in Europe. Evidence from biosystematic studies of *C. dactylon* var. *dactylon* suggested to Harlan and de Wet (1969), Harlan (1970) and Harlan et al. (1970*c*) that it was a Eurasian grass until recent times, and that a geographic area extending from western Pakistan to Turkey was a center of evolutionary activity for the taxon. Harlan (1970) stated that the aggressive weedy races now widely geographically distributed likely emerged from that center.

Variations in *C. dactylon* var. *dactylon* morphological features, distributional patterns, and associated adaptive and reproductive characteristics are documented to a fuller degree (Harlan and de Wet 1969; Harlan 1970; Harlan et al. 1970*b*, 1970*c*, 1970*d*) than are variations in DNA markers. The ability of DNA profiling to discriminate between genetically different *Cynodon* plants and estimate their degree of relatedness is documented (Caetano-Anolles et al. 1995, 1997; Caetano-Anolles 1998; Ho et al. 1997; Assefa et al. 1998; Anderson et al. 2001; Roodt et al. 2002; Zhang et al. 1999; Karaca et al. 2002). DNA profiling techniques that have been successfully used in assessing relatedness of *Cynodon* accessions include DNA fingerprinting (DAF) (Caetano-Anolles et al. 1995, 1997; Caetano-Anolles 1998; Ho et al. 1997; Assefa et al. 1998; Anderson et al. 2001), randomly amplified polymorphic DNA (RAPD) (Roodt et al. 2002), and amplified fragment length polymorphism (AFLP)

Fig. 1. AFLP fingerprints generated using primer combination e-ACT–m-CAG for 28 *Cynodon dactylon* var. *dactylon* accessions. Fragment size is indicated on the right.



(Zhang et al. 1999; Karaca et al. 2002). Though these studies have demonstrated the utility of DNA profiling in assessing the degree of relatedness of *Cynodon* members, none has focused on assessing variations within cosmopolitan *C. dactylon* var. *dactylon*. This study was conducted to quantify the genetic relatedness of *C. dactylon* var. *dactylon* accessions of disparate geographical origin based on AFLP DNA markers.

Materials and methods

Plant materials and DNA isolation

Twenty-eight *C. dactylon* var. *dactylon* clonal accessions (genotypes) originating from 11 countries encompassing 4 continents were used in the study (Table 1). Plants of each accession were grown in 15-cm diameter pots in the greenhouse. Total genomic DNA samples were isolated from fresh leaf tissue of each accession with the DNeasy plant mini kit from Qiagen Inc. (Valencia, Calif.). Before enzyme digestion, genomic DNA was diluted to a final concentration of 100 ng/μL.

AFLP DNA profiling

The AFLP analysis was performed as described by Vos et al. (1995). Lab optimization and minor modifications were made according to Bai et al. (1999). Briefly, isolated genomic DNA (approximately 300 ng) was double digested with *Eco*RI and *Mse*I restriction enzymes. AFLP adapters

Table 3. Similarity coefficients for *Cynodon dactylon* var. *dactylon* accessions based on amplified fragment length polymorphisms.

No.*	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.00												
2	0.62	1.00											
3	0.69	0.70	1.00										
4	0.63	0.74	0.83	1.00									
5	0.77	0.70	0.68	0.65	1.00								
6	0.82	0.61	0.70	0.62	0.81	1.00							
7	0.65	0.63	0.64	0.59	0.65	0.77	1.00						
8	0.66	0.66	0.69	0.61	0.68	0.75	0.82	1.00					
9	0.66	0.66	0.68	0.61	0.70	0.76	0.78	0.79	1.00				
10	0.71	0.62	0.64	0.55	0.74	0.80	0.80	0.79	0.80	1.00			
11	0.64	0.66	0.67	0.60	0.71	0.73	0.79	0.79	0.79	0.78	1.00		
12	0.54	0.72	0.58	0.75	0.62	0.55	0.53	0.57	0.55	0.53	0.54	1.00	
13	0.57	0.69	0.56	0.66	0.63	0.57	0.56	0.57	0.56	0.58	0.58	0.84	1.00
14	0.55	0.69	0.56	0.67	0.61	0.56	0.55	0.57	0.53	0.56	0.56	0.83	0.86
15	0.56	0.70	0.56	0.64	0.66	0.57	0.55	0.57	0.54	0.57	0.56	0.82	0.84
16	0.60	0.69	0.55	0.63	0.63	0.62	0.62	0.62	0.59	0.62	0.62	0.77	0.83
17	0.69	0.65	0.69	0.69	0.75	0.77	0.69	0.68	0.69	0.72	0.69	0.63	0.65
18	0.72	0.64	0.63	0.60	0.80	0.81	0.76	0.76	0.77	0.80	0.76	0.61	0.63
19	0.71	0.61	0.67	0.59	0.78	0.81	0.77	0.76	0.81	0.80	0.76	0.57	0.59
20	0.69	0.61	0.64	0.59	0.78	0.78	0.74	0.77	0.78	0.81	0.74	0.57	0.59
21	0.71	0.62	0.64	0.58	0.80	0.80	0.76	0.77	0.80	0.80	0.75	0.57	0.60
22	0.68	0.61	0.62	0.58	0.81	0.78	0.75	0.77	0.78	0.82	0.75	0.58	0.59
23	0.68	0.62	0.61	0.56	0.78	0.78	0.74	0.76	0.79	0.79	0.72	0.59	0.61
24	0.63	0.68	0.59	0.61	0.72	0.71	0.71	0.73	0.74	0.74	0.68	0.64	0.66
25	0.62	0.67	0.60	0.62	0.71	0.71	0.69	0.73	0.76	0.73	0.70	0.64	0.65
26	0.63	0.68	0.60	0.62	0.73	0.71	0.71	0.74	0.76	0.74	0.69	0.65	0.67
27	0.64	0.67	0.60	0.62	0.73	0.72	0.71	0.74	0.76	0.74	0.69	0.65	0.67
28	0.66	0.64	0.62	0.59	0.71	0.74	0.75	0.74	0.77	0.78	0.74	0.60	0.63

*Numbers correspond to those listed in Table 1 for the 28 accessions.

for both enzymes were then ligated to restriction fragments (e and m represent the sequences of preamplification primers of *Eco*RI and *Mse*I, respectively). The ligated DNA was pre-amplified using a primer combination based on the sequences of the adapters. Pre-amplification was performed for 30 cycles of 30 s at 94 °C, 1 min at 65 °C, and 1 min at 72 °C. A total of eight AFLP selective primer combinations (Table 2) with *Eco*RI primers labeled with either IRD-700 or IRD-800 infrared fluorescence dye were used for selective amplification. The following touchdown thermal profile was used in all selective amplifications: 2 min at 94 °C; 13 touchdown cycles at 94 °C for 30 s, 65 °C for 30 s (decreasing the temperature by 0.7 °C per cycle), and 72 °C for 60s; 23 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s. All PCRs were conducted on an MJ PTC-100 thermocycler. One microlitre of selective amplification PCR products and 1.2 μL of DNA size markers were loaded on a 6.5% w/*v* denaturing Long Ranger gel (BMA, Rockland, Maine) in 1× TBE buffer and run at a constant 2000 V for 3.5 h in a Li-Cor NEW® Global IR^2-4200 DNA analyzer (Li-Cor Inc., Lincoln, Nebr.).

Eight accessions were used to test the reproducibility of the AFLP procedures. Two sets of DNA samples were isolated separately from leaf tissues of each of the eight plant accessions. One set of the DNA samples was used to produce one set of selectively amplified PCR products using the previously described AFLP procedures. The second set of DNA samples was processed through the digestion and ligation to produce eight pre-amplified PCR products. The eight pre-amplified PCR products were then selectively amplified three times to produce three sets of selectively amplified PCR products. The resulting four sets of selectively amplified PCR products from the two sets of DNA samples were run on the same gel to measure reproducibility. The four PCR products from the same accessions were run in adjacent lanes of the gel.

Data analysis for genetic relationships among accessions

AFLP bands throughout the gel profiles were scored visually as present (1), absent (0) or ambiguous (9) at least twice for each accession. Data were compiled for each accession in a data matrix and were analyzed using the NTSYS (Numerical Taxonomy System) program, version 2.0 (Exeter Software, New York, N.Y.). Similarity coefficients were computed using the SIMQUAL module. Cluster analysis was performed according to UPGMA (unweighted pair-group method with arithmetic averaging) within the SAHN module of the NTSYS program. A principal coordinate analysis was performed using the DCENTER module of the NTSYSpc program.

Results and discussion

Reproducibility of AFLP

Reproducibility of the AFLP products was very high. Of the bands amplified by primer combinations e-ACT–m-CAG

14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1.00														
0.84	1.00													
0.82	0.83	1.00												
0.64	0.67	0.70	1.00											
0.62	0.63	0.68	0.73	1.00										
0.58	0.56	0.63	0.75	0.86	1.00									
0.59	0.59	0.62	0.73	0.86	0.90	1.00								
0.59	0.60	0.63	0.73	0.87	0.91	0.91	1.00							
0.59	0.60	0.63	0.73	0.88	0.90	0.92	0.91	1.00						
0.61	0.61	0.64	0.71	0.84	0.89	0.89	0.90	0.88	1.00					
0.65	0.65	0.69	0.72	0.77	0.79	0.81	0.82	0.80	0.84	1.00				
0.63	0.63	0.68	0.71	0.76	0.77	0.81	0.81	0.79	0.82	0.96	1.00			
0.65	0.66	0.69	0.71	0.77	0.78	0.80	0.82	0.79	0.81	0.96	0.95	1.00		
0.66	0.66	0.70	0.73	0.76	0.78	0.80	0.82	0.79	0.82	0.95	0.94	0.98	1.00	
0.61	0.60	0.66	0.72	0.81	0.83	0.85	0.85	0.83	0.86	0.87	0.87	0.86	0.86	1.00

and e-AAC–m-CAG (data are not provided), 99.2% were identical among the replicate DNA samples (Fig. 1). The non-repeatable bands were mainly faint bands that showed up in some PCRs, but not in others. These results are consistent with previous reports regarding the reproducibility of AFLP markers (Zhang et al. 1999; Rouf Mian et al. 2002) and further confirm that the AFLP technique generates highly reproducible DNA profiles for *C. dactylon* var. *dactylon*.

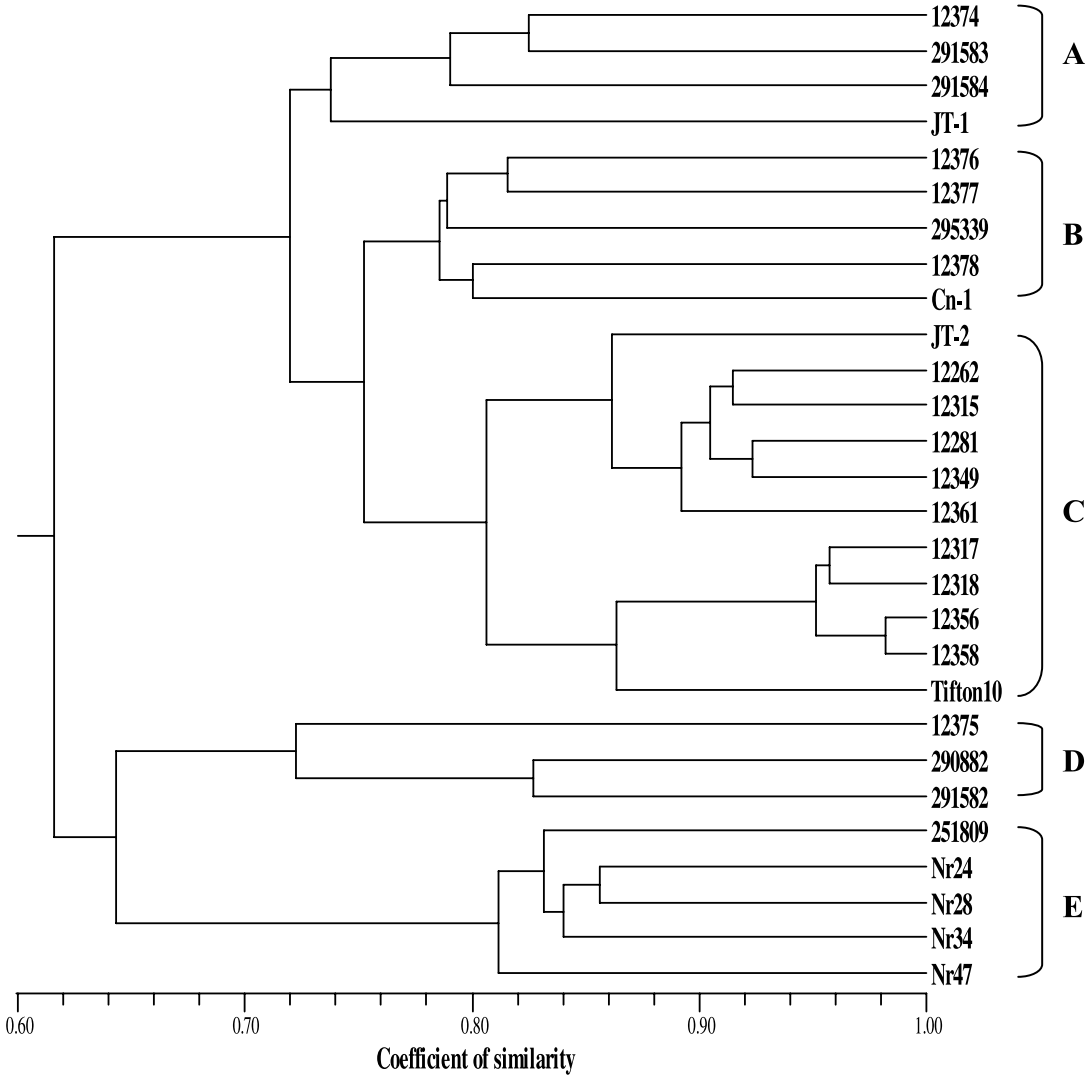
Genetic diversity and relatedness

The eight selective primer combinations amplified 590 complete bands, averaging 74 ± 26 bands per primer combination, with most bands ranging in size from 50 to 500 bp (Table 2). Of the 590 bands scored, 443 (75%) were polymorphic by virtue of their absence in at least 1 of the 28 accessions. Primer combination e-AGT–m-CAG produced the greatest total number of bands (126) and the greatest number of polymorphic bands (81), whereas e-AAC–m-CAC produced the fewest total (48) and polymorphic (36) bands (Table 2).

Genetic similarity coefficients (SC) based on the AFLP data ranged from 0.53 to 0.98 for the 28 accessions (Table 3). The highest SC (0.98) for pair wise comparisons among the 28 accessions was between accessions A12356 and A12358 from Zhejiang and Jiangsu provinces, respectively, in eastern China. The lowest SC value (0.53) was for the pairwise comparisons of Nr 28 from Spain and A12378 from Australia, A12376 from Australia and PI 251809 from Italy, and Cn-1 from Australia and PI 251809.

The UPGMA cluster tree generated by similarity coefficients grouped the 28 accessions into 5 major clusters designated A, B, C, D, and E (Fig. 2). Cluster A included four tetraploid ($2n = 4x = 36$) accessions, three of which (A12374, PI 291583, and PI 291584) were from Zimbabwe and one, JT-1, from Japan. Field collection notes of W.W. Huffine indicated that PI 291583, the commercial cultivar 'Australian Evergreen', was collected from the turf plots of Marlborough nurseries in Zimbabwe. Genetic SC values among these accessions within the cluster were very similar, ranging from 0.69 to 0.82. Cluster B comprised four tetraploid accessions (A12376, A12377, A12378, and Cn-1) from Australia and PI 295339 from Germany. Genetic SC values for accessions in cluster B ranged from 0.78 to 0.82 with a mean of 0.79 ± 0.01. The very close relationship of the four Australian accessions and PI 295339 suggests a common origin, but there is no evidence that such was the case. Records indicate that PI 295339 was collected from Ingelheim (Rheindamm), Germany, and included in the American *Cynodon* collection in 1964 (USDA, National Genetic Resources Program 2003). Cluster C consisted of tetraploid and hexaploid ($2n = 6x = 54$) accessions mainly from China. Genetic SC values for accessions in cluster C ranged from 0.76 to 0.98 with a mean of 0.85 ± 0.06. Three subgroups were evident. The first subgroup consisted only of accession JT-2, which was introduced to Japan from Dubai,

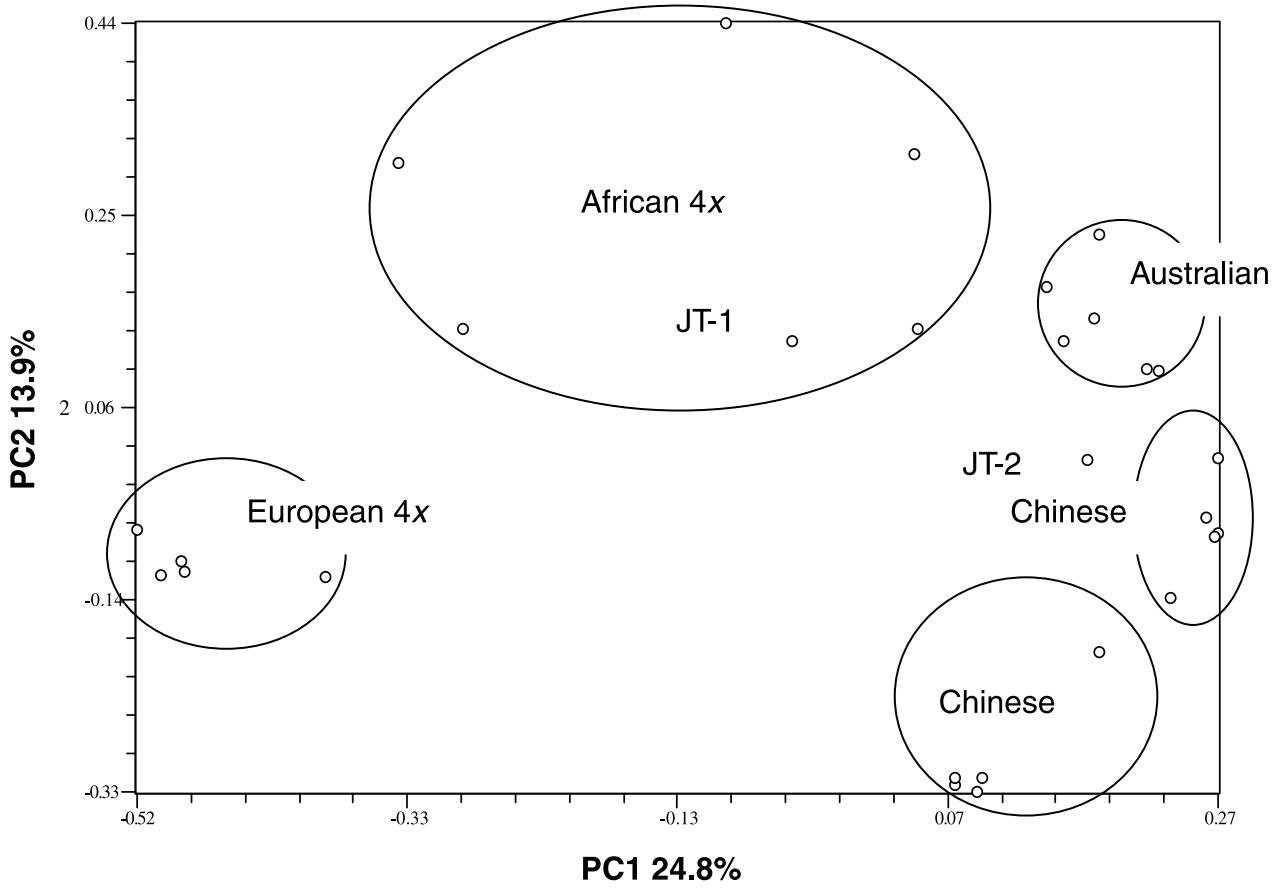
Fig. 2. UPGMA dendrogram depicting patterns of genetic diversity for 28 *Cynodon dactylon* var. *dactylon* accessions estimated by 443 AFLP markers among 28 accessions from 11 countries.



United Arab Emirates (K. Razmjoo, personal communication 1997). The second subgroup consisted of five tetraploid accessions from China (A12262, A12315, A1228, A12349, and A12361). The third subgroup comprised five hexaploid ($2n = 6x = 54$) accessions from China. Three of the five accessions, including 'Tifton 10' (Hanna et al. 1990), were collected in Shanghai and the other two from the adjacent Zhejiang (A12356) and Jiangsu (A12358) provinces. Genetic SC for the five hexaploid accessions ranged from 0.86 to 0.98, with a mean of 0.92 ± 0.05. Hexaploidy is rare in *Cynodon*, having been reported for only a few plants (Moffett and Hurcombe 1949; Powell et al. 1968; Felder 1967; Johnston 1975; Hanna et al. 1990; Malik and Tripathi 1968). Hexaploid forms were not indicated for *C. dactylon* var. *dactylon* in the taxonomic revision of the genus as listed by Harlan (1970), de Wet and Harlan (1970), and Harlan et al. (1970*a*, 1970*b*), probably because their collection contained none (Harlan and de Wet 1969). Relative to other *Cynodon dactylon* varieties, the five hexaploid accessions in this study are morphologically most similar to plants of *C. dactylon* var. *dactylon* and we accordingly consider them to best fit this taxon. Their dark bluish green color is the one morphological characteristic that distinguishes them from most other forms of *C. dactylon* var. *dactylon.*

The close genetic relationship of the tetraploid and hexaploid plants from China, as well as their sympatric existence, suggests a common ancestry. We speculate that the hexaploid race in China arose through hybridization either involving the union of an unreduced female gamete with a reduced male gamete, or the spontaneous doubling of chromosomes in a triploid zygote. Powell et al. (1968) attributed the origin of a hexaploid plant found among F_1 progeny from a tetraploid *C. dactylon* × diploid *C. transvaalensis* cross to spontaneous chromosome doubling at an early zygotic stage. Felder (1967) reported the discovery of an autohexaploid among progeny plants resulting from the self-pollination of a tetraploid *C. dactylon* plant. He hypothesized that the autohexaploid plant resulted from the union of an unreduced female gamete and a reduced male gamete. Functioning of unreduced female gametophytes (eggs) at relatively high frequency has been reported in *C. dactylon* (Harlan and de Wet 1969), and has been demonstrated in

Fig. 3. Principal coordinate map for the first and second coordinates estimated for 443 AFLP markers using the genetic similarity matrix for 28 *Cynodon dactylon* var. *dactylon* accessions.



other polyploid plant species as a mechanism causing increase in ploidy size (Harlan and de Wet 1963). Accordingly, the most likely scenario for the origin of the Chinese hexaploid forms of *C. dactylon* var. *dactylon* was fertilization of an unreduced egg with a reduced male gamete, especially assuming then, as now, a prevalence of tetraploid forms in the region.

Cluster D comprised PI 290882 from South Africa and two accessions (PI 291582 and A12375) from Zimbabwe. Genetic SC values ranged from 0.70 to 0.83, and averaged 0.76 ± 0.07. Cluster E contained five accessions, PI 251809 from Italy, Nr 24 from Bulgaria, Nr 28 from Spain, Nr 34 from Italy, and Nr 47 from France. Genetic SC values ranged from 0.77 to 0.86, and averaged 0.83 ± 0.02.

Principal coordinate analysis (PCA) (Fig. 3), in which PC1 accounted for 24.8% of total variation and PC2 accounted for 13.9%, was generally consistent with results from the cluster analysis in groupings of the accessions. Accessions originating from Australia, Asia, Africa, and Europe were placed in distinct groups. The Asia accessions from China separated into two distinct groups based on ploidy level. The PCA results indicated a closer relationship of accessions of Australian and Asian origin compared with accessions of European origin. The JT-1 accession from Japan was indicated as being closely related to African accessions. The JT-2 accession from Dubai was closely related to Chinese accessions and then to Australian accessions. It is possible that JT-1 and JT-2 were introduced into Dubai and Japan, respectively. Accessions of African origin clearly were dispersed widely in the middle of the plot and clearly constitute the most diverse set of accessions. The genetic SC values for the accessions of African origin ranged from 0.62 to 0.83 (0.70 ± 0.06), verifying their broad genetic diversity relative to the other accessions of common geographic origin.

The geographic clustering of *C. dactylon* var. *dactylon* accessions based on AFLP polymorphisms is consistent with variations found in morphological, adaptive, and cytogenetic characteristics. Clearly, geographic origin was a significant factor in their genetic differentiation. The adaptive characteristics of *C. dactylon* var. *dactylon* vary widely as a function of their evolution under different climatic and edaphic conditions (Harlan and de Wet 1969; Harlan et al. 1970*b*, 1970*c*, 1970*d*). The taxon is distributed across environments ranging from tropical to temperate and arid to humid. These forms differ in response not only to temperature and precipitation differences, but also to other biotic and abiotic stresses associated with the different climates. Harlan and de Wet (1969) observed that crossing *C. dactylon* var. *dactylon* plants of different races and (or) widely divergent geographic sources frequently resulted in reduced fertility owing to chromosomal structural differences mainly resulting from translocations and inversions. The genetic isolation of plant populations based on chromosomal structural constitu-

tion would have allowed these respective populations to be further genetically differentiated by selective forces for adaptation to specific environments.

The genetic relationship of *C. dactylon* var. *dactylon* forms with different geographic origins and the geographic differentiation pattern as indicated by the results of this study have implications relative to germplasm collection, preservation, and use. The results clearly indicate that comprehensive germplasm collection in major geographic regions such as Africa, Australia, and southeast Asia is required to sample the full extent of the available variation.

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