GENETIC CHARACTERIZATION OF *CYNODON* ACCESSIONS BY MORPHOLOGY, FLOW CYTOMETRY AND DNA PROFILING

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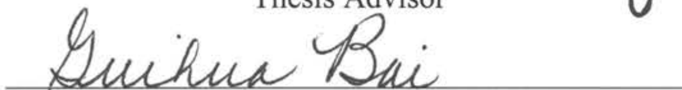

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CHAPTER I

GENERAL INTRODUCTION

Cynodon L.C. Rich. species originated in the old world where centers of diversity are found distributed in parts of Africa and Eurasia (Harlan, 1970a). The genus contains perennial and sod-forming grasses of significant importance based on their use as livestock feed, recreational turf, and soil stabilization. Beginning near the start of the 20th century and continuing until the present, research has been conducted with *Cynodon* in the areas of germplasm collection and characterization, breeding improvement, taxonomic classification, chromosome number determination, and molecular marker genetics (Taliaferro, 2003).

Taxonomy and distribution

Cynodon L. C. Rich., a genus of the tribe *Cynodonteae*, subfamily *Choridoideae*, and family *Gramineae* (*Poaceae*), comprises nine species and ten varieties in a taxonomically revised classification by J. R. Harlan and colleagues (Harlan et al. 1970a; de Wet and Harlan, 1970). The taxonomic revision was based on evidence from extensive bio-systematic studies of some 900 *Cynodon* accessions collected from many parts of the world where the plants were found, but not including China. Their studies encompassed plant morphology, geographical distribution, cytogenetic characteristics, and hybridization potential. Their classification is the same as that of the Kew Royal

Botanic gardens (Kew, 1999) except that *C. X magennisii* is omitted by Kew. There are six botanical varieties in *C. dactylon* (L.) Pers (Harlan and de Wet, 1969). *C. dactylon* var. *dactylon* is cosmopolitan and distributed across all continents and islands between about 45° N and 45° S latitudes, even penetrating to 53° N in Europe, while *C. dactylon* var. *afghanicus* Harlan et de Wet, var. *aridus* Harlan et de Wet, var. *coursii* (A. Camus) Harlan et de Wet, var. *elegans* Rendle and var. *polevansii* (Stent) de Wet et Harlan are endemic or have restricted distributions (Harlan and de Wet, 1969). The other eight species of *Cynodon* fall clearly into three groups according to geographical distribution: South Asia and Indian Ocean-South Pacific Islands (*C. arcuatus* J.S.Presl. ex C.B.Presl., *C. barberi* Rang. et Tad.); East Africa (*C. plectostachyus* (K.Schum.) Pilger, *C. aethiopicus* Clayton et Harlan, *C. nlemfuensis* Vanderyst var. *nlemfuensis,* var. *robustus* Clayton et Harlan); South Africa (*C. incompletus* Nees var. *incompletus*, var. *hirsutus* (Stent) de Wet et Harlan, *C. transvaalensis* Burtt-Davy*, C X magennisii* Hurcombe) (Harlan et al. 1970b).

Use and importance

These warm-season, sod-forming grasses are important and widely used in tropical and warmer temperate regions of the world for turf, livestock herbage, soil stabilization and spoil site remediation (Taliaferro et al. 2004; Taliaferro, 1995). The taxa of predominant turf importance are *C. dactylon* (L.) Pers. var. *dactylon* and *C. transvaalensis* Burtt-Davy. Most of the turf bermudagrasses of economic importance emanate from these two taxa. Forms of the narrowly endemic *C. X magennisii* Hurcombe and *C. incompletus* Nees var. *hirsutus* (Stent) de Wet et Harlan have had fairly wide use as turfgrasses. Taxa of minor turf importance are *C. arcuatus* J.S.Presl. ex C.B.Presl. *C.*

barberi Rang.et Tad. and *C. dactylon* var. *polevansii* (Stent) de Wet et Harlan. Other taxa have little, or no, value as turf, but in some cases will hybridize with the turf species, thereby representing potentially important contributors of genes for the breeding improvement of turf bermudagrass (Taliaferro, 2003). Bermudagrass turf is used on home and institutional lawns, parks, athletic fields, and golf course fairways, tees, putting greens and roughs, cemetery grounds, road-side right of ways, and other similar areas (Beard, 1973).

Among the *Cynodon* species, *C. arcuatus, C. barberi, C. transvaalensis, C. incompletus* var. *incompletus and* var. *hirsutus,* and *C. X magennissi* are of minor value as herbages for grazing and hay, because of very small size, little yield or endemic or limited distribution (Taliaferro et al. 2004; Harlan, 1970c). The robust East African species, *C. aethiopicus, C. plectostachyus, C. nlemfuensis* var. *nlemfuensis* and var. *robustus* are rather widely used as forage grasses because of their natural abundance and apparent productivity (Harlan, 1970c). These are called stargrasses and are used in the USA chiefly in tropical Florida. They are extensively used in Central and South America, the Caribbean and tropical Africa (Taliaferro et al. 2004). *Cynodon dactylon* is enormously variable and extremely valuable for grazing and hay production, while four of six varieties are endemic and of relatively minor value compared with other two. According to Harlan (1970c), *C. dactylon* var. *afghanicus* is a robust form that provides some pasture for livestock near irrigation projects in the lowland steppes, but it can hardly be considered a major fodder even in the local areas. *Cynodon dactylon* var. *coursii* is a robust, non-rhizomatous form endemic to Madagascar with a moderately important grazing resource on the island but of no significance elsewhere. The var.

polevansii is known from only one site near Baberspan, South Africa. The *var. elegans* provides considerable grazing in South Africa. The var. *aridus* as a fodder grass has not been characterized widely. *Cynodon dactylon* var. *dactylon*, the most widely distributed, provides an important forage resource for grazing, and the most important genetic resource for breeding improved hay and pasture grass cultivars suitable for intensive management(Burton and Hanna, 1995; Harlan,1970c).

Chromosome number and ploidy levels

Several ploidy levels and two base chromosome numbers have been reported in *Cynodon*. Hurcombe (1947) reported the basic chromosome number of the genus as 10 and that *C. transvaalensis* is the diploid form, *C. magennisii* the triploid, and *C. dactylon* the tetraploid form. She reported 2n=18 chromosomes for *C. bradleyi* (*C. incompletus*), but assumed it to be an aneuploid number. Moffett and Hurcombe (1949) reported base chromosome numbers of x=9 and x=10, *C. dactylon* with 36 somatic chromosomes, and *C. plectostachyum* plants with 18 or 54 chromosomes. Nine was later confirmed as the correct base chromosome number in *Cynodon* (Forbes and Burton, 1963; Harlan et al., 1970d).

The diploid (2n=18) species are *C. barberi, C. dactylon* var. *aridus, C. incompletus* var. *incompletus, C. plectostachyus and C. transvaalensis.* The tetraploid (2n=36) species are *C. arcuatus, C. dactylon* var. *dactylon, C. dactylon* var. *coursii, C. dactylon* var. *elegans* and *C. dactylon* var. *polevansii*, while *C. aethiopicus, C. dactylon* var. *afghanicus, C. incompletus* var. *hirsutus, C. nlemfuensis var. nlemfuensis* and *C. nlemfuensis* var. *robustus* have both tetraploid and diploid chromosome numbers (Harlan

et al. 1970d). Hexaploid (2n=6x=54) *Cynodon* plants occur rarely. Powell et al. (1968) reported a hexaploid plant among putative progeny of a cross between tetraploid *C. dactylon* var. *dactylon* by diploid *C. transvaalensis*. *C. dactylon* var. *dactylon* cv. 'Tifton 10' is a natural hexaploid plant (Burton,1991; Hanna et al. 1990). A released cultivar 'Tifton 85', a bermudagrass by stargrass (*C. nlemfuensis*) interspecific F1 hybrid, is a pentaploid (Burton et al. 1993). de Silva and Snaydon (1995) reported in Sri Lanka *Cynodon* populations from arid, dry and intermediate regions, and those from roadsides, lawns and grasslands in the hill country (pH>6.5), consisted entirely of tetraploid plants and all populations from roadsides and lawns in the wet region, and from forests in the hill country (pH<5.0), consisted entirely of diploid plants, while mixtures of diploids and tetraploids occurred only in areas with a soil pH between 6.0 and 6.5.

Nuclear DNA content is a characteristic of a species (Brown, 1999). Knowledge of genome size of a species is very useful to estimate ploidy levels and essential for assessing the coverage of a genomic library, estimating the copy number of a gene in the genome, and developing strategies for gene cloning based on genome mapping. Flow cytometry has proven to be a fast reliable means of estimating ploidy in bermudagrass. Taliaferro et al. (1997) developed a flow cytometry protocol for estimating nuclear DNA content in *Cynodon*, and reported mean nuclear DNA contents of 2X, 3X, 4X and 6X cytotypes were 1.11±0.04pg, 1.60±0.04pg, 2.25±0.13pg and 2.80±0.14pg, respectively. Arumuganathan et al. (1999) reported 2C nuclear DNA contents for one diploid African bermudagrass 'DTC 95' was 1.03±0.01pg, triploid 'Tifgreen' 1.61±0.00pg and 'Tifway' 1.37±0.01pg, tetraploid cv. 'Savannah' 1.95±0.01pg.

Germplasm collection and characterization

Collection of *Cynodon* germplasm for culture and scientific use began around the start of the 20th century in South Africa and the United States of America (Taliaferro, 2003). These germplasm pools were enlarged during the 20th century and provided genetic variations for selection and breeding. The utility and value of a germplasm collection depend largely on how well the accessions in the collection have been characterized for trait descriptors. Characterization of accessions for cytogenetic, morphological, physiological and agronomic traits increases the utility and importance of the germplasm. For *Cynodon*, characterization of accessions for traits related to turfgrass quality is especially important. Such traits include sod density and uniformity, plant texture, color, growth habit, and smoothness (Turgeon, 1996; Beard, 1973), traits that are generally visually assessed. Seed yield is very important for seed-propagated cultivars. Percent seed set and number of seed stalks are the major components determining seed yield in bermudagrass (Ahring et al. 1974; Kenna et al., 1983). Wofford and Baltensperger (1985), Coffey and Baltensperger (1989), and Cluff and Baltensperger (1991) reported heritability estimates for turfgrass characteristics in field conditions and under shade, and seed yield and seed yield components in bermudagrass, and indicated the performance of all characteristics evaluated should be improved using traditional breeding methods. The major abiotic factors are temperature extremes in which low temperature is particularly important, edaphic conditions including soil type and fertility, and precipitation, and light. The wide diversity in bermudagrass for freeze tolerance has been well documented (Beard et al., 1980; Anderson et al., 1988 and 1993). Major biotic influences include disease, nematodes and insects. Martin et al. (2001a,b) showed spring

dead spot resistance variations exist in inter-specific hybrids and among seed-propagated cultivars.

Molecular markers in genetic studies

In recent years, a number of DNA molecular techniques based on the analysis of information-rich nucleic acid molecules have been used in studying genetic diversity, relatedness, phylogeny and to identify off-types of cultivars in *Cynodon*s (Caetano-Anolles, 1998a). Caetano-Anolles et al. (1995) used DNA amplification fingerprinting (DAF) analysis, in conjunction with parsimony (PAUP) and un-weighted pair group cluster analysis using arithmetic means (UPGMA) to study the genetic variation of 13 bermudagrass cultivars by grouping them into several clusters and separating 'Tifway' from the irradiation-induced mutant 'Tifway II'. The DAF analyses readily distinguished 18 *Cynodon* cultivars available in Australia (Ho et al. 1997). The cultivars separated into two distinct groups comprising respectively *C. dactylon* x *C. transvaalensis* hybrids and the Australian bermudagrasses. The DNA profiling generally has detected substantial variation within *C. dactylon* var. *dactylon* (Caetano-Anolles et al. 1995; Ho et al. 1997; Assefa et al. 1998), a result consistent with the great adaptive and morphological diversity found in this cosmopolitan species (Taliaferro, 1995). Caetano-Anolles et al. (1997) characterized the origin of bermudagrass off-types using DAF. They showed that Tifway was genetically stable and that off-types in Tifway plantings resulted from sod contamination and not from somatic mutation (Caetano-Anolles et al. 1997). Genetic instability of Tifgreen and Tifdwarf was detected by the DAF and arbitrary signatures from amplification profiles (ASAP) (Caetano-Anolles, 1998b). The DNA fingerprinting, chromosome number and morphology combined were successfully used to distinguish

off-types in Tifway and Tifdwarf bermudagrass (Busey et al. 1996). Assefa et al. (1998) assessed genetic relatedness among 62 *Cynodon* accessions, representing eight species, using the DAF technique, UPGMA and principal coordinate analysis. The strongest species similarities were between *C. aethiopicus* and *C. arcuatus*, *C. transvaalensis* and *C. plectostachyus*, and *C. incompletes* and *C. nlemfuensis*. Intraspecific variation was least for *C. aethiopicus, C. arcuatus, and C. transvaalensis.* Accessions of like taxonomic classification were generally clustered, indicating good correlation between groupings based on DAF profiles and traditional morphological traits used in classification (Harlan, 1970a), except the cosmopolitan *C. dactylon* var. *dactylon* and *C. dactylon* var. *afghanicus.* Within taxa, accessions differing in chromosome number clustered in all instances indicating the 2x and 4x forms to be closely related (Assefa et al., 1998). Anderson et al. (2001) used the DAF technique to compare genetically putative 'U-3' bermudagrass (*C. dactylon*) with bermudagrass currently produced in Oklahoma and sold as 'U-3'. Phenetic analyses using both the UPGMA algorithm and principal coordinate analysis revealed a wide separation between the putative 'U-3' and Oklahoma 'U-3', which likely resulted from mechanical contamination (Anderson et al., 2001). Random amplified polymorphic DNA (RAPD) analyses were used to distinguish among some well-known *Cynodon* cultivars used in South Africa, such as 'Bayview', 'Cape Royal', 'Florida', 'Harrismith', 'Silverton Blue', 'Skaaplaas', Tifdwarf, and 10 new cultivars, as well as to determine the genetic variation between them by calculating genetic distances (Roodt, et al. 2002). The amplified fragment length polymorphism (AFLP) technique (Vos et al., 1995), combining the reliability of the RFLP (restriction fragment length polymorphism) with the power and ease of the PCR (polymerase chain reaction)

technique, detected enough polymorphisms to differentiate all 27 bermudagrass genotypes, even the closely related ones. The 27 genotypes were grouped into three major clusters, many of which were in agreement with known pedigrees (Zhang et al., 1999).

Objectives of the study

China is the center of origin for several important agronomic crops, notably rice (*Oryza sativa* L.) and soybean (*Glycine max* (L.) Merr.). China also is well known for its wealth of botanical diversity. Several important turfgrasses including *Zoysia japonica* Stend., *Z. tenufolia Willd. Ex Trin., Z. matrella (L.)* Merr., and *Eremochloa ophiuroides* (Munro.) Hack. originated in China (Brede and Sun, 1995). There are two *Cynodon* species, *C. dactylon* and *C. arcuatus,* and two botanical varieties, var. *dactylon* and var. *biflorus* Merino in *C. dactylon,* taxonomically recognized in China (Editorial committee of China Floral Acta of Chinese Academy, 1990). The diagnostic character of *C. dactylon* var. *biflorus*, which is not included in the Harlan' classification, is two (2) florets in one spikelet. In China, *C. dactylon* var. *dactylon* plants are widely distributed over a broad range of environments including tropical, subtropical and warm temperate climate regions south of the Yellow River, and are also scattered sparsely in Xingjiang province (Abulaiti et al., 1998), Hebei province and Beijing city. G. W. Burton surveyed turf sites in Shanghai in 1974 and collected a clonal plant from an old lawn that later was released as 'Tifton 10' (Hanna, 1990). Abulaiti et al. (1998) reported that native bermudagrasses of Xingjiang survived under -32°C in a field trial. Native bermudagrass accessions collected in east China showed great variation in morphological traits and were used in a breeding program (Liu et al., 1996).

The proposed research will focus on the characterization of a diverse group of *Cynodon* accessions for selected cytogenetic, genetic, morphological, and agronomic descriptor traits. The accessions were collected from a wide geographic expanse in China.

Specific objectives of the investigation were:

1) **To characterize the Chinese accessions for important descriptor traits including chromosome number, fertility (seed production traits), morphology, and DNA profiles.**
2) **To assess magnitude and kind of genetic variation among the Chinese *Cynodon* germplasm accessions for the various descriptor traits and compare with *Cynodon* germplasm standards.**

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CHAPTER II

PLOIDY DETERMINATION OF CHINESE CYNODON ACCESSIONS BY FLOW CYTOMETRY

ABSTRACT

Chromosome numbers varying from 2n=2x=18 to 2n=6x=54 have been reported in, bermudagrass, *Cynodon dactylon* (L.) Pers. Bermudagrass is distributed over much of the southern two-thirds of China but no information is available on chromosome number variations within this germplasm pool. Accordingly, this research was conducted to determine the ploidy level of 127 bermudagrass accessions collected over a wide geographic expanse of China. Ploidy was estimated by measuring the nuclear DNA content (pg $2C^{-1}$ nucleus) using flow cytometry. For cases where ploidy level was not definitive, somatic chromosome number was determined using a conventional cell squash technique. Four ploidy cytotypes were found among the 127 accessions, triploid (2n=3x=27), tetraploid (2n=4x=36), pentaploid (2n=5x=45) and hexaploid (2n=6x=54). The hexaploid cytotype was most prevalent (89%), had nuclear genome sizes ranging from 1.96 to 2.30 pg $2C^{-1}$ nucleus, and were distributed over the geographic range of collection. Six hexaploid and three pentaploid accessions from southeast China had respective nuclear genome size of 2.37 to 2.49 pg/2C and 2.90 to3.13 pg/2C. Three triploid (2n=3x=27) accessions with genome sizes of 1.55 to 1.65

pg/ 2C nucleus^{-1} were collected in Sichuan province, and they probably were introductions from abroad.

INTRODUCTION

Cynodon L. C. Rich. is a small genus containing nine species and ten varieties taxonomically classified by Harlan et al. (1970a), in tribe *Cynodonteae* and family *Gramineae* (*Poaceae*). While most species and varieties have restricted distributions, *C. dactylon* (L.) Pers. var. *dactylon* is cosmopolitan. It is distributed across all continents and islands between about 45° N and 45° S latitudes, even penetrating to 53° N in Europe (Harlan and de Wet, 1969; Harlan et al., 1970c). The ubiquitous and cosmopolitan species economically is the most important in the genus and because of its wide use for forage, turf, soil stabilization and remediation of spoil site in tropical and warmer temperate regions of the world (Burton, 1947; Harlan, 1970b; Taliaferro, 1995; Taliaferro, 2003).

Two basic chromosome numbers have been reported in *Cynodon*. Earlier reports indicated the basic chromosome number of *Cynodon* to be 10 (Hurcombe, 1947; Moffett and Hurcombe, 1949). However, more research results firmly established the correct basic number of *Cynodon* to be nine (Forbes and Burton, 1963; de Wet and Harlan, 1971; Harlan et al. 1970d; de Silva and Snaydon, 1995). The small size of *Cynodon* chromosomes, frequent fragments found in cell squash preparations, and chromosome satellites easily interpreted as whole chromosome may have contributed to the erroneous determination of 10 as the basic number (Forbes and Burton, 1963; de Silva and Snaydon, 1995).

Diploid (2n=2x=18), triploid (2n=3x=27), tetraploid (2n=4x=36), pentaploid (2n=5x=45), and hexaploid (2n=6x=54) have been reported in *Cynodon* (Hurcombe,

1947; Moffett and Hurcombe, 1949; Forbes and Burton, 1963; de Wet and Harlan, 1971; Harlan et al. 1970d; de Silva and Snaydon, 1995; Hanna et al., 1990). Diploid and tetraploid are the predominant cytotypes found in nature. Most of the triploid, pentaploid and hexaploid cytotypes have been discovered among the progeny of intraspecific and interspecific artificial hybridizations. Powell et al. (1968a) reported a hexaploid progeny in a cross of *C. dactylon* (2n=4x=36) by *C. transvaalensis* (2n=2x=18). Johnston (1975) observed pentaploid (2n=5x=45) hybrids in crosses of tetraploid *C. dactylon* and a hexaploid from a cross of *C. barberi* (2n=2x=18) and *C. dactylon* (2n=4x=36). 'Tifton85', an F1 hybrid of *C. dactylon* by *C. nlemfuensis*, is a pentaploid (Burton et al., 1993). Triploid hybrid cytotypes have been commonly produced via inter- and intra-specific crosses of diploid and tetraploid parents, which could belong to same taxonomical species or different species (Harlan et al. 1970d). Interspecific crosses involving tetraploid *C. dactylon* and diploid *C. transvaalensis* have been the principal breeding method for producing superior turf cultivars (Burton, 1991).

Flow cytometry has been successfully used to estimate ploidy levels by measuring the nuclear DNA content in many grass species (Keeler et al., 1987; Hopkins et al., 1996; Taliaferro et al., 1997; Huff and Palazzo, 1998; Arumuganathan et al., 1999; Johnson et al. 1998, 2001). Taliaferro et al. (1997) developed a flow cytometric protocol for estimating nuclear DNA content in *Cynodon*. Arumuganathan et al. (1999) used flow cytometry to determine ploidy in thirteen turfgrass species including three *Cynodon* cytotypes. Once a certain correlation between ploidy level and nuclear DNA content of a species has been established, flow cytometry is an easy way to help estimate ploidy level in a particular turfgrass species (Riordan et al., 1998)

There are two *Cynodon* species, *C. dactylon* and *C. arcuatus*, taxonomically recognized in China (Anonymous, 1990). In China, *Cynodon* plants are indigenous and widely distributed over a broad geographic range encompassing tropical, subtropical, and warm temperate climate regions in south of the Yellow River and east of the Tibet highlands (Anonymous, 1990; Liu et al., 1996; Wu et al. 2001). *Cynodon* is sparsely distributed in more temperate regions including Xingjiang, Hebai and Beijing (Abulaiti et al., 1998). G. W. Burton collected a clonal plant in 1974 from a Shanghai lawn that was subsequently determined to be a hexaploid and released as 'Tifton 10' (Hanna et al., 1990). No additional information on ploidy level has been reported for *Cynodon* of Chinese origin. This research was conducted to determine ploidy of 127 *Cynodon* accessions collected from aver a wide geographic range in China.

MATERIALS AND METHODS

Plant materials

Plant materials consisted of 130 *Cynodon* accessions including 127 accessions collected in China and three U.S. commercial cultivars with known chromosome number and nuclear DNA content (Table 1). The Chinese *Cynodon* accessions were collected from eleven provinces ranging from tropical Hainan Island to the temperate climate region around Beijing.

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 the time of the sampling.

Flow cytometry

Flow cytometry procedures described by Taliaferro et al. (1997) were used to measure nuclear DNA content. Flow cytometry analyses of prepared materials were conducted on a Becton-Dickinson FACSCalibur Flow Cytometer (Becton Dickinson, CA) with an argon laser emitting at 488 nm for excitation of propidium iodide (PI) at Flow Cytometry Laboratory of Oklahoma State University.

Tissue samples were prepared for flow cytometry by collecting 20-25 young shoots and excising the meristematic regions from just beyond the last visible node. The excised tissue was placed in a 60 by 15 mm plastic petri dish on ice. The leaf tissue was

then chopped into chips as thin as possible with a razor blade in about 0.4 ml of ice-cold buffer solution (10 mM Tris buffer pH 7.6, 1 mM dihydrate sodium citrate, 2 mM anhydrous $MgCL_2$). Approximately 200 µl of nuclei extraction solution was then passed through a 35 µm nylon mesh filter. The extracted samples were then injected with 6 µl of PI (Sigma, St. Louis, Missouri) stain solution (2.4 mg PI /ml) and 2 µl of a 9:1 dilution of RNAase (R-4642; Sigma, St. Louis, Missouri). Deionised water was used as the solvent for the PI stain and the diluent for RNAase. Samples were then kept in an ice chest at least 15 min before flow cytometric measurement. The mean nuclear DNA content of each plant sample, measured in picograms (pg), was based on 5000 scanned nuclei. Channel catfish (*Ictalurus punctatus*) or domestic swine (*Sus scrofa*) blood cells were used as internal standards. Nuclear DNA contents of blood cells of channel catfish and domestic swine were 2.0 and 5.67 pg/2C, respectively (Taliaferro et al. 1997). For every plant sample at least three measurements (replicates) were obtained in three or more different runs (dates). Sample DNA content was calculated with dividing the mean value of the sample channel by mean value of the internal standard channel, then multiplying the result by DNA content of internal standard used.

Cytology

Somatic chromosome number was determined for all accessions having DNA content outside the reported range for respective ploidy levels (Taliaferro et al., 1997; Arumuganathan et al., 1999). Additionally, somatic chromosome number was determined for selected accessions having DNA content within the reported range to confirm the accuracy of the flow cytometry data. Somatic chromosome numbers were determined using conventional squashes of shoot tip somatic cells under a light microscope equipped

with a digital camera system. The somatic cell squashes were prepared from shoot apical meristem tissues as described by Powell (1968b) with minor modifications. Briefly, actively growing shoot tips were stripped, and the stripped shoot tips were cut longitudinally to expose the apical meristem. As a pretreatment to soften tissues, the split shoot tips were placed into a saturated solution of monobromonaphthalene for 2 hours. Then the pretreated shoot tips were fixed in 3:1 ethanol: acetic acid solution for 24 hours at room temperature. The fixed shoot tips were digested in cytolase (PCL5, DSM Food Specialties, Charlotte, NC) solution at 37°C for 40, 60, and 70 min for triploid, tetraploid and pentaploid, and hexaploid samples, respectively. Chromosomes were stained with acetocarmine.

RESULTS AND DISCUSSION

The nuclear DNA contents of the 127 Chinese *Cynodon* accessions and three commercial cultivars are presented in Table 1. The standard deviations of DNA content measurements ranged from 0.01 to 0.08, demonstrating that flow cytometry was very precise. These results are consistent with previous reports regarding the precision of flow cytometry in *Cynodon* plants (Taliaferro et al., 1997; Arumuganathan et al., 1999). The DNA contents of the Tifton 10, Tifgreen and Uganda used as external standards highly agreed with the previous reported results by Taliaferro et al. (1997) and Arumuganathan et al. (1999).

Nuclear genome size among the 127 Chinese accessions ranged from 1.55 pg /2C to 3.13 pg /2C nucleus (Table 1). Based on previously reported data (Taliaferro et al., 1997; Arumuganathan et al., 1999), diploid, triploid, tetraploid, and hexaploid cytotypes were indicated by respective nuclear genome sizes of 1.03 to 1.14, 1.37 to 1.62, 1.95 to 2.36, and 2.64 to 2.93 pg DNA $2C^{-1}$ nucleusBased on these ranges two accessions were classified as triploid, 116 accessions as tetraploid, and one accessions as hexaploid (see Inferred ploidy in Table 1).

Nine accessions had genome sizes outside the previously reported ranges. Somatic chromosome counts determined for these nine accessions indicated one triploid (A12260), three pentaploids (A12348, A12352 and A12365), and five hexaploids (A12317, A12318, A12319, A12356 and A12358) (Table 2; Fig. 1). Somatic chromosome counts for A12272, A12282, A12257 and A12360 confirmed that the three inferred ploidy levels based on nuclear DNA contents were correct (Table 2).

Chinese literature indicates only two species of *Cynodon*, namely C. *dactylon* and *C. arcuatus* taxonomically recognized in China (Anonymous, 1990). All but three of the 127 accessions are considered to belong to the taxon *C. dactylon* var. *dactylon* as described by Harlan et al. (1969, 1970a). Pentaploid and hexaploid accessions are included in this group based on similarity of morphological features compared to tetraploid accessions. The descriptions of *C. dactylon* var. *dactylon* by Harlan et al. (1969, 1970a) do not indicate it to contain other than tetraploid cytotypes, but this may have resulted from absence of such plants in the collection on which studies were conducted leading to the revision of the genus by Harlan and colleagues. The three triploid accessions were collected in Sichuan province and are morphologically similar to hybrids from *C. dactylon* var. *dactylon* by *C. transvaalensis* crosses. Triploid turf-type *Cynodon* cultivars have been widely commercially imported into this region (Wu et al., 2001).

The 116 tetraploid *Cynodon* accessions (89.23%) originated from 11 provincial regions clearly indicating this to be the predominant cytotype over the geographic expanse represented by the collection. In three western provinces (Sichuan, Yunnan and Chongqing) 95 of 98 accessions collected were tetraploids. In the other eight eastern and southern provincial regions 20 of the 29 accessions collected samples were tetraploids. Obviously, tetraploids were the dominant ploidy level in China. The wide distribution of the tetraploid cytotype in China is consistent with previous reports (Harlan and de Wet, 1969; Harlan et al. 1970a) that tetraploid *C. dactylon* var. *dactylon* is truly cosmopolitan being distributed over a wide geographic expanse.

Six of 14 accessions (43%) collected from Shanghai, Jiangsu and Zhejiang provinces were hexaploids. Tifton 10 is a hexaploid and was collected as a clonal plant in Shanghai in 1974 by G.W. Burton (Hanna et al., 1990). The hexaploid cytotype may therefore be relatively more prevalent in this geographic region than in the other regions, though a larger sampling of plants is needed to accurately assess its relative prevalence. The fact that only 6 of the 127 accessions (4.72%) were hexaploids indicates that the cytotype is sparsely distributed over the entire geographic region of collection relative to the tetraploid cytotype. Only a few hexaploid plants have previously been reported in *Cynodon* (Moffett and Hurcombe, 1949; Powell et al., 1968; Felder, 1967; Johnston, 1975; Malik and Tripathi, 1968).

This is the first report of pentaploid *Cynodon* plants occurring naturally. The pentaploids were collected in subtropical environments, two from Hannan Island and the third from Fujian province. All three pentaploid accessions had poor winter survival in Stillwater, Oklahoma (data not shown). The only previous report of pentaploid *Cynodon* plants was by Johnston (1975) who found three pentaploid plants among progeny from a hexaploid plant (derived from interspecific hybridization of *C. barberi* and *C. dactylon*) crossed with a tetraploid *C. dactylon* plant.

Polyploid polymorphism in grass is not unusual. However Harlan et al. (1970) considered *C. dactylon* var. *dactylon* to be exclusively of the tetraploid cytotype based on the absence of hexaploid cytotypes in their *Cynodon* collection. The degree of polyploid polymorphism found in this study provides new information relative to the extent of polyploidy polymorphism in *Cynodon dactylon* var. *dactylon.* Additional systematic

collections and cytogenetic studies of the polyploids may provide insight in the species evolution.

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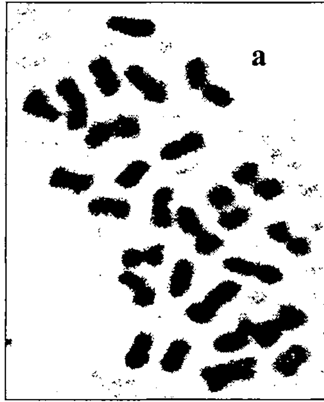
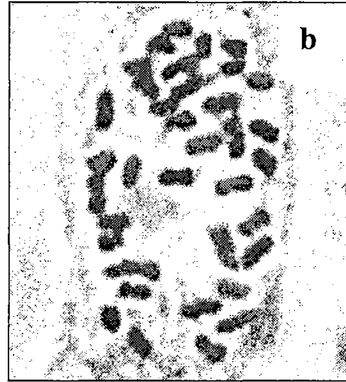
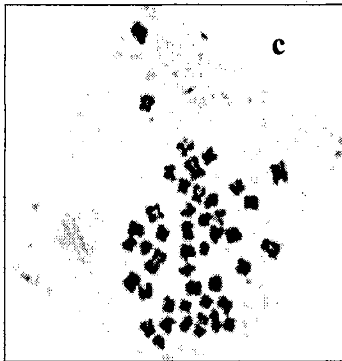
Fig. 1. Photomicrographs of the four observed ploidy levels in Chinese *Cynodon* accessions. a:A12260, 2n=3x=27; b:A12257,2n=4x=36; c:A12348, 2n=5x=45; d:A12319, 2n=6x=54.







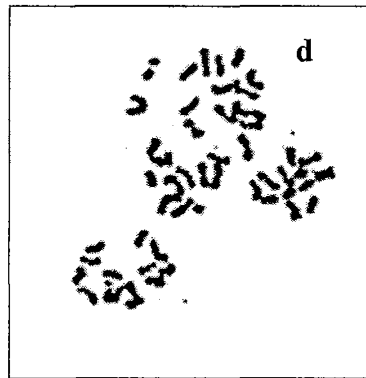


Table 1. Identification, source and nuclear DNA content of 127 Chinese *Cynodon* accessions and three commercial cultivars.

Identification	Origin/ Reference	DNA Content Mean±SD ----pg/2C---	Inferred Ploidy (2n)	Identification	Origin/ Reference	DNA content Mean±SD ----pg/2C---	Inferred Ploidy (2n)
A12253	Sichuan	2.15±0.07	36	A12296	Sichuan	2.13±0.05	36
A12254	Sichuan	2.05±0.06	36	A12297	Sichuan	2.08±0.02	36
A12255	Sichuan	2.04±0.03	36	A12298	Chongqing	2.06±0.06	36
A12257	Chongqing	1.96±0.07	36	A12299	Chongqing	2.22±0.05	36
A12258	Chongqing	2.09±0.04	36	A12300	Sichuan	2.04±0.07	36
A12259	Sichuan	2.01±0.04	36	A12301	Sichuan	2.05±0.04	36
A12260	Sichuan	1.65±0.01	-----	A12302	Sichuan	2.02±0.05	36
A12262	Yunnan	2.09±0.03	36	A12303	Sichuan	2.04±0.04	36
A12263	Yunnan	2.03±0.05	36	A12304	Sichuan	2.09±0.05	36
A12264	Sichuan	2.07±0.01	36	A12305	Sichuan	2.08±0.04	36
A12265	Sichuan	2.02±0.02	36	A12306	Sichuan	2.05±0.03	36
A12266	Sichuan	2.17±0.04	36	A12307	Sichuan	2.13±0.06	36
A12267	Sichuan	2.10±0.01	36	A12308	Sichuan	2.07±0.05	36
A12268	Sichuan	2.07±0.04	36	A12309	Sichuan	2.12±0.02	36
A12269	Sichuan	2.05±0.02	36	A12310	Sichuan	2.07±0.03	36
A12270	Sichuan	2.03±0.04	36	A12311	Sichuan	2.13±0.05	36
A12271	Sichuan	2.05±0.03	36	A12312	Sichuan	2.08±0.05	36
A12272	Sichuan	1.61±0.01	27	A12313	Sichuan	2.04±0.05	36
A12273	Sichuan	2.09±0.06	36	A12314	Chongqing	2.06±0.06	36
A12274	Sichuan	2.11±0.01	36	A12315	Shanghai	2.15±0.06	36
A12275	Sichuan	1.99±0.04	36	A12316	Shanghai	2.09±0.03	36
A12276	Sichuan	2.22±0.05	36	A12317	Shanghai	3.12±0.05	-----
A12277	Sichuan	2.02±0.02	36	A12318	Shanghai	2.99±0.05	-----
A12278	Sichuan	2.01±0.07	36	A12319	Shanghai	3.08±0.06	-----
A12280	Sichuan	2.03±0.05	36	A12321	Sichuan	2.03±0.05	36
A12281	Sichuan	2.20±0.03	36	A12322	Sichuan	2.17±0.04	36
A12282	Sichuan	1.55±0.03	27	A12323	Sichuan	2.00±0.04	36
A12283	Sichuan	2.15±0.04	36	A12324	Sichuan	2.08±0.07	36
A12284	Sichuan	2.12±0.04	36	A12325	Sichuan	2.15±0.02	36
A12285	Sichuan	2.08±0.07	36	A12326	Yunnan	1.99±0.06	36
A12286	Sichuan	2.03±0.05	36	A12327	Sichuan	2.02±0.06	36
A12287	Sichuan	2.11±0.05	36	A12328	Sichuan	2.07±0.08	36
A12288	Sichuan	2.00±0.06	36	A12329	Sichuan	2.26±0.03	36
A12289	Sichuan	2.10±0.04	36	A12330	Sichuan	2.07±0.05	36
A12290	Sichuan	2.08±0.06	36	A12331	Sichuan	2.07±0.07	36
A12291	Sichuan	2.11±0.05	36	A12332	Sichuan	2.06±0.06	36
A12292	Chongqing	2.04±0.06	36	A12333	Sichuan	2.00±0.05	36
A12293	Chongqing	2.10±0.05	36	A12334	Sichuan	2.21±0.02	36
A12294	Sichuan	2.12±0.05	36	A12335	Sichuan	2.09±0.05	36
A12295	Sichuan	2.11±0.04	36	A12336	Sichuan	2.05±0.03	36
A12337	Sichuan	2.05±0.05	36	A12362	Fujian	2.03±0.06	36
A12338	Sichuan	2.10±0.05	36	A12363	Jiangsu	2.02±0.06	36
A12339	Sichuan	2.30±0.03	36	A12364	Zhejiang	2.07±0.05	36
A12340	Sichuan	2.14±0.06	36	A12365	Fujian	2.49±0.03	-----
A12341	Sichuan	2.01±0.05	36	A12366	Fujian	2.12±0.04	36
A12342	Sichuan	2.06±0.06	36	A12367	Shandong	2.09±0.07	36
A12343	Sichuan	2.17±0.07	36	A12368	Beijing	2.01±0.01	36
A12344	Sichuan	2.05±0.02	36	A12370	Hainan	2.15±0.05	36

A12345	Sichuan	2.12±0.02	36	A12371	Hainan	2.10±0.05	36
A12346	Sichuan	2.12±0.01	36	A12372	Guangdong	2.00±0.02	36
A12347	Sichuan	2.12±0.03	36	93-138	Yunnan	2.02±0.06	36
A12348	Hainan	2.45±0.04	-----	93-139	Yunnan	2.05±0.06	36
A12349	Hainan	2.16±0.07	36	93-140	Yunnan	2.05±0.06	36
A12350	Guangdong	2.14±0.07	36	93-141	Yunnan	2.04±0.02	36
A12351	Hainan	2.08±0.02	36	93-142	Yunnan	2.11±0.05	36
A12352	Hainan	2.37±0.02	-----	93-143	Yunnan	2.04±0.04	36
A12353	Guangdong	2.04±0.05	36	93-144	Yunnan	2.02±0.06	36
A12354	Guangdong	2.03±0.03	36	93-145	Yunnan	2.05±0.03	36
A12355	Zhejiang	2.13±0.06	36	93-146	Yunnan	2.25±0.05	36
A12356	Zhejiang	3.13±0.05	-----	93-147	Yunnan	2.03±0.05	36
A12357	Jiangsu	2.02±0.07	36	93-148	Yunnan	2.10±0.05	36
A12358	Jiangsu	2.98±0.05	-----	93-149	Yunnan	2.08±0.03	36
A12359	Jiangsu	2.02±0.06	36	Tifton 10	Hanna et al., 1990	2.90±0.08	54
A12360	Jiangsu	2.90±0.08	54	Tifgreen	Hanson, 1972	1.61±0.03	27
A12361	Jiangsu	2.01±0.07	36	Uganda	Hanson, 1972	1.05±0.02	18

Table 2. Cytologically observed chromosome number of nine unpredicted and four predicted Chinese *Cynodon* plants.

Identification	Observed chromosome number (2n)	Observed cell number
A12257	36	8
A12260	27	11
A12272	27	9
A12282	27	8
A12317	54	6
A12318	54	16
A12319	54	11
A12348	45	16
A12352	45	15
A12356	54	5
A12358	54	5
A12360	54	9
A12365	45	11

CHAPTER III

AFLP DIVERSITY OF CHINESE CYNODON ACCESSIONS

ABSTRACT

Cynodon dactylon (L.) Pers. is indigenous and widely distributed in China. However, very little is known of the genetic diversity of the Chinese *Cynodon* germplasm pool. Accordingly, this study was conducted to quantify the genetic variation and to characterize the variation pattern and the genetic relatedness of 119 *C. dactylon* accessions collected from eleven provinces in China using a fluorescence-labeled amplified fragment length polymorphism (AFLP) DNA profiling procedure. Based on the 466 polymorphic AFLP bands produced with thirteen selective amplification primer combinations, the accessions were grouped into five clusters. Genetic similarity coefficients of two clusters containing cv. 'Tifway' and 'Tifgreen' ranged from 0.97 to 0.99, suggesting the triploid plants most probably were introduced cultivars from the US. Within Chinese indigenous accessions, genetic similarity coefficients (SC) ranged from 0.65 to 0.99. Genetic variation pattern and groupings for the Chinese accessions were associated with their geographic origin and ploidy level. Tetraploid genotypes had the greatest genetic variation with genetic similarity coefficients ranging from 0.69 to 0.99, while pentaploids had the least with SC values ranging from 0.95 to 0.98. The pentaploid, hexaploid, and tetraploid accessions of similar geographic origin were grouped together

both by cluster and principal coordinate analysis, suggesting a common ancestry among the cytotypes. Fully sampling the genetic diversity of *Cynodon* in China will require more comprehensive collection throughout its distribution.

INTRODUCTION

Cynodon L. C. Rich., a genus of the tribe *Cynodonteae*, subfamily *Choridoideae*, and family *Gramineae* (*Poaceae*), comprises nine species and ten varieties in a taxonomically revised classification by J. R. Harlan and colleagues (Harlan et al. 1970; de Wet and Harlan, 1970). Harlan and de Wet (1969) indicated that *Cynodon dactylon* (L.) Pers var. *dactylon*, the most widely distributed taxon, is enomously variable and found across all continents and islands between about 45° N and 45° S latitudes, even penetrating to 53° N in Europe. As an economically important and widely used warm-season grass, *C. dactylon* var. *dactylon* is extremely valuable as a pasture, turf, and soil conservation grass (Harlan, 1970; Burton and Hanna, 1995; Taliaferro, 1995).

There are two taxonomically recognized *Cynodon* species, *C. dactylon* and *C. arcuatus*, and two botanical varieties, var. *dactylon* and var. *biflorus* Merino in *C. dactylon* in China (Anonymous, 1990). The diagnostic character of *C. dactylon* var. *biflorus*, which is not included in Harlan's classification, is two (2) florets in one spikelet. In China, *C. dactylon* var. *dactylon* plants are widely distributed in a broad range including tropical, subtropical and warm temperate climate regions south of the Yellow River, and are also scattered sparsely in Xingjiang (Abulaiti et al., 1998), Hebei and Beijing. Glenn W. Burton surveyed turf sites in Shanghai in 1974 and collected a clonal plant from an old lawn that later was released as 'Tifton 10' (Hanna, 1990).

The PCR-based DNA fingerprinting techniques based on the analysis of information-rich nucleic acid molecules used in studying genetic diversity, relatedness, phylogeny and in identifying off-types of cultivars in *Cynodon* (Caetano-Anoles, 1998a) are the randomly amplified polymorphic DNA (RAPD) (Roodt, et al. 2002) and DNA

amplified fingerprinting (DAF) (Caetano-Anolle et al. 1995; Caetano-Anolle et al. 1997; Ho et al. 1997; Assefa et al., 1998; Anderson et al. 2001). Recently, the amplified fragment length polymorphism (AFLP) (Vos et al., 1995) has been used in *Cynodon,* to differentiate bermudagrass genotypes (Zhang et al., 1999) and to detect the genetic diversity among forage bermudagrass cultivars (Karaca et al., 2002).

Cynodon grasses are indigenous in China. However, very little is known of the genetic diversity of Chinese *Cynodon* or of its genetic relationship to *Cynodon* from other regions of the world. Quantification of this variation will provide invaluable information regarding the potential for improvement of *C. dactylon* var. *dactylon* and related taxa. The objectives of the study are to quantify the genetic variation within and genetic relatedness among the Chinese Cynodon accessions based on AFLP markers. Selected standard commercial *Cynodon* cultivars used in the U.S. were included for comparison.

MATERIALS AND METHODS

Plant materials and DNA isolation

The Chinese *Cynodon* germplasm collection consisted of 119 clonal accessions collected from eleven provinces ranging from tropical Hainan Island to the temperate climate region around Beijing (Table 1). 'Tifway', 'Tifgreen', and 'Tifton10' standard U.S. commercial cultivars were included to determine their relatedness to the Chinese accessions. The Chinese *Cynodon* collection included six triploid (2n=3x=27), 103 tetraploid (2n=4x=36), three pentaploid (2n=5x=45) and seven hexaploid (2n=6x=54) plants (Table 1). The *Cynodon* clonal plants were grown in the greenhouse at Oklahoma State University in 15 cm diameter pots containing a standard soil mix (Scotts-Sierra Horticultural Products Co., Marysville, OH). The plants were maintained in a healthy condition by daily watering and biweekly fertilization with M-77 Peatlite Special water-soluble fertilizer (Sctts-Sierra Horticultural Products Co., Marysville, OH). DNA samples were isolated from fresh leaf tissues of the experimental potted plants with DNeasy plant mini kit from QIAGEN Inc. (Valencia, CA 91355).

AFLP procedures

The AFLP analysis was performed as described by Vos et al. (1995) with a few minor modifications (Bai et al., 1999). Before the AFLP analysis of 122 experimental *Cynodon* DNA samples, DNA from eight accessions were used to test the reproducibility of the optimized AFLP procedures described below. Two sets of DNA samples were isolated separately from 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 AFLP procedures. The second set of DNA samples was used for three separate PCR reactions.

The resultant four sets of selectively amplified PCR products from the two sets of DNA samples were run in adjacent lanes on the same gel to evaluate reproducibility.

The AFLP procedures are mainly comprised of DNA sample preparation, digestion, ligation, pre-amplification, selective amplification and electrophoresis. Genomic DNA was diluted to a final concentration of 100 ng / μl for AFLP analysis. The diluted genomic DNA (approximately 300 ng) was double digested with *Eco*RI and *Mse*I restriction enzymes at 37°C for two hours. AFLP adapters for both enzymes were then ligated to restriction fragments at 20 °C overnight. 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 30s at 94°C, 1 min at 65°C, and 1 min at 72 °C and PCR products of pre-amplification were checked in a 1% agarose gel. A total of 13 AFLP selective primer combinations (Table 2) with *Eco*RI primers being 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 30s, 65°C for 30s (-0.7°C per cycle), and 72°C for 60s; 23 cycles at 94°C for 30s, 56°C for 30s, and 72°C for 60s. All PCR reactions were conducted on a MJ PTC-100 thermocycler. Approximate one μl of the selective amplification PCR products and 1.0 μl of DNA size standard were loaded onto a 6.5% denaturing Long Ranger gel (BMA, Rockland, ME) and run in 1X TBE buffer at 1500V and 40 W for 3.5 h in a Li-Cor's IR^2-4200 DNA Analyzer (Li-Cor Inc. Lincoln, NE).

Data profiling and analysis

Electrophoresis bands were visually scored twice as present (1), absent (0) or ambiguous (9) for each accession. Data of polymorphic bands were compiled for each replicated experiment in a data matrix and analyzed by using the NTSYS (Numerical Taxonomy System) version 2.0 (Exeter Software, New York, NY). Similarity coefficients of pair-wise comparisons among the experimental *Cynodon* accessions were computed with the SIMQUAL module. Cluster analysis was performed according to the un-weighted pair-group mean algorithm (UPGMA) within the SAHN module of the NTSYS program. A principal coordinate analysis to construct a two-dimensional array of eigenvectors was performed using the DCENTER module of the NTSYS program.

RESULTS AND DISCUSSION

Reproducibility of AFLP procedures

Eight accessions (Tifton10, 12317, 12364, 12355, Tifgreen, 12349, 12259 and 12326) were used to evaluate the reproducibility of the AFLP procedures by employing two selective primer combinations: eACT/mCAG (Fig. 1) and eAAC/mCAG. In 62 of the 64 replicated gel lanes, DNA banding patterns of the replicated PCR products were identical. 1.2% of total bands evaluated were different between replicates. The differences of replicated banding patterns were in level of intensity and present mainly in the bands of size larger than 450 bp. This high reproducibility (98.8%) agrees with the reproducibility previously reported (Zhang et al. 1999) further confirming the AFLP procedure to be highly reliable for DNA profiling of bermudagrass.

DNA profiling

Thirteen AFLP selective amplification primer combinations totally produced 763 bands among the 122 *Cynodon* genotypes (Table 1), with an average of 58.7±11.4 bands per primer combination (Table 2). Of the 763 bands scored, 466 were polymorphic accounting for 61.1%, averaging 35.9±12.4 bands per primer combination. The primer combinations eAGT/mCAC and eGCT/mCAG amplified the largest and smallest numbers of total bands and polymorphic bands per gel, respectively (Table 2).

Genetic diversity and relatedness

The genetic diversity was relatively high among the accessions in this study. The genetic similarity coefficients among the 122 accessions ranged from 0.55 to 0.99. The lowest similarity coefficient (0.55) was between Tifgreen and 12351, and Tifgreen and

12261. Tifgreen is a triploid hybrid between tetraploid *C. dactylon* and diploid *C. transvaalensis* accessions from Africa (Juska and Hanson, 1964). Accessions12351 and 12261 are from China. The highest similarity coefficient was 0.99 detected between accessions 12279 and 12280. The two accessions were collected from very close sites in Aba, Sichuan Province of China.

Cluster analysis based on the genetic similarity coefficient clearly separated the 122 *Cynodon* accessions into five distinct major groups: A, B, C, D and E (Fig.2.). The variation patterns and groupings of the *Cynodon* accessions appear to be associated with their ploidy level and geographic origin. Cluster A contains Tifgreen and two triploid accessions (Fig. 2.). The genetic similarity coefficients among the three genotypes ranged from 0.97 to 0.99 indicating that they are genetically very similar. The morphological traits of accessions 12256 and 12369 grown in greenhouse and field plots were very similar, but different from Tifgreen in leaf length and sod density. Accessions 12256 and 12369 probably were cultivars introduced to China and possibly mutational variants of Tifgreen or 'Tifdwarf'. Caetano-Anolles (1998b) reported the genetic instability of Tifgreen and Tifdwarf detected by DAF and ASAP analysis. Off-types of Tifgreen were phenotypically distinct from the wild type, but genetically close to each other.

Cluster B consists of Tifway, 12260, 12272 and 12282. The four accessions genetically are very similar with SC ranging from 0.97 to 0.99 and averaging 0.98±0.01. Accessions 12260, 12272 and 12282 were morphologically uniform, but distinct from Tifway in foliage color and developmental characteristics, particularly in the late growing season. The Tifway tended to have darker green color and fewer inflorescences than the other three accessions in the fall. Caetano-Anolles et al. (1997) indicated that Tifway

was genetically stable, thus, it is less likely that the three Chinese accessions are mutational variants of Tifway. However, the three triploids were collected at three locations in Sichuan province where triploid *Cynodon* cultivars have been introduced and widely used (Wu et al., 2001).

Cluster C in Fig. 2 contains only two accessions 12261 and 12257. Interestingly, Accession 12261 is a triploid collected from Neijiang, Sichuan. Accession 12257 is a tetraploid from Tongjiaxi, Chongqing. The genetic similarity of pair-wise comparison between the two accessions is 0.92. Morphological traits (color, leaf texture, internode length, plant height) of the triploid genotype are similar to those of tetraploid common bermudagrass. However, seed set data of a replicated field experiment in 2002 indicated that averaged seed set rate of 12261 was 0.07%, much lower than those of common bermudagrass genotypes.

Cluster D contained 109 accessions making it the largest group. The cluster contained the majority of the tetraploid cytotypes and all of the hexaploid cytotypes of the Chinese accessions (Fig. 2). Similarity coefficients in the cluster averaged 0.82±0.05, and ranged from 0.69 to 0.99. The eight hexaploid cytotypes were separated into three subgroups. One subgroup contained 12317, 12318, 12320, 12356 and 12358. Accessions 12317 and 12318 were collected from Shanghai, and 12356 and 12358 from Zhejiang and Jiangsu, respectively. Field notes indicated that accession 12320 was a fine textured plant from Hongya, Sichuan, but the plant used in this study had relatively coarse texture suggesting that it was not the true 12320 accession. Mechanical mixture of the two accessions may have occurred because the original nursery plots in China of the two accessions were adjacent to each other. The similarity coefficient between 12318 and

12320 was 0.99 further suggesting that the accession identified as 12320 in this study was a contaminant. The other three Hexaploid genotypes (Tifton10, 12319 and 12360) clustered into two subgroups. Tifton10 and 12319 were from Shanghai, and 12360 from Jiangsu. In cluster D, 101 of the tetraploid genotypes scattered into various sub-groupings (Fig. 2). Collectively, the accessions from the same geographic locations or nearby regions tended to have higher genetic similarity and to cluster into same subgroups or neighbor subgroups. As shown in Fig. 1, most accessions that originated in the western provinces of Sichuan, Chongqing and Yunnan were included in the same sub-groupings or in neighboring sub-groupings, and most accessions from eastern and southern provinces including Shanghai, Jiangsu, Zhejiang, Fujian, Guangdong and Hainan, tended to group into the same or adjacent groupings. However, seven accessions (12364, 12357, 12361, 12363, 12354, 12367 and 12359) from eastern and southern regions formed sub-groupings with accessions from west regions, while two accessions (12344 and 12258) from western regions formed sub-groupings closer to groupings containing predominantly eastern and southern origin accessions.

Cluster E contains one tetraploid and three pentaploid accessions (Fig. 2). Two pentaploid accessions 12348 and 12352 and the tetraploid accession 12351 were collected from Xinlong, Wanquanhe and Haikou in Hainan Island. Another pentaploid accession, 12365, originated in Minghou, Fujian. Among the pentaploid genotypes, genetic similarity coefficients averaged 0.96±0.01, ranging from 0.95 to 0.98, indicating very low genetic diversity among the pentaploid accessions.

Principal coordinate analysis clearly indicated that Tifgreen and Tifway and the accessions respectively clustering with them were widely separated from the Chinese

accessions (Fig. 3). Both Tifgreen and Tifway are interspecific F_1 hybrids from tetraploid *C. dactylon* by diploid *C. transvaalensis* crosses with parents of African origin. Among the Chinese accessions, the pentaploid genotypes separated from tetraploid and hexaploid accessions (Fig. 3). However, the hexaploid accessions were grouped together with the tetraploid accessions from closer geographic regions. Genetic similarity analyses (Table 3) among the ploidy levels also demonstrated that genetic distance between tetraploids and hexaploids is somewhat closer (0.78) than the distance (0.71) between tetraploids and pentaploids. Within the ploidy levels, the range of similarity coefficients and the average similarity coefficient of tetraploids was larger than those of the hexaploids and pentaploids, showing that the Chinese tetraploid *Cynodon* contained much wider genetic diversity compared to the pentaploid and hexaploid cytotypes.

A dendrogram from the cluster analysis (Fig. 2) showed that hexaploid accessions and pentaploid accessions formed clusters or sub-clusters in mixture with tetraploid accessions that originated in the same geographic region as the pentaploids and hexaploids. The spread of tetraploid, pentaploid and hexaploid forms in 2-dimensional plot of principal coordinate analysis (Fig. 3) in which PC1 and PC2 accounted for 10.4% and 7.2% of total variation, respectively, is basically consistent with the indication of the dendrogram. The close genetic relatedness of the three different ploidy forms indicated they have a common ancestry. Harlan and de Wet (1969) pointed out that *C. dactylon* var. *dactylon* is the only truly cosmopolitan taxon in *Cynodon*, and in their revised classification of genus *Cynodon*, only the tetraploid cytotype is indicated for *C. dactylon* var. *dactylon* (Harlan, 1970; Harlan et al. 1970; de Wet and Harlan, 1970). Hexaploidy and pentaploidy are rare in *Cynodon*. Powell et al. (1968) reported a hexaploid clone

from a cross of tetraploid × diploid parents, and speculated that the doubling of chromosome number at an early zygote stage probably accounted for its occurrence. Another hexaploid plant was identified in the progeny of a self-pollinated plant of tetraploid *C. dactylon* (Felder, 1967). He hypothesized that the autohexaploid plant resulted from the union of an unreduced female gamete and a reduced male gamete. Accordingly, the origin of the Chinese hexaploid forms most likely resulted from either chromosome doubling in a young zygote or from the union of reduced and unreduced gametes of tetraploid parents. The pentaploid forms probably originated from the union of one normal gamete from a tetraploid parent with another normal gamete from a hexaploid parent.

It is evident that the AFLP differentiation of the Chinese *Cynodon* genotypes in cluster analysis corresponds well with their geographical patterns. Obviously geographic origin plays a significant role in their genetic differentiation. This is because adaptation and evolution of *Cynodon* plants is a function of natural selection under local environmental factors, which include abiotic and biotic conditions. Harlan and de Wet (1969) indicate that there are reasonably consistent variation patterns, which can be discerned on the basis of appearance, adaptation, and geographic distribution. The finding of geographic variation patterns has significance in collection of the full range of genetic diversity, which must cover the different geographic regions.

Although the *Cynodon* accessions in this study were collected from two distinct geographic regions in China, discrete genetic differentiation within a region is not observed and overlap existed between the two separate regions in the dendrogram (Fig.2) and principal coordinate plot (Fig.3). This might be explained by the open pollination

behavior of *Cynodon* plants. The predominant mode of sexual reproduction in *C. dactylon* is outcrossing due to cross-pollination and self-incompatibility (Burton, 1947; 1965; Taliaferro and Lamle, 1997). The high probability of cross-pollination results in frequent gene flows between natural populations in close geographic regions, which probably prevent formation of differentiated genetic groups within a region. Presumably, as the geographic distance between natural *Cynodon* populations increases the frequency of gene flow would decrease. Harlan and de Wet (1969) observed that crossing of *C. dactylon* var. *dactylon* plants of close geographic origins resulted in hybrids with higher fertility than crosses of parents of disparate geographic origins. We believe that genetic variation and differentiation pattern of Chinese *Cynodon* germplasm pool in the main distribution regions is of a gradual mode for tetraploid form without apparent genetic barrier. It will be interesting to examine agronomic and adaptive traits of the Chinese accessions to compare the AFLP diversity with the variation on the traits.

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Table 1. Chinese *Cynodon* plants and three commercial cultivars used in this study.

No.	Identification	Ploidy (2n)	Original Location / Source	No.	Identification	Ploidy (2n)	Original Location / Source
1	12256	27	Shanghai	62	12328	36	Sichuan
2	12260	27	Sichuan	63	12329	36	Sichuan
3	12261	27	Sichuan	64	12330	36	Sichuan
4	12272	27	Sichuan	65	12331	36	Sichuan
5	12282	27	Sichuan	66	12332	36	Sichuan
6	12369	27	Hainan	67	12333	36	Sichuan
7	Tifway	27	G.W. Burton, Tifton, GA	68	12334	36	Sichuan
8	Tifgreen	27	G.W. Burton, Tifton, GA	69	12335	36	Sichuan
9	12253	36	Sichuan	70	12336	36	Sichuan
10	12254	36	Sichuan	71	12337	36	Sichuan
11	12255	36	Sichuan	72	12338	36	Sichuan
12	12259	36	Sichuan	73	12339	36	Sichuan
13	12264	36	Sichuan	74	12340	36	Sichuan
14	12265	36	Sichuan	75	12341	36	Sichuan
15	12266	36	Sichuan	76	12342	36	Sichuan
16	12267	36	Sichuan	77	12343	36	Sichuan
17	12268	36	Sichuan	78	12344	36	Sichuan
18	12269	36	Sichuan	79	12345	36	Sichuan
19	12270	36	Sichuan	80	12346	36	Sichuan
20	12271	36	Sichuan	81	12347	36	Sichuan
21	12273	36	Sichuan	82	12257	36	Chongqing
22	12274	36	Sichuan	83	12258	36	Chongqing
23	12275	36	Sichuan	84	12292	36	Chongqing
24	12276	36	Sichuan	85	12293	36	Chongqing
25	12277	36	Sichuan	86	12298	36	Chongqing
26	12278	36	Sichuan	87	12299	36	Chongqin
27	12279	36	Sichuan	88	12314	36	Chongqing
28	12280	36	Sichuan	89	12324	36	Chongqing
29	12281	36	Sichuan	90	12262	36	Yunnan
30	12283	36	Sichuan	91	12263	36	Yunnan
31	12284	36	Sichuan	92	12326	36	Yunnan
32	12285	36	Sichuan	93	12349	36	Hainan
33	12286	36	Sichuan	94	12351	36	Hainan
34	12287	36	Sichuan	95	12371	36	hainan
35	12288	36	Sichuan	96	12350	36	Guangdong
36	12289	36	Sichuan	97	12353	36	Guangdong
37	12290	36	Sichuan	98	12354	36	Guangdong
38	12291	36	Sichuan	99	12372	36	Guangdong
39	12294	36	Sichuan	100	12362	36	Fujian
40	12295	36	Tongnan, Sichuan	101	12366	36	Fuzhou, Fujian

41	12296	36	Sichuan	102	12355	36	Zhejiang
42	12297	36	Sichuan	103	12357	36	Jiangsu
43	12300	36	Sichuan	104	12364	36	Zhejiang
44	12301	36	Sichuan	105	12359	36	Jiangsu
45	12302	36	Sichuan	106	12361	36	Jiangsu
46	12303	36	Sichuan	107	12363	36	Jiangsu
47	12304	36	Sichuan	108	12315	36	Shanghai
48	12305	36	Sichuan	109	12316	36	Shanghai
49	12306	36	Sichuan	110	12367	36	Shandong
50	12307	36	Sichuan	111	12368	36	Beijing
51	12308	36	Sichuan	112	Tifton10	54	Shanghai, W.W. Hanna, Tifton, GA
52	12309	36	Sichuan	113	12317	54	Shanghai
53	12310	36	Sichuan	114	12318	54	Shanghai
54	12311	36	Sichuan	115	12319	54	Shanghai
55	12312	36	Sichuan	116	12320	54	Sichuan
56	12313	36	Sichuan	117	12348	45	Hainan
57	12321	36	Sichuan	118	12352	45	Hainan
58	12322	36	Sichuan	119	12356	54	Zhejiang
59	12323	36	Sichuan	120	12358	54	Jiangsu
60	12325	36	Sichuan	121	12360	54	Jiangsu
61	12327	36	Sichuan	122	12365	45	Fujian

Table 2. Selective primer pairs, scored total and polymorphic bands in Chinese *Cynodon* AFLP profiling.

Selective amplification Primer pairs	Total Bands	Polymorphic Bands	% polymorphic Bands
eAAC/mCAG†	63	25	39.68
eAAC/mGAC	40	17	42.50
eACT/mCAC	49	42	85.71
eACT/mCAG	61	45	73.77
eACT/mGAC	62	38	61.29
eACT/mCTG	63	46	86.79
eAGT/mCAA	62	27	43.55
eAGT/mCAC	72	61	84.72
eAGT/mCAG	58	37	63.79
eAGT/mCAT	69	41	59.42
eAGT/mGAC	53	25	47.17
eAGT/mCTG	76	45	59.21
eGCT/mCAG	35	17	48.57
Total	763	466	
Average	58.69±11.38	35.85±12.40	61.07

† : e is the pre-amplification primer sequence for *Eco*RI site (5-GACTGCGTACCAATTC) without any selective nucleotides and m is the pre-amplification primer sequence for *Mse*I site (5-GATGAGTCCTGAGTAA).

Table 3. Genetic similarity coefficients within and among three ploidy levels of Chinese *Cynodon* accessions.

Ploidy	No of Accessions	Genetic similarity coefficient			
		Within ploidy (Range)	Among ploidy		
			4x	5x	6x
4x	103	0.80 (0.69-0.99)	1.00	0.71	0.78
5x	3	0.96 (0.95-0.98)		1.00	0.73
6x	8	0.85 (0.77-0.99)			1.00

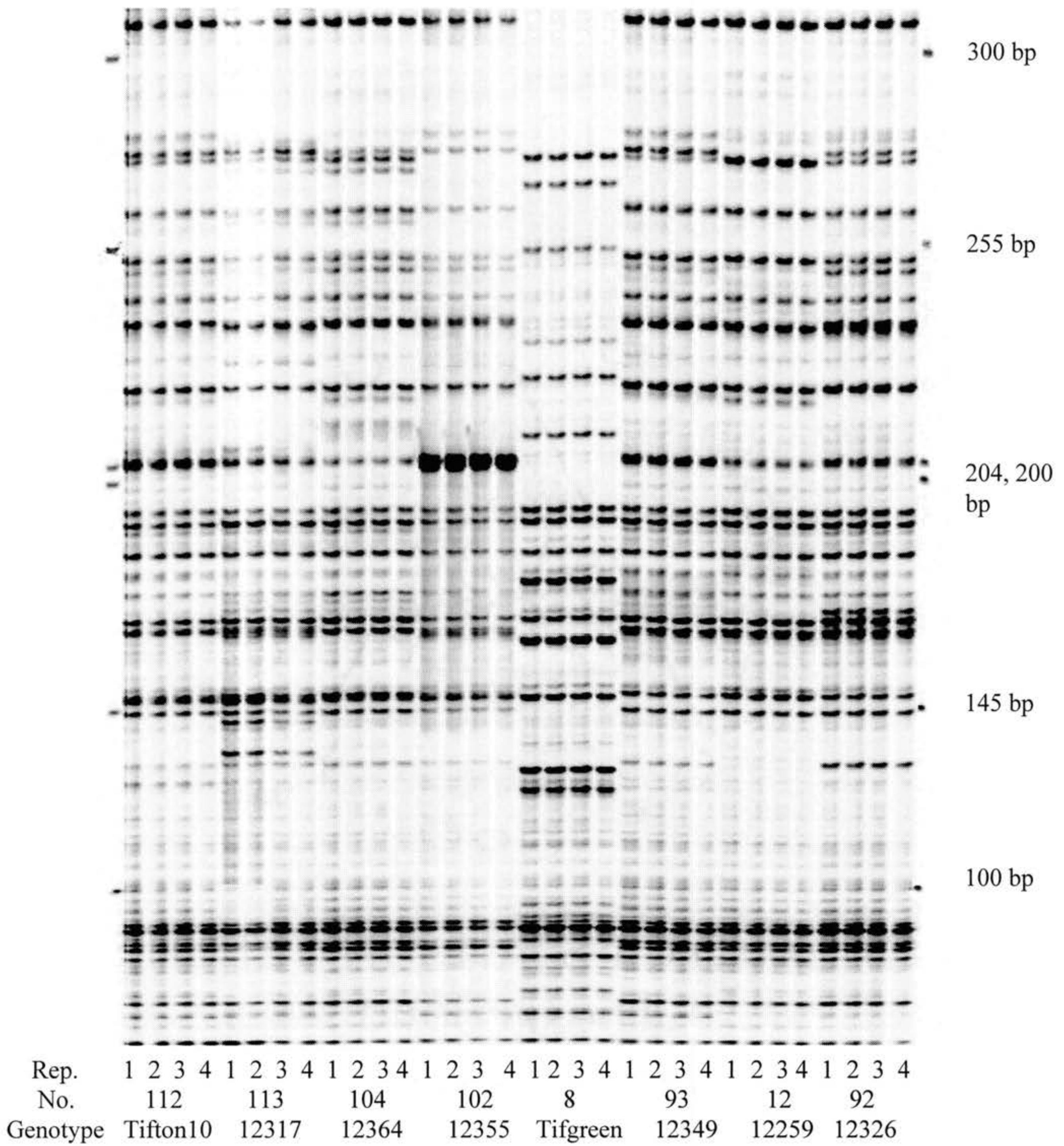

Fig.1. Replicated AFLP DNA patterns generated using primer-pair eACT/mCAG for eight genotypes. Fragment size is indicated on the right.

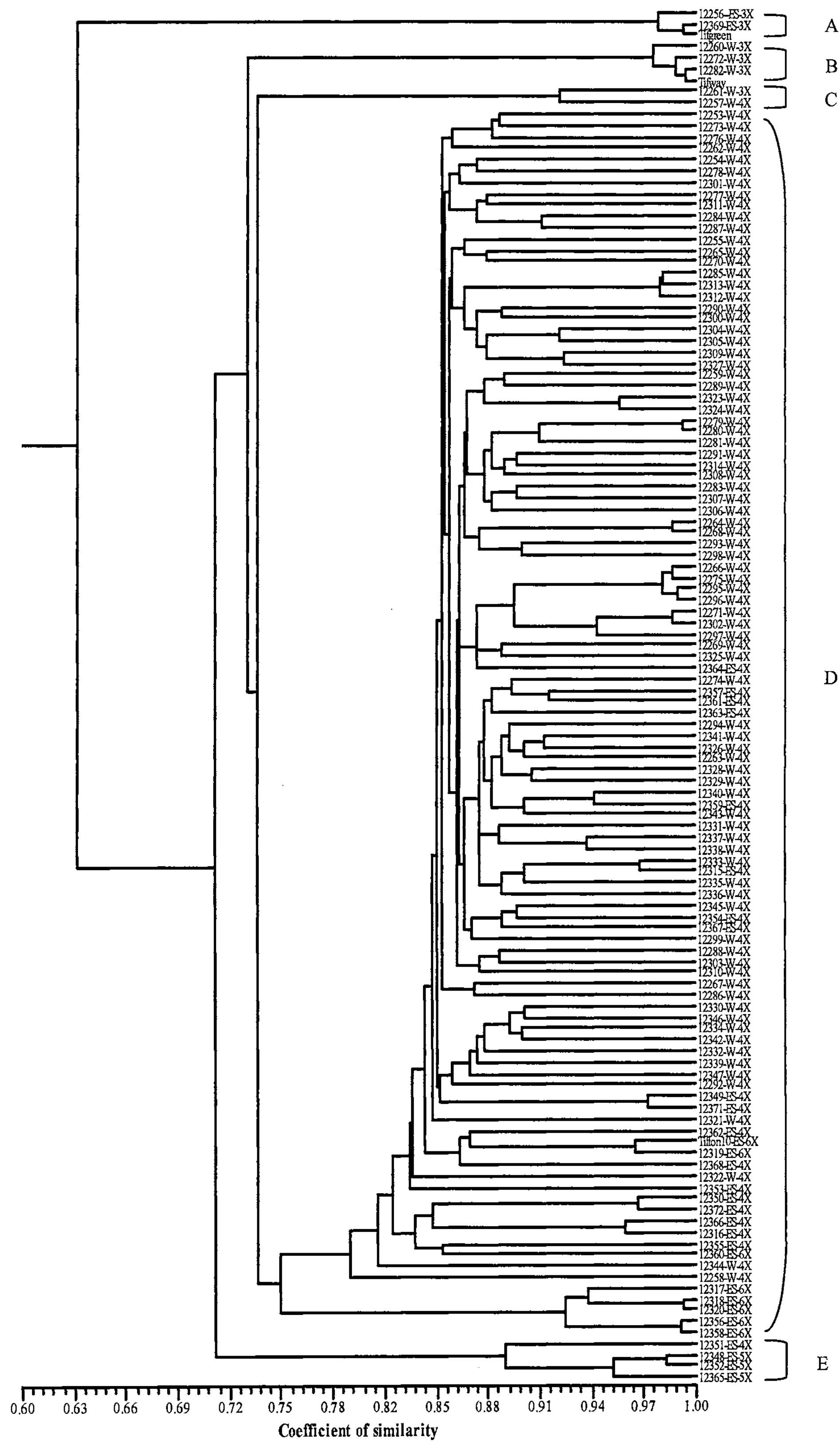

Fig. 2. Cluster analysis tree and similarity coefficients of 122 *Cynodon* accessions based on AFLP DNA profiling.

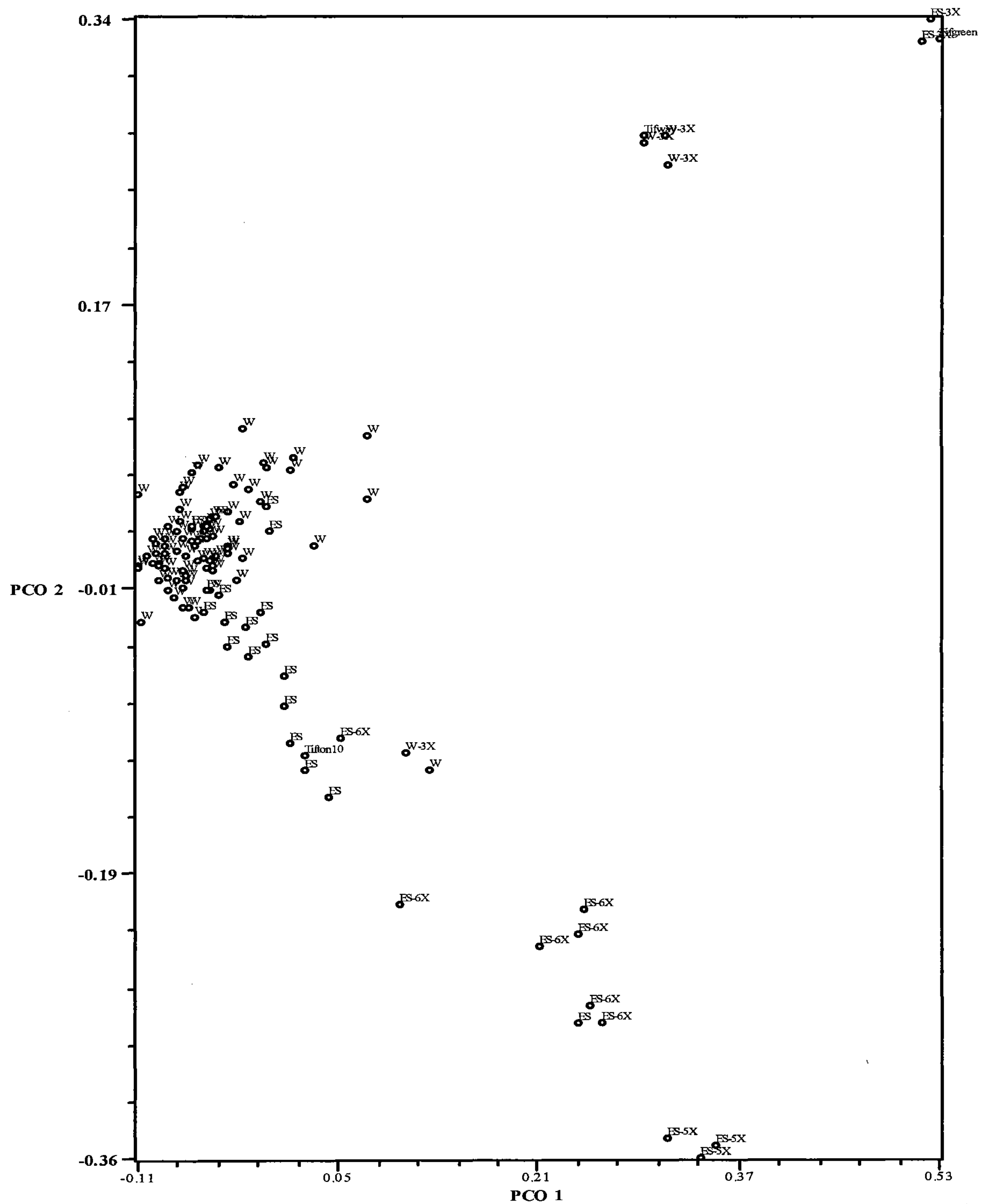

Fig. 3. Principal coordinate plot indicating variation pattern and geographic origin for 122 accessions using 466 AFLP markers. Note W stands for the west region and ES for east and south region and ploid also indicated.

CHAPTER IV

AFLP ANALYSIS OF CYNODON DACTYLON (L.) PERS. VAR. DACTYLON GENETIC VARIATION

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ABSTRACT

Cynodon dactylon (L.) Pers. var. *dactylon* (common bermudagrass) is geographically widely distributed between about 45°N and 45°S latitudes, penetrating to about 53°N latitude 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 twenty-eight *C. dactylon* var. *dactylon* accessions originating from 11 countries encompassing four 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 eight 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

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, containing 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 45°N and 45°S latitudes, and penetrates to approximately 53°N latitude in Europe. Evidence from biosystematic studies of *C. dactylon* var. *dactylon* suggested to Harlan and de Wet (1969), Harlan (1970) and Harlan et al. (1970c) that it was a Eurasian grass until recent times, and that a geographic area extending from West 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* for 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. 1970b, c, 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-Annolle et al. 1995, 1997; Caetano-Annolle 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-Annolle et al. 1995, 1997; Caetano-Annolle 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) (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 the 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 four 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 DNeasy plant mini kit from QIAGEN Inc. (28159 Avenue Stanford, Valencia, CA). Prior to enzyme digestion, genomic DNA was diluted to a final concentration of 100ng/ul.

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 for both enzymes were then ligated to restriction fragments. 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 30s 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 being 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 30s, 65°C for 30s (-0.7°C per cycle), and 72°C for 60s; 23 cycles at 94°C for 30s, 56°C for 30s, and 72°C for 60s. All PCR reactions were conducted on a MJ PTC-100 thermocycler. One µl of the selective amplification PCR products and

1.2 µl of DNA size markers were loaded on a 6.5% denaturing Long Ranger gel (BMA, Rockland, ME) in 1X TBE buffer and run at a constant 2000V for 3.5 hr in a Li-Cor's NEW® Global IR^2-4200 DNA Analyzer (Li-Cor Inc. Lincoln, NE).

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 producing 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, NY). Similarity coefficients were computed by using the SIMQUAL module. Cluster analysis was performed according to the unweighted pair-group mean algorithm (UPGMA) 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 procedures

Reproducibility of the AFLP products was very high. Of the primer combinations e-ACT/m-CAG and e-AAC/m-CAG amplified bands (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 PCR reactions, 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 *Cynodon dactylon* var. *dactylon*.

Genetic diversity and relatedness

The eight selective primer combinations totally amplified 590 bands, averaging 74±26 SD 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 one of the 28 accessions. The primer combination e-AGT/m-CAG produced the greatest total number of bands (126) and the greatest number of polymorphic bands (81), while 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, respectively from Zhejiang and Jiangsu provinces in east China. The lowest SC value (0.53) was for the

pair-wise 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 five major clusters designated as A, B, C, D, and E (Fig. 2). Cluster A included four tetraploid (2n=4x=36) accessions, three (A12374, PI 291583 and PI 291584) from Zimbabwe and 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 was comprised of four tetraploid accessions (A12376, A12377, A12378, 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 U.S. *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 sub-groups were evident. The first subgroup comprised only accession JT-2, which was introduced to Japan from Dubai, 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 sub-group was comprised of 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 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. (1970a,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 variety *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, and their sympatric existence, suggest 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* x 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 other polyploidy plant species as a mechanism effecting 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 was comprised of 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, 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 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 Australia 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, clearly constituting the most diverse set

of accessions. The genetic SC values for the African origin accessions 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 adaptional 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. 1970b, c, 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 climatic conditions. 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 due to chromosomal structural differences mainly resulting from translocations and inversions. The genetic isolation of plant populations based on chromosomal structural constitution 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 of different geographic origins and the geographic differentiation pattern as indicated by the results of this study have implications relative to germplasm collection, preservation, and utilization. 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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Table 1. *C. dactylon* var. *dactylon* accessions analyzed for amplified fragment length polymorphisms.

No.	Identification: 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

Pre-amplification primers		
*Eco*RI	GACTGCGTACCAATTC	
*Mse*I	GATGAGTCCTGAGTAA	
Selective amplification 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' and 'm' represent the sequences of pre-amplification primers of *Eco*RI and *Mse*I, respectively.

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

† 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	28
1.00																											
0.62	1.00																										
0.69	0.70	1.00																									
0.63	0.74	0.83	1.00																								
0.77	0.70	0.68	0.65	1.00																							
0.82	0.61	0.70	0.62	0.81	1.00																						
0.65	0.63	0.64	0.59	0.65	0.77	1.00																					
0.66	0.66	0.69	0.61	0.68	0.75	0.82	1.00																				
0.66	0.66	0.68	0.61	0.70	0.76	0.78	0.79	1.00																			
0.71	0.62	0.64	0.55	0.74	0.80	0.80	0.79	0.80	1.00																		
0.64	0.66	0.67	0.60	0.71	0.73	0.79	0.79	0.79	0.78	1.00																	
0.54	0.72	0.58	0.75	0.62	0.55	0.53	0.57	0.55	0.53	0.54	1.00																
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															
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	1.00														
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	0.84	1.00													
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	0.82	0.83	1.00												
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	0.64	0.67	0.70	1.00											
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	0.62	0.63	0.68	0.73	1.00										
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	0.58	0.56	0.63	0.75	0.86	1.00									
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	0.59	0.59	0.62	0.73	0.86	0.90	1.00								
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	0.59	0.60	0.63	0.73	0.87	0.91	0.91	1.00							
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	0.59	0.60	0.63	0.73	0.88	0.90	0.92	0.91	1.00						
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	0.61	0.61	0.64	0.71	0.84	0.89	0.89	0.90	0.88	1.00					
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	0.65	0.65	0.69	0.72	0.77	0.79	0.81	0.82	0.80	0.84	1.00				
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	0.63	0.63	0.68	0.71	0.76	0.77	0.81	0.81	0.79	0.82	0.96	1.00			
0.63	0.68	0.60	0.62	0.73	0.71	0.71	0.74	0.76	0.74	0.69	0.65	0.67	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		
0.64	0.67	0.60	0.62	0.73	0.72	0.71	0.74	0.76	0.74	0.69	0.65	0.67	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	
0.66	0.64	0.62	0.59	0.71	0.74	0.75	0.74	0.77	0.78	0.74	0.60	0.63	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

† numbers correspond to those listed in Table 1 for the 28 accessions.

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.

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.

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.

Figure 1.

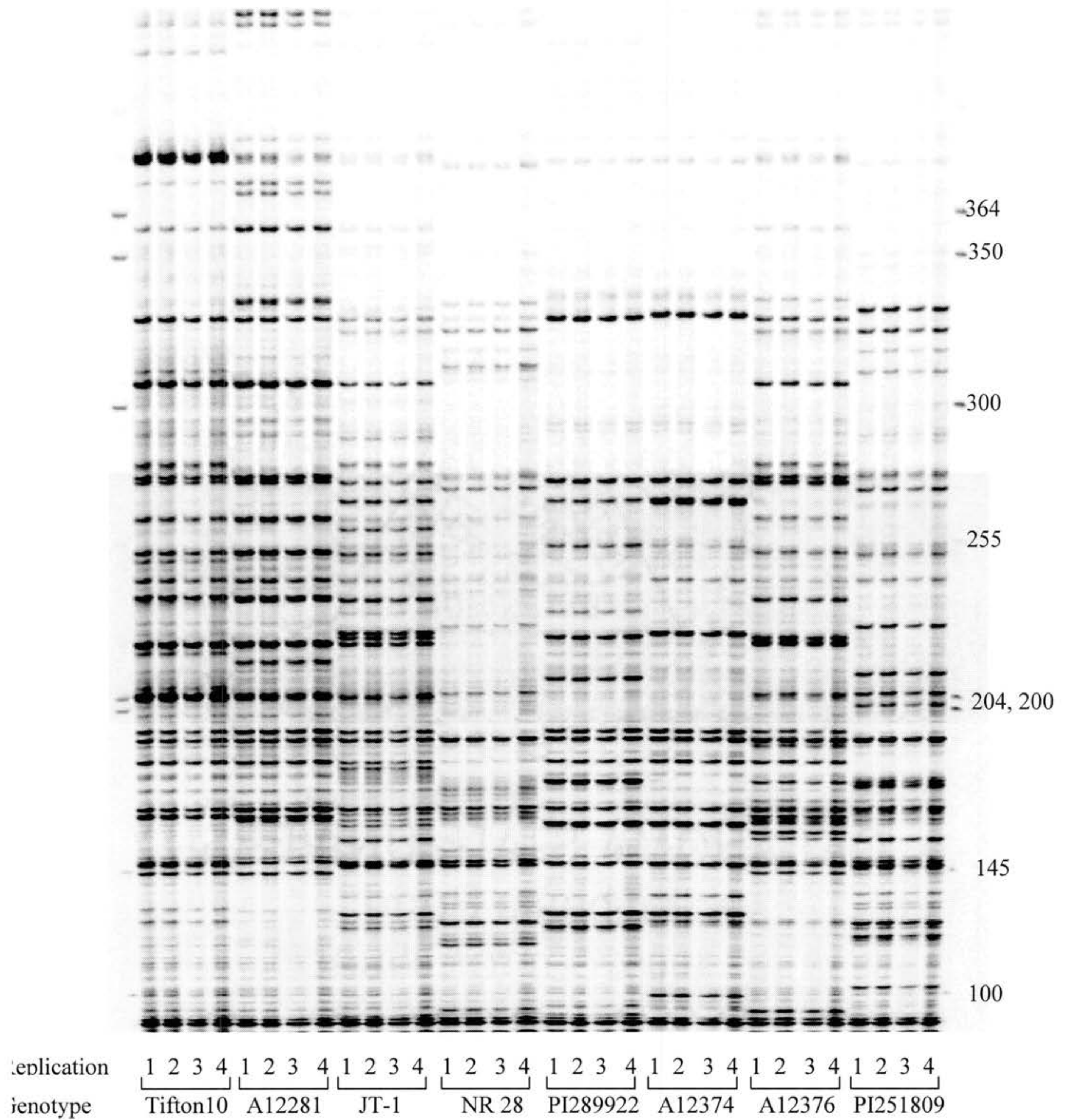


Fig. 2.

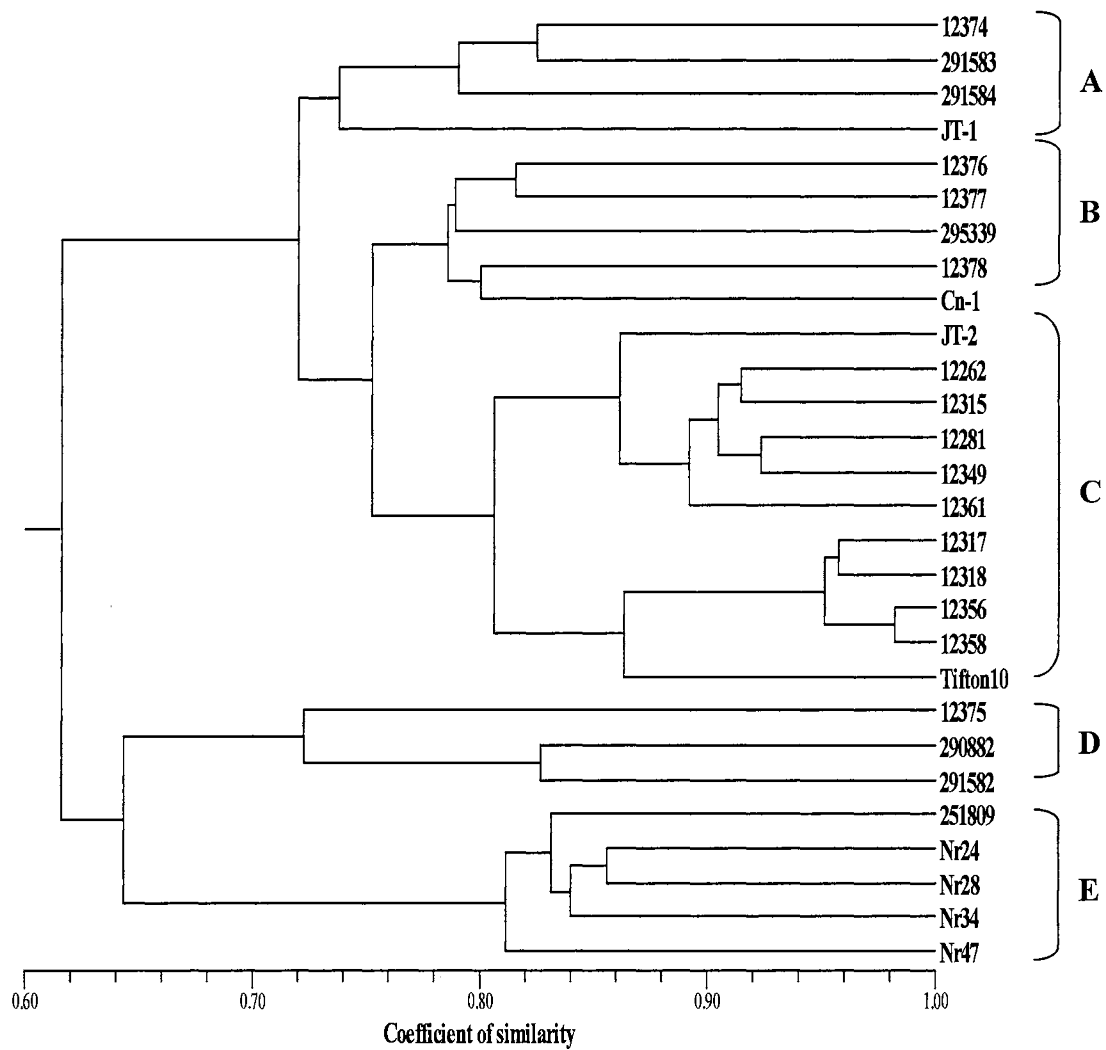
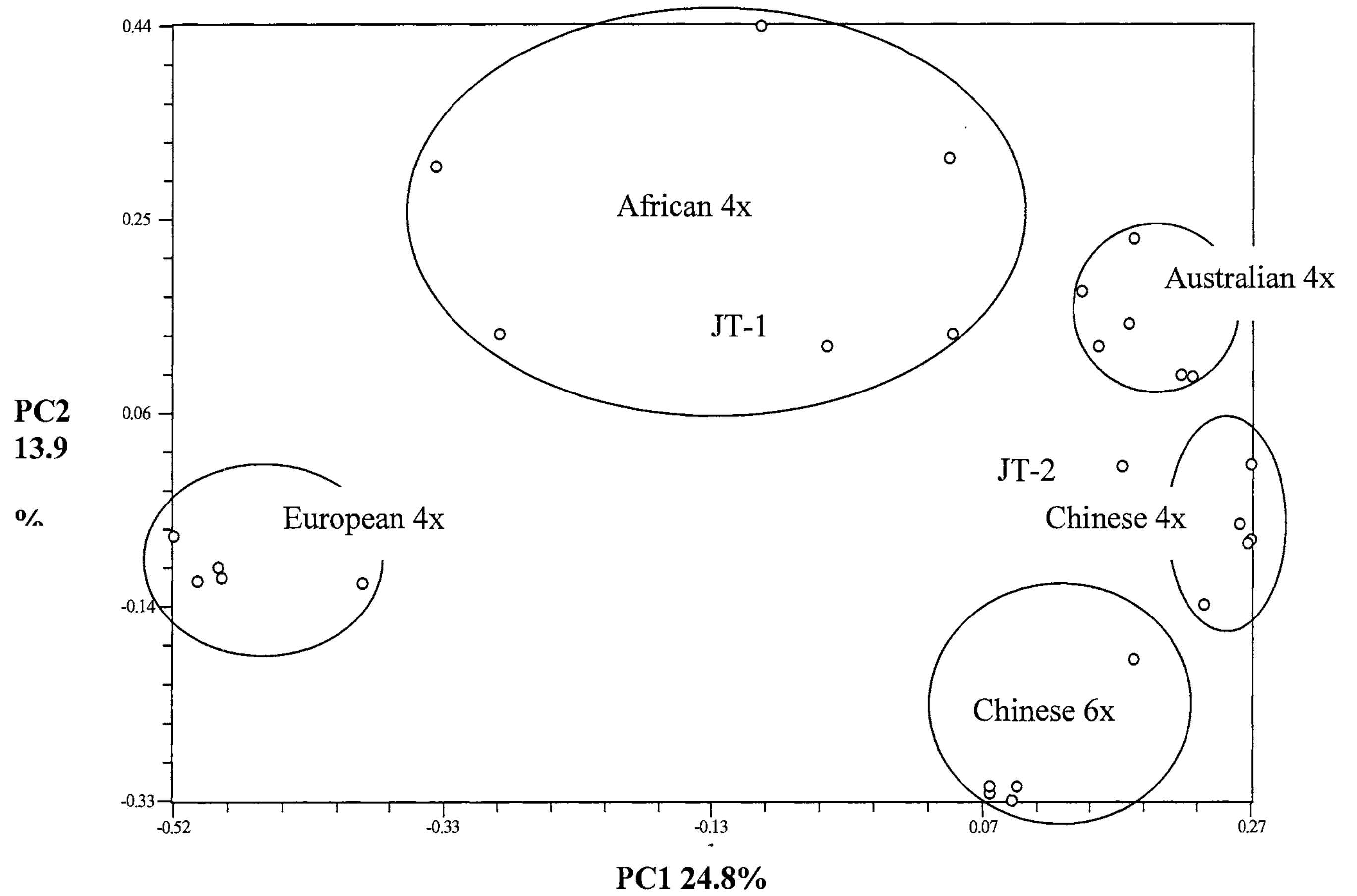


Fig. 3.



CHAPTER V

GENETIC DIVERSITY OF *CYNODON TRANSVAALENSIS* BURTT-DAVY AND ITS RELATEDNESS TO HEXAPLOID *C. DACTYLON* (L.) PERS. AS INDICATED BY AFLP MARKERS

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

EXECUTIVE SUMMARY

Cynodon transvaalensis is used as turf and has been hybridized with *C. dactylon* var. *dactylon* to produce high quality turf bermudagrass cultivars. Little information exists on the magnitude of genetic variation within the South Africa indigenous species. Wu et al. (××××-××××) demonstrated variation among 14 *C. transvaalensis* accessions for AFLP DNA markers and characterized their relatedness to two *C. dactylon* var. *dactylon* accessions and three derivative interspecific hybrids. The results suggest that there is sufficient genetic variation within *C. transvaalensis* for genetic improvement.

Short title: ***Cynodon transvaalensis* Genetic Diversity**

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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 inter-specific breeding improvement.

INTRODUCTION

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. 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, and erect narrow leaves, and 2-4 racemes per inflorescence with the spikelets loosely arranged on the racemes (Harlan et al., 1970b). *C. 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 ($\geq$38°C), and intolerance to sustained very low ($\leq$3.2mm) mowing heights (Juska and Hanson, 1964).

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

Information on the magnitude of variation within *C. transvaalensis* is limited. *C. 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

randomly 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 Karaca et al. (2002) respectively differentiated between 27 and 31 *Cynodon* genotypes using amplified fragment length polymorphism (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 based on 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* based on coarser foliage morphology and chromosome number (2n=3x=27). Chromosome number was determined using 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 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 pre-amplified using a primer combination based on the sequences of the adapters. The sequences of *Eco*RI and *Mse*I primers were 5-GACTGCGTACCAATTC 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°C and 72 °C. A 1% agarose gel was used to check the products of pre-amplification. 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 10X PCR buffer, 1.0 µl 25mM $MgCl_2$, 0.2 µl 10mM dNTP, 0.4 µl labeled *EcoR*I 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 µl of the selectively amplified PCR products were loaded on a 6.5% denaturing Long Ranger gel (BMA, Rockland, ME) and run in 1X TBE buffer at 1500V for 3.5 hr in a Li-Cor automated sequencer (Li-Cor Inc., Lincoln, NE). 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 8 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. In order to insure 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 cophenetic matrix to 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.

The 19 accessions clustered into two major groups and sub-clustered into hierarchy sub-groups based on the UPGMA tree of similarity coefficients (Fig. 2). The cophenetic correlation coefficient (r) of the cophenetic value matrix with the

dissimilarity matrix was high, with an r v alue 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 principal coordinate analysis (Figs. 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 sub-group was comprised of T572, T574, T576, T577, all from Lesotho, and PI 290874. The same Lesotho accessions were studied by Zhang et al. (1999) and they clustered into one distinct group. 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 in Cairo, Egypt as PI 183551(Juska and Hanson, 1964). Mahdi (1955) indicated that 'Ugandagrass' had been grown in Egypt for 50 years, 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 based on evaluations for turf quality. Selected accessions include PIs 289922 (Howick), 290905 (Frankenwald Fine), 290874 (Uganda), 290894 (Sekaapploss 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. Based on 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 (Figs. 2 & 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 to 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.

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 potential exists for discovery of *C. transvaalensis* plants with superior combining ability when hybridized with *C. dactylon* var. *dactylon* plants.

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

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

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

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
eAAC/mCAA†	62	42
eAAC/mCAC	44	39
eAAC/mCAT	46	20
eAAC/mGAC	33	26
eACT/mCAA	60	32
eACT/mCAC	44	30
eACT/mCAG	47	37
eACT/mGAC	54	43
eAGT/mCAA	62	12
eAGT/mCAC	53	31
eAGT/mCAG	66	45
eAGT/mCAT	46	12
eGCTG/mCAA	54	12
Total	671	381
Average	51.6±9.4	29.3±12.1

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

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.

	Genotype		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	290894	Ctr	0.00																		
2	290897	Hyb	0.48	0.00																	
3	290905	Ctr	0.16	0.47	0.00																
4	291591	"	0.15	0.48	0.01	0.00															
5	289922	"	0.23	0.49	0.09	0.10	0.00														
6	290812	"	0.29	0.49	0.26	0.27	0.27	0.00													
7	290813	"	0.33	0.49	0.29	0.30	0.32	0.13	0.00												
8	290665	"	0.17	0.49	0.04	0.04	0.12	0.28	0.28	0.00											
9	4048	"	0.21	0.51	0.13	0.14	0.16	0.23	0.27	0.12	0.00										
10	2747	"	0.21	0.48	0.13	0.13	0.14	0.22	0.28	0.14	0.08	0.00									
11	290874	"	0.24	0.52	0.18	0.19	0.17	0.25	0.32	0.19	0.15	0.11	0.00								
12	T572	"	0.26	0.51	0.24	0.25	0.23	0.31	0.34	0.24	0.23	0.22	0.13	0.00							
13	T574	"	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						
14	T576	"	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					
15	T577	"	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				
16	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			
17	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		
18	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	
19	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

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.

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

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

Fig. 1

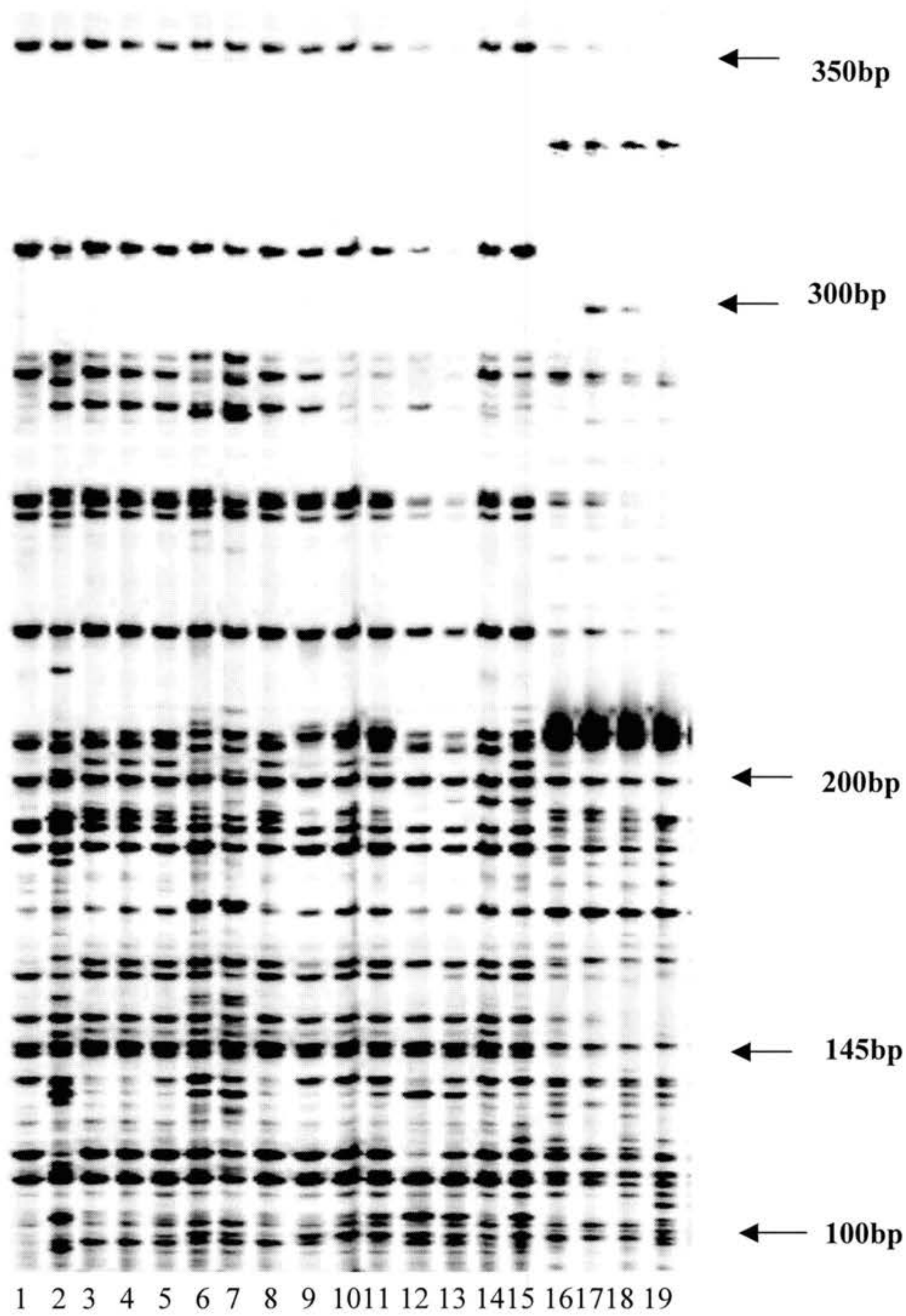


Fig. 2

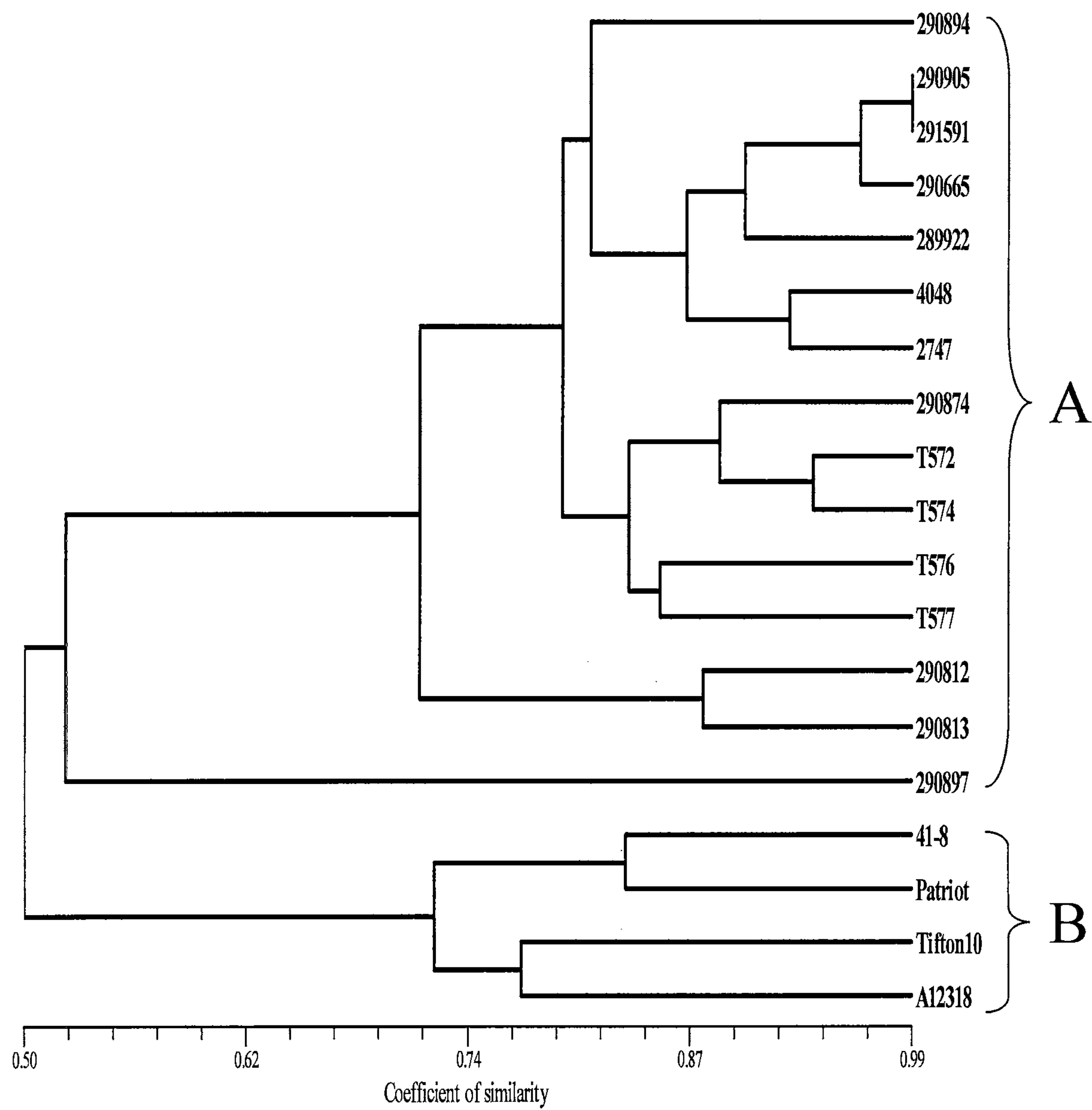
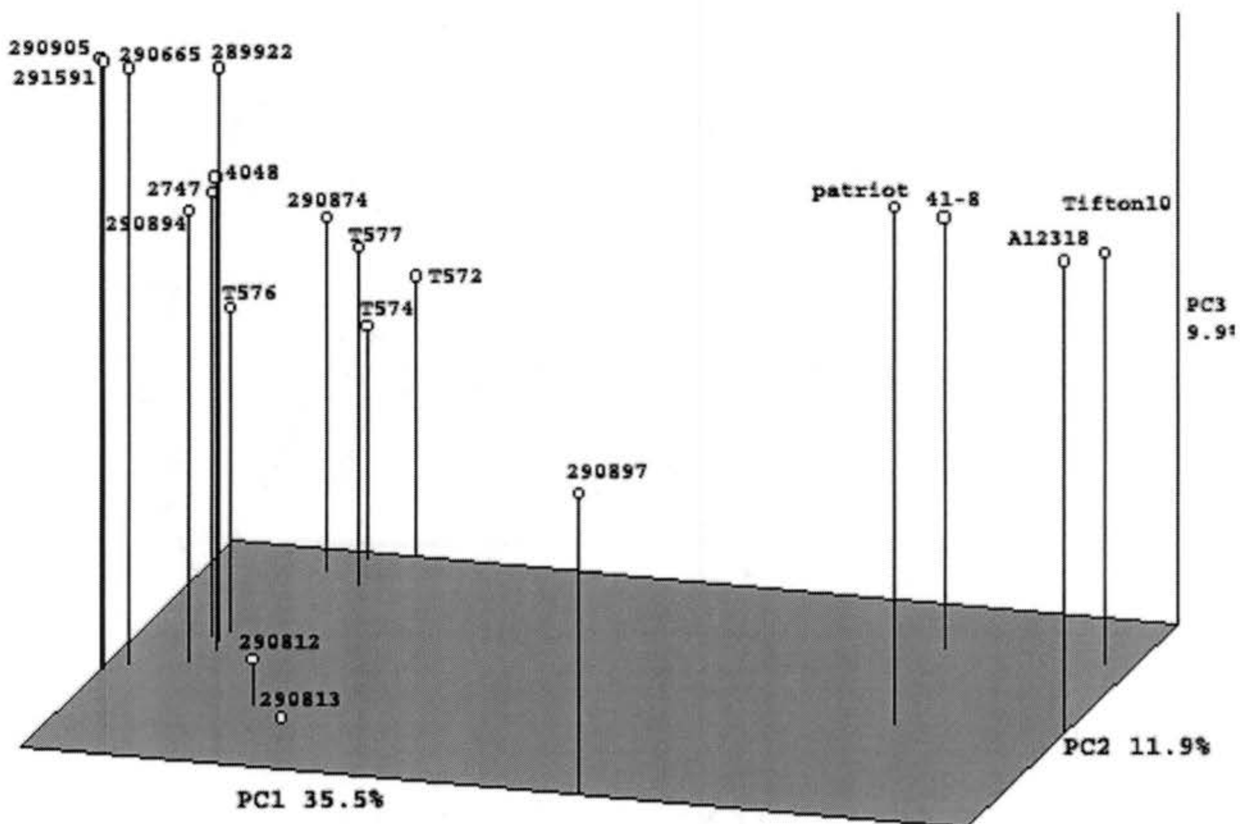


Fig. 3.



CHAPTER VI

GENETIC CHARACTERIZATION OF CHINESE *CYNODON* ACCESSIONS BY FIELD EXPERIMENTATIONS:

I. Genetic Variability in Seed Yield and its Components

ABSTRACT

Cynodon dactylon (L.) Pers. is indigenous and widely distributed in China but no information is available on the diversity of the indigenous germplasm for seed yields and related traits. Accordingly, we conducted two field experiments in 2002 and 2003 at Stillwater, Oklahoma to characterize the genetic variability among 120 *C. dactylon* accessions from china for seed yield and its components, and to determine relationships among seed yield and its components. Experiment A consisted of 120 clonal Chinese *C. dactylon* accessions (genotypes), while experiment B consisted of half-sib families from 56 of the 120 accessions. The field plot design for both experiments was a randomized complete block design with three replications. Highly significant differences ($P>0.01$) existed in both experiments for seed yield, seed head prolificacy, seed set percentage, seed number seedhead^{-1}, raceme number seedhead^{-1}and raceme length seedhead^{-1}. However, significant ($P<0.01$) year and genotype ×year interactions for seed yield, seed head prolificacy, seed set percentage and seed number seedhead^{-1}, and large magnitudes of variances of year and genotype ×year interactions occurred in both experiments

indicating important environmental influences on those traits. Phenotypic correlation analyses indicated that correlations of seed yield with seed head prolificacy, seed set percentage, and seed number seedhead^{-1} were substantial and positive ($P<0.01$) in both experiments, while correlations of seed yield with raceme number and raceme length seedhead^{-1} were negligible. Path coefficient analyses indicated that seed head prolificacy and seed set percentage had the highest direct effects on seed yield. The enormous amount of genetic variability of seed yield and related components in Chinese *C. dactylon* demonstrated the potential of the germplasm for developing seeded cultivars. Seed head prolificacy and seed set percentage would be the good indirect predictors for selection of seed yield in early stage of breeding.

INTRODUCTION

Cynodon dactylon (L.) Pers. is widely used for turf, forage, soil stabilization and soil remediation (Harlan, 1970; Taliaferro, 1995, 2003). This species is highly cross-pollinated and self-incompatible (Burton, 1947; 1965). Burton and Hart (1967) reported the open-pollinated seed set of single-cross combinations of six *C. dactylon* plants ranged from 0.5 to 37.0 % in a field experiment in Arizona, while the self-pollinated seed set for the same clones ranged from 0.05 to 2.3% in laboratory. Richardson et al. (1978) reported open-pollination seed set ranging from 2.8 to 43.2% and self-pollinated seed set ranging from 0.10 to 8.09% among eight selected clones. Taliaferro and Lamle (1997) reported that bermudagrass self-pollination resulted in greatly reduced growth rate of pollen tubes with very few reaching the micropyle as compared to cross-pollination. Cluff and Baltensperger (1991) reported that the realized heritabilities for seed yield and its components were from 0.33 to 0.94, and seed yield was partially (42%) determined by additive gene action and could be increased by phenotypic selection methods. From the more than 700 *Cynodon* accessions originally assembled at the Oklahoma Agricultural Experiment Station by Harlan and de Wet (1969), several cold tolerant genotypes were discovered that had good fertility and good seed production potential (Ahring et al., 1974). From their study of single-cross bermudagrass seed production under different management conditions, Ahring et al. (1982) indicated the feasibility of using available germplasm in their collection to produce new single-cross seed propagated cultivars. Sustained efforts of bermudagrass breeding aimed at improvement of seed production and cold adaptation resulted in the release of the first seeded bermudagrass cultivar

'Guymon' in 1982 (Taliaferro et al. 1983). In the subsequent 20 years, the interest in breeding seeded bermudagrass cultivars substantially increased, especially in development of new turf-type cultivars by private seed companies, and dramatic improvement in seed yield and turf quality related traits were achieved (Baltensperger and Klingenberg, 1994; Taliaferro, 2003).

Correlation coefficients have been commonly used to measure the closeness of the relationships of related characteristics in plants. However, a correlation coefficient only furnishes information of relationship between two examined traits without considering multiple associations with other characters. As more variables are considered in correlation analysis, relationships among variables become complex. Path coefficient analysis partitions a correlation coefficient into direct and indirect effects, and permits a critical examination of the specific forces acting to produce a given correlation and measures the relative importance of each variable (Dewey and Lu, 1959). Studies on seed yield and its components and biomass yield and its components (Dewey and Lu, 1959; Board et al., 1997; Das et al., 2004) have demonstrated that path coefficient analysis provides more useful information by dissecting correlation coefficients into direct and indirect effects.

C. dactylon is indigenous to China where it is widely distributed in tropical, subtropical and warm temperate regions. It is most prevalent south of the Yellow river, bit is scattered sparsely in northern parts of China including the far northwest and northeast regions (Anonymous, 1990). There is no published report available on seed yield and seed yield components of Chinese native bermudagrass. The objectives of the present study were to (i) characterize the genetic variability of seed yield and its

components, and (ii) determine relationships among seed yield and its components by phenotypic correlation and path coefficient analysis in Chinese *C. dactylon*.

MATERIALS AND METHODS

The study consisted of two field experiments conducted in 2002 and 2003. In experiment A, 120 Chinese *Cynodon* clonal accessions and four U.S. commercial cultivars as controls were used (Table 1). Experiment B comprised half-sib families from 56 of the 120 clonal accessions and the same four commercial cultivars as in experiment A (Table 1). The Chinese *Cynodon* accessions were collected from eleven provinces of China ranging from tropical Hainan Island to the temperate climate region around Beijing. The two experiments were conducted in neighboring fields on the Oklahoma State University Agronomy Research Station, Efaw Farm, Stillwater.

The experimental design for each of the two field experiments was a randomized complete block with three replications. Plot size was 2.5×2.5 m^2 with 0.5 m alleys between neighboring plots. The soil type was Renfrow loam (a member of the fine, mixed, thermic family of Udertic Paleustolls). Based on the results of soil analyses by the Soil, Water& Forage Analytical laboratory, Plant and Soil Sciences Department, Oklahoma State University, nitrogen and phosphorous fertilizers were incorporated during seed bed preparation prior to planting by applying a 28-28-0 fertilizer mix at a rate of 400 kg ha^{-1} in May 2001. The pH of the soil was 6.0. Plants for the two experiments were started and grown in a greenhouse at the Agronomy Research Station, Oklahoma State University, Stillwater, Oklahoma. Six plants from each clonal accession and each half-sib family accession were started in January 2001 by planting in cells containing Metro-Mix 250 growing medium (Scotts-Sierra Horticultural Products Co., Marysville, OH). Plants of the clonal accessions were started from vegetative propagules and plants of half-sib families were from seed. The plants grew under favorable conditions for

approximately four months in the greenhouse. 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 healthy and trimmed to approximately 6 cm height before transplanting into field plots on June 5 (Experiment A) and June 8 (Experiment B). Greenhouse plants were transplanted to field plots on June 5 and June 8, 2001 for experiments A and B, respectively. Two plants were spaced 1 m apart in the center of each plot. Immediately after transplanting to the field, a pre-emergence herbicide was applied to control annual weeds. Supplemental irrigation was provided during the first 2-weeks to facilitate growth. In August 2001, nitrogen was applied at a rate of 10 g m^{-1} to increase plant growth. Nitrogen fertilizer was applied thereafter on April 30, 2002 and in first week of May 2003 at a rate of 112 kg N ha^{-1}. In early spring of each year prior to the initiation of bermudagrass growth, Roundup® (glyphosate, N-phosphonomethyl glycine), 2,4-D [(2,4-dichlorophenoxy) acetic acid] and a pre-emergence herbicide [Barricade® (prodiamine)] were applied to control weeds. The major winter weeds were henbit (*Lamium amplexicaule*), dandelion (*Taraxicum officinale*) and plantain (*Plantago* spp.). The 0.5 m alleys between plots were cleaned with applications of Roundup as needed during the growing seasons to separate the plots. On March 14, 2002, the plots were burned. In the first week of May 2003, all plots were mowed to 5 cm height. In late August of 2002 and 2003, the above ground biomass of the plots was mowed to a height of 5 cm.

Plant samples and data were collected from both experiments in 2002 and 2003 using identical methods. Seed head prolificacy of each plot was visually assessed in August each year, using a rating scale from one to nine, where one represents no

inflorescence, three a few, five some, seven many, and nine the most abundant on a comparative basis. The number of racemes seedhead^{-1}, raceme length (mm) seedhead^{-1} and number of seed seedhead^{-1} were determined for five random mature seed head samples from each plot. The number of seed seedhead^{-1} was counted under dissecting microscopes after the seed heads were soaked in 15-20% (v: v) bleach solution for four to 12 h. The spikelet number seedhead^{-1} was estimated with a linear formula derived from 212 random inflorescence samples by regressing spikelet number on the raceme length of same seed head: $Y=8.4+0.79X$ ($r^2=0.68$, $P<0.01$). Percent of open-pollinated seed set for a plot was calculated by dividing the number of caryopses seedhead^{-1} by the number of spikelets seedhead^{-1} and multiplying the result by 100 and averaging over the five seedheads from each plot. Seed yield was estimated by harvesting all biomass from a 0.09m^2 area from each plot in August each year. Pure seed per sample was obtained by isolating and hand rubbing the seed heads in each sample, then separating pure seed and inert fractions by screening followed by air separation with a Model B South Dakota seed blower. Seed weight plot^{-1} was determined using an electronic balance.

Among the120 Chinese accessions, 56 half-sib families and four commercial cultivars used in two field experiments, three accessions and two half-sib families did not survive in at least in one plot over the 2001 winter. Accordingly, those entries were not included into the data analysis. Plot means for seed set, seedhead prolificacy, seed number seedhead^{-1}, racemes seedhead^{-1} and raceme length seedhead^{-1} were used for statistical analyses. A randomized complete block design was used with entries as fixed effects, while year and replication were considered as random effects. Log transformation for data in seed yield was used to help correct non-normality for the trait. The PROC

Mixed procedure of SAS (SAS Inst., 1999) was used for analysis of variance (ANOVA) and to estimate variance components. The PROC CORR procedure was used to perform phenotypic correlation (subsequently referred to simply as correlation) analyses. Path coefficient analyses were conducted using standard methods (Fig.1) (Dewey and Lu, 1959; Das et al., 2004).

RESULTS AND DISCUSSIONS

Variability of Seed yield and its components

There were highly significant differences ($P<0.01$) among accessions for all traits in Experiment A (Table 2). For this experiment, effects of year on seed yield and yield components were also significant ($P<0.01$). Genotype × year interactions were significant for all traits examined; therefore, means and ranges for all traits are presented by year in Table 3. Mean seed yield in 2003 was three fold higher than in 2002. Seed head prolificacy, seed set, and seed number seedhead^{-1}were higher in 2003 compared to 2002, while racemes seedhead^{-1} and raceme length seedhead^{-1} were lower in 2003 than in 2002. Ranges of the seed yield components were, however, similar between years.

Interestingly, seed was obtained from six triploids (12260, 12261, 12272, 12282, Tifsport and Tifway), three pentaploids (12348, 12352 and 12365) and seven hexaploids (12317, 12318, 12319, 12320, 12356, 12358 and 12360), except for three triploids (12272, 12282 and Tifsport) in 2002. Although seed was not detected in several seed yield samples (0.9 M^2) of the triploid genotypes, one or more seed was found in all of the seed head samples (5 heads plot^{-1}) used to assess set percentage. Seed set percentages of the triploids ranged from 0.1 to 0.5. Seed yield of the pentaploid accessions was relatively low (8-65 kg ha^{-1}), but all seed yield samples and seed head samples contained seed, with percent seed set ranging from 9.5 to 21.7. For the seven hexaploids, seed yield and seed set percentages were in the ranges of 3.0 to198 kg ha^{-1} and 3.0 to 33.8 %, respectively.

For experiment B, analyses of variance of seed yield and yield components indicated significant differences ($P<0.01$) due to genotypes (Table 4). Effect of year was

significant ($P<0.01$) for seed yield, seed head prolificacy, seed set, and seed number seedhead^{-1}, but not significant for raceme number seedhead^{-1}and raceme length. Genotype × year interactions were significant for seed yield and seed head prolificacy and nonsignificant for other traits measured (Table 4). Therefore, means and ranges for seed yield and seed head prolificacy are presented by year and for the other traits over years in Table 5. Mean seed yield in 2003 was about six fold higher than in 2002. Bermudagrass seed production typically increases after the 1st post establishment year. The results from both experiments clearly indicate enormous variation in seed yield and seed yield components among the Chinese bermudagrass genotypes. The ranges of seed yield and seed set percentage in these experiments were similar to the ranges reported by Ahring et al. (1974, 1982), Richardson et al (1978) and Kenna et al. (1983).

Estimates of variance components and associated standard errors for seed yield, seed head prolificacy, percent seed set, seed number seedhead^{-1}, raceme number seedhead^{-1}and raceme length seedhead^{-1} for experiment A are presented in Table 6. Estimates of genotypic variance (δ^2_G) and genotype × year variance ($\delta^2_{G\times Y}$) for seed yield and five seed yield components were significant ($P<0.01$). Additionally, variance estimates for genotype × block for seed head prolificacy, seed number seedhead^{-1}, raceme number seedhead^{-1}and raceme length seedhead^{-1} were also significant ($P<0.05$ or $P<0.01$). The magnitude of δ^2_G for seed yield was higher than $\delta^2_{G\times Y}$, δ^2_B and $\delta^2_{G\times B}$, but lower than δ^2_Y. These results indicated substantial genetic variability in seed yield among the Chinese bermudagrass genotypes, but also clearly indicated that environmental effects and genotype × environment interactions were large. Variance estimates for seed yield components also indicated large magnitudes of genetic variability

among Chinese bermudagrass genotypes (Table 6). Magnitudes of variance estimates for $\delta^2_{G\times Y}$, δ^2_B, $\delta^2_{G\times B}$, and δ^2_Y relative to that of δ^2_G were larger for seed prolificacy, seed set, and seed number seedhead^{-1}than for raceme number per head and raceme length per head, indicating that the former three traits are more sensitive to environment than the latter.

For experiment B, variance component estimates for seed yield, seed head prolificacy, percent seed set, seed number seedhead^{-1}, raceme number seedhead^{-1}and raceme length seedhead^{-1} are presented in Table 7. Highly significant ($P<0.01$) estimates were obtained for δ^2_G (seed yield and the five seed yield components), $\delta^2_{G\times Y}$ (seed yield, seed head prolificacy, seed set percentage and seed number seedhead^{-1}), and $\delta^2_{G\times B}$ (seed head prolificacy and raceme length seedhead^{-1}). For seed yield, the magnitude of δ^2_G was lower than $\delta^2_{G\times Y}$ and δ^2_Y, indicating substantial effects due to environment and interaction of genotype and environment. As in experiment A, the magnitude of δ^2_G for seed head prolificacy in experiment B was larger than all other main effect variance components, suggesting that this trait was not as sensitive as seed yield to environmental conditions. For seed set percentage and seed number seedhead^{-1}, the variance components attributable to δ^2_Y were larger than those of δ^2_G indicating the importance of environment during time of flowering. For raceme number seedhead^{-1}and raceme length seedhead^{-1}, magnitudes of δ^2_G were larger than those of $\delta^2_{G\times Y}$, δ^2_Y, $\delta^2_{G\times B}$ and δ^2_B, indicating the relatively greater importance of genotype for these traits.

Correlation and path coefficient analysis

Correlation coefficients for seed yield and yield components in experiment A and B are given in Table 8. Correlation coefficients for same pair-wise comparisons were basically similar in both experiments. Seed yield and seed head prolificacy had significantly positive correlations for both experiments. These results indicated that genotypes with more seed heads tended to produce higher seed yields. For both experiments, seed yield was significantly positively correlated with seed set and seed number seedhead^{-1} (Table 8). These results indicated that higher seed set and higher seed number seedhead^{-1} both contributed significantly to increased seed yields. The results are in congruence with those of Kenna et al. (1983) who reported positive and significant correlation ($r=0.52$) between open-pollinated seed set percentage and seed yield. Ahring et al. (1974) also reported that high seed set contributed significantly to seed yield. Correlations of seed yield with raceme number seedhead^{-1} and raceme length seedhead^{-1} were negligible, even though the correlation between seed yield and raceme length in experiment A was significant ($P<0.01$). This indicated that raceme number seedhead^{-1}and raceme length seedhead^{-1}had no substantial effects on seed yield. Ahring et al. (1974) pointed out that the relative numbers of component parts of the inflorescence (i.e., racemes seedhead^{-1}, florets raceme^{-1}, and florets seedhead^{-1}) were apparently of only minor importance in determining seed yield. The highest correlation coefficients among seed yield components was between seed set percentage and seed number seedhead^{-1} in both experiments (Table 8). This indicated that genotypes bearing more seeds seedhead^{-1} had higher fertility. The correlations between raceme length and raceme number

seedhead^{-1} were positive and significant in both experiments. This indicated that it is possible to have higher raceme number and longer racemes in the same genotype. Plants with more racemes per seed head normally had more spikelets, although these two characters did not contribute greatly to seed yield. The correlations of seed head prolificacy with seed set and seeds seedhead^{-1} were low but positive and significant in both experiments.

Path coefficient analysis partitioned correlation coefficients among seed yield and its components into direct and indirect effects (Tables 9 and 10). Seed head prolificacy had the largest direct positive effect on seed yield in both experiments. The magnitudes of these direct effects were not much different from the magnitudes of correlation between these two traits. The path coefficient and correlation analyses both indicated that direct selection for higher seed head prolificacy would indirectly select for higher seed yield in bermudagrass populations having seed production capability. In both experiments, seed set had the second highest direct positive effect on seed yield, again agreeing with correlation analyses that together indicate the importance of seed set as a determinant of seed yield. Correlations between seed number seedhead^{-1} and seed yield were positive in both experiments, however, the direct effects of seed number seedhead^{-1} on seed yield were negligible. This indicated that indirect effects resulted in the positive correlation. It is evident from Tables 9 and 10 that seed number had a high indirect effect on seed yield via seed set. This indicates seed set may be used as a criterion for indirect selection for seed yield. In phenotypic path coefficient analysis, a large residual effect usually indicates that there are traits other than those included in pathways that contribute to the dependent variables (Wang et al., 1999). In the present study, residual effects for

experiments A and B were 0.642 and 0.601, respectively (Table 9 and 10). These large residual effects indicated that there were either other unmeasured traits contributing to seed yield or there was substantial sampling error.

The enormous amount of genetic variability for seed yield and seed yield components among the Chinese *C. dactylon* genotypes in this study supports the findings on genotypes from other origins (Richardson et al., 1978; Harlan and de Wet, 1969). Seed yield is heavily affected by environment and genotype by environment interactions. In this study, average seed yields in 2003 were approximately six times and three times higher than those in 2002 for experiments A and B, respectively. These differences may be due to age of stand, or environmental conditions during the respective flowering periods, or both. Excellent summer seed yields were obtained under dryland conditions followed by a moist and dry weather sequence (Ahring et al., 1974). Ahring et al. (1982) further indicated mild stress and growth promotion cycles resulted in progressive "flushes" of inflorescence production.

In summary, the results of the two field experiments demonstrate the existence of enormous genetic variability in seed yields and seed yield components in the Chinese *C. dactylon* collection. The results from correlation and path coefficient analyses indicate that selection for increased seed head prolificacy and seed set should be the best indirect selection traits for improvement of seed yield.

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Table 1. Identification and origin of 120 Chinese *Cynodon* accessions and four commercial cultivars.

Identification	Origin/ Reference	Ploidy (2n)	Identification	Origin/ Reference	Ploidy (2n)
A12253†	Sichuan	36	A12315	Shanghai	36
A12254†	Sichuan	36	A12316	Shanghai	36
A12255†	Sichuan	36	A12317	Shanghai	54
A12256	Shanghai	27	A12318	Shanghai	54
A12257†	Chongqing	36	A12319	Shanghai	54
A12258†	Chongqing	36	A12320	Shanghai	54
A12259†	Sichuan	36	A12321	Sichuan	36
A12260	Sichuan	27	A12322	Sichuan	36
A12261	Sichuan	27	A12323	Sichuan	36
A12262	Yunnan	36	A12324	Sichuan	36
A12263	Yunnan	36	A12325	Sichuan	36
A12264†	Sichuan	36	A12326	Yunnan	36
A12265†	Sichuan	36	A12327†	Sichuan	36
A12266†	Sichuan	36	A12328†	Sichuan	36
A12267†	Sichuan	36	A12329†	Sichuan	36
A12268†	Sichuan	36	A12330†	Sichuan	36
A12269†	Sichuan	36	A12331†	Sichuan	36
A12270†	Sichuan	36	A12332	Sichuan	36
A12271†	Sichuan	36	A12333†	Sichuan	36
A12272	Sichuan	27	A12334†	Sichuan	36
A12273†	Sichuan	36	A12335†	Sichuan	36
A12274†	Sichuan	36	A12336†	Sichuan	36
A12275†	Sichuan	36	A12337†	Sichuan	36
A12276†	Sichuan	36	A12338	Sichuan	36
A12277†	Sichuan	36	A12339	Sichuan	36
A12278†	Sichuan	36	A12340	Sichuan	36
A12279†	Sichuan	36	A12341	Sichuan	36
A12280†	Sichuan	36	A12342	Sichuan	36
A12281†	Sichuan	36	A12343	Sichuan	36
A12282†	Sichuan	27	A12344†	Sichuan	36
A12283†	Sichuan	36	A12345	Sichuan	36
A12284†	Sichuan	36	A12346	Sichuan	36
A12285†	Sichuan	36	A12347	Sichuan	36
A12286	Sichuan	36	A12348	Hainan	45
A12287	Sichuan	36	A12349	Hainan	36
A12288†	Sichuan	36	A12350†	Guangdong	36
A12289	Sichuan	36	A12351†	Hainan	36
A12290	Sichuan	36	A12352	Hainan	45
A12291†	Sichuan	36	A12353†	Guangdong	36
A12292	Chongqing	36	A12354	Guangdong	36
A12293†	Chongqing	36	A12355	Zhejiang	36

A12294†	Sichuan	36	A12356	Zhejiang	54
A12295	Sichuan	36	A12357	Jiangsu	36
A12296	Sichuan	36	A12358	Jiangsu	54
A12297	Sichuan	36	A12359	Jiangsu	36
A12298	Chongqing	36	A12360	Jiangsu	54
A12299†	Chongqing	36	A12361	Jiangsu	36
A12300	Sichuan	36	A12362	Fujian	36
A12301†	Sichuan	36	A12363	Jiangsu	36
A12302	Sichuan	36	A12364	Zhejiang	36
A12303†	Sichuan	36	A12365	Fujian	45
A12304†	Sichuan	36	A12366	Fujian	36
A12305†	Sichuan	36	A12367	Shandong	36
A12306†	Sichuan	36	A12368	Beijing	36
A12307†	Sichuan	36	A12369	Hainan	27
A12308	Sichuan	36	A12370	Hainan	36
A12309†	Sichuan	36	A12371	Hainan	36
A12310†	Sichuan	36	A12372	Guangdong	36
A12311	Sichuan	36	Tifway	Burton, 1966	27
A12312	Sichuan	36	Tifsport	Hanna, et al., 1997	27
A12313†	Sichuan	36	Midland	Hein, 1953	36
A12314†	Chongqing	36	Tifton 44	Burton and Monson, 1978	36

†Half-sib progenies derived from the accession used for experiment B.

Table 2. Analyses of variance for seed yield (kg ha^{-1}) and five yield components of bermudagrass genotypes in experiment A.

Source	df	Mean squares					
		Seed yield (kg ha^{-1})	Seed head prolificacy	Seed set	Seed number $seedhead^{-1}$	Raceme number $seedhead^{-1}$	Raceme length (mm) $seedhead^{-1}$
Year	1	3950650**	150.33**	26721**	39399**	5.60**	102621**
Block	2	131930*	0.45	21.17	459.79	0.32*	4266.02**
Genotype	120	90461**	16.31**	2558.19**	10078**	0.78**	7616.05**
Genotype × Year	120	44123**	2.61**	382.82**	1763.92**	0.10**	887.50**
Genotype × Block	240	30010	1.26*	216.19	1126.98**	0.08*	632.02*
Residual	240	26180	0.94	180.62	823.90	0.06	481.86

*, ** Indicate significance at the 0.05 and 0.01 levels of probability, respectively.

Table 3. Mean and range of seed yield and its components for experiment A in 2002 and 2003.

Descriptor	2002		2003	
	Mean ± Std	Range	Mean ± Std	Range
Seed yield (kg/ha)	74.44±143.33	0.00-466.30	222.58±249.18	0.00-864.44
Seed head prolificacy	4.43±1.94	1.00-9.00	5.33±2.00	2.00-9.00
Seed set (%)	31.20±23.94	0.00-88.72	43.43±26.22	0.05-96.16
Seed No./ head	67.40±53.34	0.00-206.10	81.74±49.82	0.01-190.50
Racemes/ head	4.68±0.46	3.70-5.70	4.48±0.43	3.50-5.90
Raceme length/head (mm)	217.67±47.48	131.60-345.20	191.98±37.51	119.60-329.40

Table 4. Analyses of variance for seed yield (kg ha^{-1}) and five yield components For the 56 half-sib families in experiment B.

Source	df	Mean squares					
		Seed yield (kg ha^{-1})	Seed head prolificacy	Seed set	Seed number seedhead^{-1}	Raceme number seedhead^{-1}	Raceme length (mm) seedhead^{-1}
Year	1	7732622**	118.40**	54736**	211729**	0.09	389.36
Block	2	88733	7.64*	3519.86**	17008**	0.06	3465.72*
Genotype	58	96648**	12.67**	1131.08**	5075**	0.47**	6578.73**
Genotype ×Year	58	58851**	3.32**	341.68	1730	0.09	854.46
Genotype × Block	116	33829	2.53**	288.65	1655	0.13**	974.09**
Residual	116	31721	0.95	266.16	1238	0.08	600.33

*, ** Indicate significance at the 0.05 and 0.01 levels of probability, respectively.

Table 5. Means and ranges of seed yield and its components for experiment B in 2002 and 2003.

Descriptor	2002		2003	
	Mean ± Std	Range	Mean ± Std	Range
Seed yield (kg/ha)	52.51±77.99	0.00-194.30	344.82 ± 298.62	1.00-1005.92
Seed head prolificacy	4.49 ±1.99	3.00-9.00	5.64±1.93	3.00-9.00

	Over two years	
	Mean ± Std	Range
Seed set (%)	34.79±24.41	0.00-101.96
Seed No. / seed head	72.10±52.46	0.00-270.60
Racemes/ seed head	4.58±0.40	4.10-5.30
Raceme length/seed head (mm)	206.85±42.13	168.40-269.30

Table 6. Estimates of variance components and their associated standard errors for seed yield and its components for 120 clonal accessions.

Descriptor	Variance components					
	δ^2_G	δ^2_{GxY}	δ^2_Y	δ^2_{GxB}	δ^2_B	δ^2_{Res}
Seed yield (kg/ha)	7054.48± 2282.19 **	6183.66± 2084.89 **	10953± 15664	2101.89± 1821.29	474.99± 601.25	25987± 2371.84 **
Seed head prolificacy	2.2431± 0.3587 **	0.5568± 0.1165 **	0.4055± 0.5837	0.1598± 0.0715 *	0.00	0.9411± 0.0857 **
Seed set (%)	362.75± 56.70 **	67.40± 17.64 **	76.36± 109.54	18.04± 13.03	0.00	179.79± 16.65 **
Seed No. Per head	1359.22± 224.98 **	314.47± 81.16 **	106.42± 157.61	157.34± 64.58 **	0.00	817.94± 75.66 **
Racemes Per head	0.1140± 0.0177 **	0.01199± 0.0048 **	0.0170± 0.0244	0.0094± 0.0048 *	0.0011± 0.0014	0.0639± 0.0059 **
Raceme length per head (mm)	1119.82± 169.11 **	132.45± 41.69 **	305.40± 435.26	74.26± 37.32 *	17.1756± 19.8644	484.54± 45.12 **

*, ** Indicate significance at the 0.05 and 0.01 levels of probability, respectively.

Table 7. Estimates of variance components and their associated standard errors for seed yield and its components for 56 half-sib families in experiment B.

Descriptor	Variance components					
	δ^2_G	δ^2_{GxY}	δ^2_{Year}	δ^2_{GxB}	δ^2_{Block}	$\delta^2_{Residual}$
Seed yield (kg/ha)	5477.01± 3769.04 **	9602.52 ± 4054.22 **	42767± 60970	1319.31± 3035.75	488.54± 780.94	31468± 4106 **
Seed head prolificacy	1.3028 ± 0.4128 **	0.7919± 0.2111 **	0.6501 ± 0.9454	0.8005± 0.1785 **	0.0421± 0.0637	0.9436 ± 0.1236 **
Seed set (%)	129.09± 39.78 **	29.2100± 25.2412 **	303.72± 432.37	13.4135± 25.7078	28.0111± 30.4985	264.10 ± 34.47 **
Seed No. / seed head	486.67 ± 181.41 **	184.52± 125.88 **	1166.68 ± 1664.40	218.84± 135.67	133.74± 147.98	1229.61± 160.57 **
Racemes /seed head	0.0588 ± 0.0159 **	0.0026± 0.0068	0.0004± 0.0013	0.0213± 0.0101 **	0.00	0.0826 ± 0.0110 **
Raceme length/seed head (mm)	918.24± 213.49 **	84.4349± 58.9818	0.00	191.05± 75.38	22.6733± 31.0527	597.61± 78.15 **

** Indicate significance at 0.01 level of probability.

Table 8. Phenotypic correlation coefficients among seed yield and seed yield components for 120 *C. dactylon* clonal accessions in experiment A (above diagonal) and 56 half-sib families in experiment B (below diagonal).

Characters	Seed yield	Seed head prolificacy	Seed set	Seed number/ seed head	Racemes /seed head	Raceme length/ seed head
Seed yield	---	0.506**	0.379**	0.346**	0.038	-0.099**
Seed head prolificacy	0.517**	---	0.143**	0.143**	0.226**	0.030
Seed set	0.476**	0.241**	---	0.954**	-0.067	-0.162**
Seed number/ seed head	0.444**	0.198**	0.952**	---	0.108**	0.055
Racemes /seed head	0.034	0.000	0.017	0.186**	---	0.743**
Raceme length/ seed head	-0.014	-0.085	0.014	0.224**	0.720**	---

** Indicate significance at probability of 0.01.

Table 9. Path coefficients of seed yield components indicating direct and indirect effects on seed yield for 120 Chinese *C. dactylon* accessions of experiment A in 2002 and 2003.

	Direct effect	Indirect effect via					Correlation With seed yield
		SP	SS	SN	RN	RL	
Seed head prolificacy (SP)	0.463	--	0.060	-0.017	0.001	-0.001	0.506
Seed set (SS)	0.419	0.066	--	-0.114	0.000	0.007	0.378
Seed number /head (SN)	-0.119	0.066	0.400	--	0.001	-0.002	0.345
Raceme number /head (RN)	0.006	0.105	-0.028	-0.013	--	-0.032	0.038
Raceme Length (RL)	-0.043	0.014	-0.068	-0.007	0.004	--	-0.099

Residual effect=0.642

Table 10. Path coefficients of seed yield components indicating direct and indirect effects on seed yield for 56 *C. dactylon* half-sib families of experiment B in 2002 and 2003.

	Direct effect	Indirect effect via					Correlation With seed yield
		SP	SS	SN	RN	RL	
Seed head prolificacy (SP)	0.427	--	0.089	0.000	0.000	0.001	0.517
Seed set (SS)	0.371	0.103	--	0.002	0.001	0.000	0.476
Seed number /head (SN)	0.002	0.085	0.353	--	0.006	-0.001	0.444
Raceme number /head (RN)	0.032	0.000	0.006	0.000	--	-0.004	0.034
Raceme Length (RL)	-0.006	-0.036	0.005	0.000	0.023	--	-0.014

Residual effect=0.601

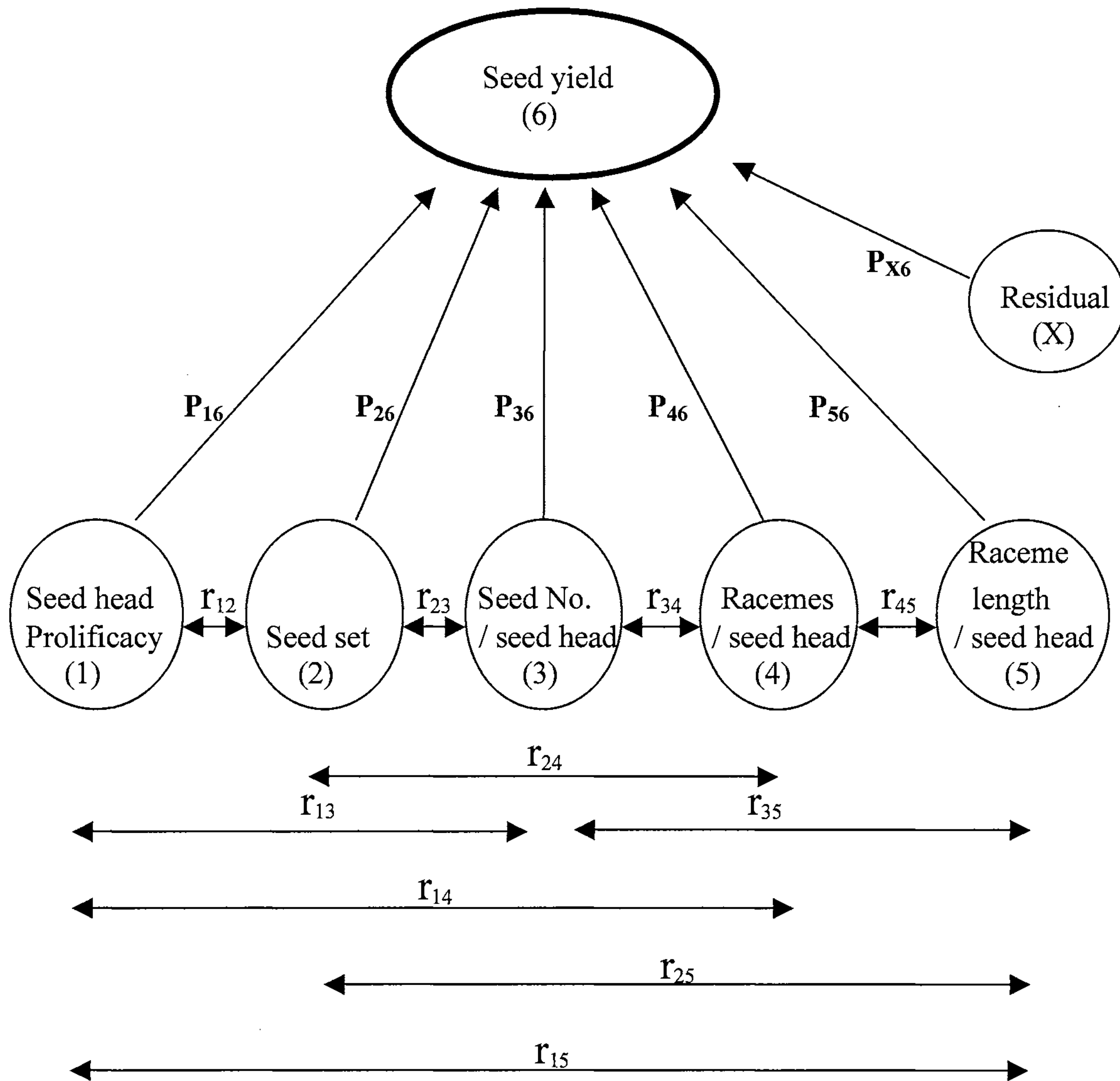

Fig. 1. A path diagram showing cause and effect relationships of five seed yield components and seed yield in bermudagrass. One-directional arrows (⟶) represent direct path (P), and two-directional arrows (⟷) represent mutual correlations.

CHAPTER VII

GENETIC CHARACTERIZATION OF CHINESE *CYNODON* ACCESSIONS BY FIELD EXPERIMENTATIONS:

II. Genetic Variability in Traits Related to Turf

ABSTRACT

Bermudagrasses, *Cynodon* spp., are warm-season, sod-forming, perennial plants widely used for turf in warmer regions of the world. Although bermudagrass is indigenous to and widely distributed in China no information is available on genetic within this pool for characters related to turf performance. Accordingly, two field experiments were conducted in 2002 and 2003 to assess variation among 120 clonal accessions (Experiment 1) and half-sib progeny families of 56 of the 120 clonal accessions (Experiment 2) collected in China. 'Tifway' and 'TifSport' were included in each experiment as standards. Eleven and 12 traits were respectively assessed by visual rating and by measurement in both experiments using a randomized complete block design with three replications. Clonal accessions in Experiment 1 differed significantly ($P<0.05$) for all traits. Half-sib family accessions in Experiment 2 differed significantly ($P<0.05$) for most of the traits. Cluster analysis was used to group the accessions based on similarity of phenotypic traits. Magnitude of genetic variance estimate for each of all traits in the accessions was larger than that in half-sib families. Compared to cv 'Tifway'

and 'Tifsport', the Chinese accessions were higher in ratings for green up, but lower in winter color retention. Seven phenotypic clusters were formed among the accessions, and a core sub-collection of forty-seven accessions was selected based on both the cluster analysis and the diversity of ploidy and geographic origin. The evaluated characters should be improved by classical breeding procedures.

INTRODUCTION

Cynodon dactylon (L.) Pers. var. *dactylon*, common bermudagrass, is ubiquitous and cosmopolitan (Harlan et al., 1970). The taxon is found on every continent and most islands between about 45°N and 45°S latitudes. It penetrates to approximately 53°N in Europe and is found reaching around 3,000 m elevation in South Asia, and below sea level in East Asia and North Africa (Harlan et al., 1969). In warmer regions of the world, the warm-season, sod-forming, perennial grass is widely used for turf, forage, and soil stabilization and remediation (Harlan et al., 1969; Beard, 1973; Taliaferro, 1995; 2003). In the southern U.S., bermudagrass is the principal introduced forage and turfgrass species.

Natural variation within the taxon is enormous, ranging from very small, fine textured plants used as turfgrasses to large, leafy robust plants used as pasture grasses (Harlan et al., 1969). In the U.S., many superior turf bermudagrass cultivars were derived from bermudagrass germplasm imported primarily from Africa during the last century (Taliaferro, 1995; 2003). Juska and Hanson (1964) reported the morphological characteristics related to general turf use of twenty-four bermudagrass cultivars, introductions and selections, and their results provided some indications of the wide range in morphological traits within turf-type bermudagrass. Wofford and Baltensperger (1985), and Coffey and Baltensperger (1989) reported heritability estimates for selected turfgrass characters in bermudagrass. Their results indicated that variation for various morphological and turf performance characters were heritable, and several traits including leaf length and leaf width had moderate to high narrow sense heritability values

(Wofford and Baltensperger, 1985; Coffey and Baltensperger, 1989). However, the full range of genetic variability in *C. dactylon* var. *dactylon* is likely not represented in current germplasm collections and much potential exists to add valuable new germplasm to the collected pool (Taliaferro, 2003). Harlan (1970) noted that little of the enormous variability that exists in the taxon had been used in breeding improvement programs up to that time. The notation remains currently true.

Cynodon dactylon is indigenous to China. The species is widely distributed in the southern provinces, ranging from tropical Hainan Island to a line connecting the northern edge of Sichuan province eastward to Shanghai. *C. dactylon* is also found in northern China, especially in the regions south of the Yellow River (Anonymous,1990). In the far northwest region, *C. dactylon* is found in the southern and northern oasis plains in Xinjiang (Abulaiti et al., 1998). In southwestern China, it is widely distributed including sites at approximately 3000 m in elevation in Yunnan, but only reaches around 1700 m elevation in the western mountainous regions in Sichuan. No information exists on genetic variation for turf related characters in Chinese native bermudagrass. The objectives of the study were to quantify genetic variability for morphological and adaptive characters related to turf performance in Chinese *Cynodon* accessions.

MATERIALS AND METHODS

The plant accessions and experimental design used in this study were the same as described in the previous chapter. Sampling and data collection were in 2001 through 2003, and methods were identical for both field experiments.

The characters examined, year and date of evaluation, and method of data collection in this study are given in Table 1. First year ground coverage, winter color retention, greenup, genetic color, sod density, slime mold, leaf spot and weed abundance were visually rated in field as described in Table 1. Rate of plant establishment (stolon growth), plant height with seedhead and height without seedhead were measured in field. In August of 2002 and 2003, five full-length stems were collected randomly in each plot, and were kept in a plastic bag which was stored at -20°C in a freezer for measurements of morphological traits (Table 1).

The PROC Mixed procedure of SAS (SAS Inst., 1999) was used to perform analysis of variance (ANOVA), and to estimate variance components. Clonal accessions or half-sib family accessions were treated as fixed effects and years and replications were treated as random effects. Plot mean values for all traits were used in the statistic analyses. Arcsine and square root transformation of data in percentages were used for visually estimated estimates of ground coverage and winter-kill. For cluster analyses, standardization of the accession mean for each trait by year in experiment A (clonal accessions) was performed within STAND module, and similarity coefficients among the accessions were calculated using SIMINT module of NTSYS program version 2.0 (Exeter Software, New York, NY). Then cluster analysis was performed according to the

un-weighted pair-group mean algorithm (UPGMA) within SAHN module of the NTSYS program by using the standardized data.

RESULTS AND DISCUSSIONS

Genetic variability of turf related traits in experiment A

Analyses of variance (ANOVA) for 11 visually rated and 12 measured traits in the 117 Chinese *Cynodon* accessions are given in Table 2. Significant differences ($P<0.01$) among genotypes were observed for each of the 23 traits (Table 2). Variation due to years and genotype × year interactions were significant ($P<0.05$) for 16 of 18 traits measured over years. Accordingly, means and ranges of the 16 traits are given by year in Table 3. Standard deviations (Std) and ranges indicated phenotypic variation was enormous for each of the 23 traits (Table 3). Estimates of the variance components and associated standard errors for traits are presented in Table 4. Genotypic variance (δ^2_G) components were significant ($P<0.05$) for all traits. Genotype by year interaction components (δ^2_{GxY}) were significant ($P<0.05$) for all traits except the two leaf blade width traits (i.e.1st leaf blade width and 2nd leaf blade width on 3rd node). Variance component estimates associated with year (δ^2_Y), genotype by block interaction (δ^2_{GxB}) and block (δ^2_B) were not significant. Magnitudes of δ^2_G for winter color retention, genetic color, sod density, winter-kill, greenup I, 2nd internode length, 2nd internode diameter, 3rd internode diameter were smaller than δ^2_{GxY} or δ^2_Y, suggesting large environment effects and genotype by environment interactions for those traits. Accordingly, accurate assessment of these traits requires testing in more than one environment, with emphasis on testing two or more years. Magnitudes of δ^2_{GxY}, δ^2_Y for plant height with seedhead, height without seedhead, greenup II and greenup III, average internode length, 3rd internode length, leaf blade width and leaf blade length were lower

than those of δ^2_G, indicating higher heritability values for those traits. These results are in congruence with those of Wofford and Baltensperger (1985) based on the magnitudes of their reported heritability estimates for leaf length, leaf width, and internode length. Magnitudes of δ^2_G for stolon growth, ground coverage, slime mold and leaf spot, which were measured only in one year, are larger than the summation of δ^2_B and δ^2_{Res}, suggesting high genetic effects on phenotypic variation for those traits. However, slime mold and leaf spot only discernibly occurred in 2002, indicating environmental effects are critical. The occurrence of weeds in significant numbers was only in 2003 because when no control measures were taken. The magnitude of δ^2_G for weed abundance was larger that of δ^2_B, indicating weed abundance in plots largely is decided by genotypes. Weed abundance was greatest in plots of genotypes with a more open sod due either to grown habit or to adaptational weakness (e.g. winter injury).

Means and ranges of visually rated and measured traits for Tifway and Tifsport standard are presented in Table 5. Comparison of the data in Table 5 with that in Table 3 for the Chinese accessions is informative relative to the potential value of the Chinese germplasm in trait improvement. For instance, none of the Chinese accessions retained late season color as well as Tifway or Tifsport. Alternatively, many of the Chinese accessions had earlier spring greenup compared to the two cultivars. Some of the Chinese accessions had darker green color than the two cultivars as indicated in the upper range for genetic color. None of the Chinese accessions had the textural fineness of Tifway and TifSport as indicated by values for leaf blade width and internode diameter.

Genetic variability of turf related traits in Experiment B

The ANOVA mean squares for visually rated and measured characters for the 54 half-sib families are given in Table 6. Significant differences ($P<0.05$) among half-sib families were found for sod density, height with seedhead, winter kill, greenup II, greenup III, average internode length, 2nd internode length, 3rd internode length, 3rd internode diameter, 1st leaf blade width on 3rd node, 1st leaf blade length on 3rd node, 2nd leaf blade width on 3rd node, 2nd leaf blade length on 3rd node, stolon growth rate, slime mold, leaf spot and weed abundance. No significant differences ($P>0.05$) were found among the half-sib families for genetic color, height without seedhead, greenup I, 3rd internode diameter and winter color retention. Significant differences among the half-sib families suggest that additive genetic effects constitute a major portion of the total genetic variation for those traits. Breeding methods that capitalize on additive genetic variation should result in improvement for those traits (Wofford and Baltensperger, 1985). Genotype by year interactions were significant for six and two of the visually rated and physically measured traits, respectively. Means and ranges of means for all traits are presented in Table 7. Standard deviations and ranges of the means for all traits examined in 2001 through 2003 were smaller than those values in experiment A (Table 3), except four descriptors (two internode diameters and two leaf blade lengths) in 2003 in Experiment B (Table 7). These results are in agreement with the previous reported (Wofford and Baltensperger, 1985). The genetic variance component estimates for the 54 half-sib families are given in Table 8. All δ^2_G variance components except that for sod density in Table 8 were smaller than the corresponding values in Table 4. The results are expected since the variance component estimates from the half-sib families were the additive portion of the total genetic variation (Wofford and Baltensperger, 1985).

Cluster Analysis of the Chinese *Cynodon* accessions

Cluster analysis resulted in a dendrogram of nine clusters based on the phenotypic data (Fig. 1). Accessions with co-phenetic distances smaller than the subjective value 1.28 were included in the same cluster. Cluster one consisted of Midland and Tifton-44, both pasture-type cultivars of similar morphology. Cluster four contained Tifsport and Tifway. The Chinese accessions were included in seven different clusters (2, 3, and 5 to 9). Cluster nine had eight distinct sub-groupings. The number of accessions in each of the seven clusters and their geographical origins and ploidy levels are given in Table 9. Tetraploid accessions were grouped into each of the seven clusters, indicating this cytotype to have a greater range of phenotypic variation than the other cytotypes. The three pentaploids were grouped into one cluster indicating the smallest range of phenotypic variation relative to other cytotypes. Hexaploid and triploid Chinese cytotypes grouped into three and two separate clusters, respectively. Phenotypic similarity of the majority of accessions from Sichuan province is indicated by 76 of 117 being included in group nine. The cluster analysis provided a basis for developing a core collection of the Chinese accessions comprised of 47 accessions (Table 10). The accessions in the core from cluster nine include one to five accessions from the cluster or subclusters proportional to the number in respective groupings. Accessions included in the core were selected to represent cytotypes and the various regions of geographic origin. Triploid cytotypes were not included in the core groupings. Since most of the morphological traits are very sensitive to environment and genotype by environment interactions, the core selections would be of high value in regions climatically similar to

Oklahoma. In such environments, phenotypic expression and variability should be similar to the kinds and levels exemplified by respective groupings (Casler, 2001).

In summary, significant genetic variability existed among the clonal and half-sib Chinese *Cynodon* accessions for most traits. Magnitudes of genetic variance for all traits in the clonal accessions was higher than that of half-sib families. Traits indicated as having moderate to high levels of genetic variation as indicated by genetic variance components should respond to selection using traditional breeding methods. Those characters having moderate to high narrow sense heritability values should be improved using classical phenotypic recurrent selection procedures, while characters with low heritability could be improved by traditional breeding procedures with progeny testings. Seven phenotypic clusters were formed among the Chinese *Cynodon* accessions. Tetraploid and pentaploid cytotype accessions contained the largest and smallest amounts of phenotypic variation, respectively. A core sub-collection of 47 accessions was selected from each Chinese *Cynodon* cluster based on the diversity of ploidy levels and geographic origins.

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Table 1. Year and date of data or sample collection, and evaluation methods of traits related to turf performance of Chinese *Cynodon* accessions and half-sib families.

Year	Date for data or sample collection	Character	Method
2001	Aug. 13	Stolon growth (cm)	Length measurements of 10 longest stolons per plot
	Sept. 21	Ground coverage (%)	Visual estimates of ground coverage of plants in a plot
	Dec.03	Winter color retention	1 brown, 9 completely green
2002-2003	Apr.09-Mar.29[†]	Greenup I	1 brown, 9 completely green
	Apr.19-Apr.16[†]	Greenup II	As previously stated
	Apr.30-Apr.27[†]	Greenup III	As previously stated
	Apr.30-May01[†]	Winter kill (%)	Percentage of ground cover damaged
	May 10-May25[†]	Genetic color	1 light green, 9 dark green
	May 12-May25[†]	Sod density	1 least dense, 9 most dense
	Aug.08-Aug.02[†]	Height with seedhead (cm)	Measurements of 5 random plant heights with seedhead per plot
	Aug.09-Aug.02[†]	Height without seedhead (cm)	Measurements of 5 random plant heights without seedhead per plot
	Aug.13-Aug.16[†]	Average internode length (mm)	Measurements on 5 random stems
	” ”	2nd internode length (mm)	” ”
	” ”	3rd internode length (mm)	” ”
	” ”	2nd internode diameter (mm)	” ”
	” ”	3rd internode diameter (mm)	” ”
	” ”	1st leaf blade width (mm) on 3rd node	” ”
	” ”	1st leaf blade length (mm) on 3rd node	” ”
	” ”	2nd leaf blade width (mm) on 3rd node	” ”
	” ”	2nd leaf blade length (mm) on 3rd node	” ”
	Nov.15-Nov.12[†]	Winter color retention	As previously stated
	July 10[‡]	Slime mold	1 no slime mold, 9 most infected
	July 13[‡]	Leaf spot	1 no leaf spot, 9 most infected
	Aug.01[‡]	Weed abundance	1 least weeds, 9 most weeds

[†] Represents the traits were examined both 2002 and 2003, the date before dash (-) for 2002, and after dash (-) for 2003.

[‡] Represents data of the traits were available in one year, slime mold and leaf spot occurred in 2002, and weed in 2003.

Table 2. Analyses of variance for 23 turf related traits of Chinese *Cynodon* accessions in experiment A.

Mean squares for two-year data

Source	df	GC†	SD†	HWSH†	HNSH†	WK†	GU I†	GU II†	GU III†	AINL†	2IL†
Genotype (G)	116	0.9**	2.8**	154.8**	110.6**	0.25**	2.9**	6.2**	7.5**	168.8**	92.3**
Year (Y)	1	277.0**	25.6**	75.7	238.2*	13.16**	65.7**	16.5**	8.4	6094.9**	4254.0**
Rep (R)	2	0.3	14.3**	119.0**	95.7**	0.25**	8.0**	16.8**	15.4**	360.0**	102.1**
G × Y	116	0.5**	1.5**	30.5**	39.7**	0.10**	1.9**	2.2**	2.6**	26.9**	17.5*
G × R	132	0.2	0.4	17.7	15.2	0.02	0.4	0.6	0.7	15.9	10.2
Residual	234	0.2	0.4	17.8	14.3	0.02	0.4	0.6	0.6	17.9	12.9

Mean squares for two-year data (cont.)

Source	df	3IL†	2ID†	3ID†	1LW†	1LL†	2LW†	2LL†
Genotype	116	162.6**	0.08**	0.09**	1.12**	1503**	0.94**	1626**
Year	1	99.2*	10.80**	11.07**	12.97**	40931**	11.11**	52364**
Rep	2	118.4**	0.02	0.01	0.03	839**	0.38	961**
G × Y	116	21.2	0.02*	0.02**	0.20	321**	0.17**	422**
G × R	132	14.2	0.01	0.01	0.21*	179	0.13*	203
Residual	234	17.2	0.01	0.01	0.16	160	0.10	204

Mean squares for three-year data

Source	df	WCR†
Genotype	116	1.24**
Year	2	501.48**
Rep	116	1.60**
G × Y	232	0.81**
G × R	232	0.22
Residual	468	0.22

Mean squares for one year data

Source	df	SGR†	GCR†	SM†	LS†	WD†
Genotype	116	1573.8**	0.27**	9.3**	5.5**	0.43**
Rep	2	2015.5**	0.43**	11.2**	5.7*	0.21
Residual	231	247.8	0.03	1.7	1.2	0.10

†: GC stands for genetic color, SD for sod density, HWSH for height with seedhead (cm), HNSH for height without seedhead (cm), WK for winter kill, GU I for greenup I, GU II for greenup II, GU III for greenup III, AINL for average internode length (mm), 2IL for 2nd internode length (mm), 3IL for 3rd internode length (mm), 2ID for 2nd internode diameter (mm), 3ID for 3rd internode diameter (mm), 1LW for 1st leaf blade width on 3rd node (mm), 1LL for 1st leaf blade length on 3rd node (mm), 2LW for 2nd leaf blade width on 3rd node (mm), 2LL for 2nd leaf blade length on 3rd node (mm), WCR for winter color retention, SGR for stolon growth rate (cm), GCR for ground cover, SM for slime mold, LS for leaf spot, and WD for weed abundance

Table 3. Means and ranges for the 23 turf related traits in experiment A

	2001		2002		2003	
Descriptor	Mean ± Std	Range	Mean ± Std	Range	Mean ± Std	Range
Stolon growth (cm)	88.7±26.4	32.2-202.1				
Ground coverage	0.71±0.24	0.10-1.00				
Winter color retention	1.7±0.7	1.0-4.0	1.1±0.4	1.0-2.0	3.4±0.9	1.0-6.0
Genetic color			6.9±0.5	5.0-8.0	5.6±0.8	3.0-8.0
Sod density			5.2±1.0	3.0-8.0	4.8±1.1	2.0-7.0
Height with seedhead (cm)			32.5±7.0	9.0-65.0	33.1±6.2	18.0-54.0
Height without seedhead (cm)			24.6±6.3	6.0-45.0	25.8±5.6	12.0-46.0
Winter kill			0.59±0.27	0.10-0.99	0.35±0.20	0.03-0.98
Greenup I			2.7±1.4	1.0-6.5	2.1±0.6	1.0-4.0
Greenup II			3.5±1.6	1.0-8.0	3.8±1.1	1.0-7.0
Greenup III			3.9±1.6	1.5-8.5	4.2±1.3	1.0-8.0
Average internode length (mm)			32.4±7.7	16.6-60.5	26.4±5.5	14.5-45.2
2nd internode length (mm)			22.5±5.8	11.2-51.2	17.5±4.3	8.8-39.2
3rd internode length (mm) †			22.7±6.4	11.2-60.8		
2nd internode diameter (mm)			1.03±0.18	0.56-1.65	0.78±0.13	0.39-1.13
3rd internode diameter (mm)			1.04±0.18	0.58-1.65	0.78±0.14	0.36-1.19
1st leaf blade width on 3rd node (mm) †			2.87±0.58	1.76-11.00		
1st leaf blade length on 3rd node (mm)			54.2±21.1	15.8-125.2	69.6±19.8	28.4-135.0
2nd leaf blade width on 3rd node (mm)			3.00±0.47	1.67-4.86	2.74±0.56	1.80-7.66
2nd leaf blade length on 3rd node (mm)			54.8±22.0	15.8-138.2	72.2±21.9	22.0-159.0
Slime mold			4.0±2.1	1.0-9.0		
Leaf spot			3.9±1.6	1.0-9.0		
Weed abundance					3.5±1.8	1.0-9.0

†: Over-year data for mean ± std and range.

Table 4. Estimates of variance components and their associated standard errors for the 23 turf related traits in experiment A.

Descriptor	Variance components					
	δ^2_G	δ^2_{GxY}	δ^2_Y	δ^2_{GxB}	δ^2_B	δ^2_{Res}
Winter color retention	0.05±0.02**	0.20±0.03**	1.43±1.43	0.00±0.00	0.00±0.00	0.22±0.01**
Genetic color	0.08±0.02**	0.08±0.02**	0.79±1.12	0.00±0.02	0.00±0.00	0.23±0.02**
Sod density	0.22±0.07**	0.35±0.07**	0.07±0.10	0.00±0.00	0.06±0.06	0.44±0.03**
Height with seedhead (cm)	21.12±3.55**	4.41±1.48**	0.14±0.33	0.04±1.16	0.45±0.53	17.70±1.64**
Height without seedhead (cm)	11.41±2.64**	9.30±1.93**	0.58±0.99	0.52±0.97	0.34±0.40	14.24±1.32**
Winter kill	0.024±0.006**	0.028±0.005**	0.037±0.053	0.002±0.002	0.001±0.001	0.020±0.002**
Greenup I	0.17±0.08**	0.50±0.08**	0.18±0.26	0.00±0.00	0.03±0.03	0.40±0.03**
Greenup II	0.66±0.14**	0.54±0.10**	0.04±0.07	0.01±0.04	0.07±0.07	0.56±0.05**
Greenup III	0.80±0.17**	0.66±0.12**	0.02±0.03	0.03±0.04	0.06±0.07	0.63±0.06**
Average internode length (mm)	23.65±3.74**	3.32±1.24**	17.86±25.37	0.00±0.00	1.50±1.57	16.88±1.11**
2nd internode length (mm)	12.62±2.07**	1.97±0.80**	12.20±17.32	0.00±0.00	0.43±0.48	11.51±0.76**
3rd internode length (mm)	23.64±3.60**	1.88±0.99*	0.23±0.42	0.00±0.00	0.47±0.54	15.67±1.03**
2nd internode diameter (mm)	0.011±0.002**	0.002±0.001*	0.031±0.045	0.000±0.000	0.000±0.000	0.012±0.001**
3rd internode diameter (mm)	0.012±0.002**	0.002±0.001**	0.032±0.046	0.000±0.000	0.000±0.000	0.012±0.001**
1st leaf blade width on 3rd node (mm)	0.164±0.026**	0.000±0.000	0.039±0.056	0.009±0.011	0.000±0.000	0.175±0.013**
1st leaf blade length on 3rd node (mm)	195.9±34.2**	54.3±15.0**	116.8±166.5	9.7±11.2	3.2±4.0	159.7±14.8**
2nd leaf blade width on 3rd node (mm)	0.142±0.023**	0.012±0.008	0.034±0.048	0.003±0.008	0.001±0.002	0.112±0.011**
2nd leaf blade length on 3rd node (mm)	201.7±37.4**	75.6±19.9**	148.9±212.3	0.0±13.5	3.5±4.4	203.0±18.9**
Stolon growth (cm) †	442.45±69.37**				15.12±17.24	247.8±23.05**
Ground coverage†	0.080±0.012**				0.003±0.004	0.029±0.003**
Slime mold†	2.53±0.41**				0.08±0.10	1.69±0.16**
Leaf spot†	1.40±0.24**				0.04±0.05	1.25±0.16**
Weed abundance†	1.09±0.24**				0.36±0.37	2.02±0.19**

† Traits had one-year data.

*, ** Mean square associated with variance component estimate was significant at the 0.05 and 0.01 probability levels, respectively.

Table 5. Means and ranges for the 23 traits of Tifsport and Tifway by combining data from experiment A and B by year.

	2002		2003	
Descriptor	Mean ± Std	Range	Mean ± Std	Range
Winter color retention	2.2±0.4	2.0-3.0	6.0±0.0	6.0-6.0
Genetic color	5.7±0.5	5.0-6.0	6.2±0.4	6.0-7.0
Sod density	7.0±0.4	6.5-8.0	7.8±0.4	7.0-8.0
Height with seedhead (cm)	27.3±4.4	20.0-33.0	26.7±1.9	25.0-30.0
Height without seedhead (cm)	22.2±6.0	12.0-30.0	19.3±1.0	18.0-20.0
Winter kill	0.86±0.14	0.65-0.98	0.26±0.05	0.20-0.30
Greenup I	1.6±0.6	1.0-2.5	1.7±0.5	1.0-2.0
Greenup II	1.8±0.4	1.0-3.5	4.3±0.5	4.0-5.0
Greenup III	2.4±1.4	1.5-4.5	4.7±0.5	4.0-5.0
Average internode length (mm)	29.5±2.8	25.9-33.1	27.4±1.2	25.3-28.9
2nd internode length (mm)	18.2±2.9	13.8-22.6	11.8±3.2	9.2-17.6
3rd internode length (mm)	21.0±3.9	16.2-25.2	17.1±5.7	10.2-26.2
2nd internode diameter (mm)	0.58±0.14	0.43-0.86	0.47±0.03	0.42-0.52
3rd internode diameter (mm)	0.59±0.17	0.40-0.86	0.44±0.02	0.42-0.46
1st leaf blade width on 3rd node (mm)	2.14±0.16	2.00-2.36	1.92±0.09	1.76-2.02
1st leaf blade length on 3rd node (mm)	47.6±13.9	21.8-62.2	51.0±6.9	40.2-60.0
2nd leaf blade width on 3rd node (mm)	2.08±0.13	1.96-2.34	1.97±0.10	1.80-2.10
2nd leaf blade length on 3rd node (mm)	47.4±12.8	22.6-57.8	53.4±5.7	49.0-63.5
Slime mold	1.8±1.1	1.0-3.0		
Leaf spot	5.0±2.6	2.0-8.0		
Weed abundance			2.0±1.0	1.0-4.0

Table 6. Analyses of variance for 23 turf related traits of 54 Chinese bermudagrass half-sib families in experiment B.

Mean squares for two-year data

Source	df	GC†	SD†	HWSH†	HNSH†	WK†	GU I†	GU II†	GU III†	AINL†	2IL†
Genotype (G)	53	0.68	2.28**	28.79*	21.10	0.086**	1.35	2.44**	2.73**	97.64**	67.90**
Year (Y)	1	182.81**	36.00**	241.96**	627.78**	4.88**	83.01**	1.92	1.49	5038.76**	3793.88**
Rep (R)	2	0.17	0.04	27.63	0.52	0.10*	0.64**	1.07	0.75	29.86	38.94
G × Y	53	0.40**	0.46	13.01	11.16	0.04**	0.78**	0.94**	1.15**	12.03	17.99
G × R	106	0.32*	0.59*	18.60	14.84	0.03*	0.59**	0.57	0.74**	18.08	25.15
Residual	108	0.21	0.40	16.05	11.07	0.02	0.38	0.54	0.42	13.84	22.51

Mean squares for two-year data (cont.)

Source	df	3IL†	2ID†	3ID†	1LW†	1LL†	2LW†	2LL†	WCR†
Genotype	53	62.17**	0.05	0.06*	0.24**	1404**	0.24**	1434**	0.27
Year	1	391.67**	2.45**	3.46**	3.51**	23019**	1.84**	29688**	15.56**
Rep	2	43.92	0.11**	0.16**	0.03	755	0.02	603	0.06
G × Y	53	17.11	0.03**	0.03*	0.06	207	0.06	244	0.24**
G × R	106	24.28*	0.02**	0.03*	0.09**	374**	0.09**	378**	0.12
Residual	108	17.29	0.01	0.02	0.06	175	0.06	190	0.09

Mean squares for one year data

Source	df	SGR†	GCR†	SM†	LS†	WD†
Genotype	116	527.4*	0.08**	5.3**	3.87**	2.15*
Rep	2	52.4	0.02	6.3	1.21	9.38**
Residual	231	357.5	0.04	2.1	2.15	1.31

†: GC stands for genetic color, SD for sod density, HWSH for height with seedhead (cm), HNSH for height without seedhead (cm), WK for winter kill, GU I for greenup I, GU II for greenup II, GU III for greenup III, AINL for average internode length (mm), 2IL for 2nd internode length (mm), 3IL for 3rd internode length (mm), 2ID for 2nd internode diameter (mm), 3ID for 3rd internode diameter (mm), 1LW for 1st leaf blade width on 3rd node (mm), 1LL for 1st leaf blade length on 3rd node (mm), 2LW for 2nd leaf blade width on 3rd node (mm), 2LL for 2nd leaf blade length on 3rd node (mm), WCR for winter color retention, SGR for stolon growth rate (cm), GCR for ground cover, SM for slime mold, LS for leaf spot, and WD for weed abundance .

Table 7. Means and ranges for traits related to turf performance of 54 Chinese bermudagrass half-sib families in experiment B.

	2001		2002		2003	
Descriptor	Mean ± Std	Range	Mean ± Std	Range	Mean ± Std	Range
Stolon growth (cm)	95.4±20.2	58.6-170.8				
Ground coverage	0.75±0.17	0.20-1.00				
Winter color retention	1.7±0.5	1.0-2.0	1.1±0.3	1.0-2.0	3.4±0.8	2.0-6.0
Genetic color			7.2±0.4	6.0-8.0	5.7±0.7	4.0-8.0
Winter kill			0.53±0.21	0.10-0.99	0.30±0.16	0.10-0.80
Greenup I			3.1±1.1	1.0-6.5	2.1±0.4	1.0-3.0
Greenup II			3.9±1.1	1.5-6.5	4.0±0.7	2.0-7.0
Greenup III			4.2±1.2	1.5-7.0	4.1±0.8	2.0-7.0
2nd internode diameter (mm)			1.04±0.16	0.70-1.40	0.86±0.16	0.51-1.20
3rd internode diameter (mm)			1.07±0.16	0.77-1.52	0.86±0.18	0.44-1.26
Slime mold			3.5±1.8	1.0-9.0		
Leaf spot			4.8±1.6	2.0-9.0		
Weed abundance					2.8±1.3	1.0-7.0
			Over years			
			Mean ± Std		Range	
Sod density			5.05±0.9		3.0-8.0	
Height with seedhead (cm)			33.9±4.3		20.0-50.0	
Height without seedhead (cm)			25.8±3.7		9.0-36.0	
Average internode length (mm)			28.4±5.4		11.4-46.0	
2nd internode length (mm)			22.8±5.4		9.0-45.6	
3rd internode length (mm)			23.0±5.2		9.0-45.3	
1st leaf blade width on 3rd node (mm)			2.98±0.32		2.20-4.02	
1st leaf blade length on 3rd node (mm)			57.6±21.2		17.2-137.4	
2nd leaf blade width on 3rd node (mm)			2.97±0.32		2.12-4.00	
2nd leaf blade length on 3rd node (mm)			59.7±21.6		16.2-140.7	

Table 8. Estimates of variance components and their associated standard errors for traits related to turf performance for 54 Chinese *Cynodon* half-sib families in experiment B.

Descriptor	Variance components					
	δ^2_G	δ^2_{GxY}	δ^2_Y	δ^2_{GxB}	δ^2_B	δ^2_{Res}
Winter color retention	0.00±0.00	0.05±0.01**	0.10±0.14	0.02±0.01	0.00±0.00	0.09±0.01**
Genetic color	0.03±0.03	0.06±0.03*	1.14±1.61	0.05±0.03*	0.00±0.00	0.21±0.03**
Sod density	0.27±0.08**	0.02±0.03	0.22±0.31	0.09±0.05*	0.00±0.00	0.40±0.05**
Height with seedhead (cm)	1.70±1.02*	0.00±0.00	1.40±2.11	1.77±1.53	0.08±0.26	15.05±1.68**
Height without seedhead (cm)	1.07±0.88	0.03±0.88	3.81±5.48	1.75±1.25	0.00±0.00	11.07±1.51**
Winter kill	0.006±0.003*	0.007±0.003**	0.029±0.043	0.004±0.002*	0.001±0.001	0.017±0.002**
Greenup I	0.06±0.05	0.13±0.05**	0.51±0.72	0.11±0.05**	0.00±0.01	0.38±0.05**
Greenup II	0.244±0.086**	0.135±0.066*	0.006±0.016	0.017±0.054	0.005±0.010	0.536±0.073**
Greenup III	0.212±0.098*	0.241±0.077**	0.002±0.013	0.157±0.058**	0.000±0.007	0.424±0.058**
Average internode length (mm)	13.26±3.19**	0.00±0.00	31.02±43.99	2.42±1.44*	0.11±0.28	13.24±1.48**
2nd internode length (mm)	7.13±2.27**	0.00±0.00	23.29±33.12	2.06±2.09	0.13±0.36	21.03±2.34**
3rd internode length (mm)	6.31±2.09**	0.00±0.00	2.31±3.42	3.53±1.92*	0.18±0.41	17.23±1.92**
2nd internode diameter (mm)	0.002±0.002	0.006±0.002**	0.015±0.021	0.005±0.002**	0.001±0.001	0.013±0.002**
3rd internode diameter (mm)	0.004±0.002*	0.003±0.002*	0.021±0.030	0.004±0.002*	0.001±0.001	0.018±0.003**
1st leaf blade width on 3rd node (mm)	0.025±0.008**	0.001±0.005	0.021±0.031	0.017±0.007	0.000±0.000	0.056±0.008**
1st leaf blade length on 3rd node (mm)	166.4±46.9**	10.5±15.6	140.8±200.9	99.6±28.4**	3.5±7.0	175.4±23.9**
2nd leaf blade width on 3rd node (mm)	0.026±0.008**	0.000±0.004	0.011±0.016	0.015±0.007*	0.000±0.000	0.056±0.008**
2nd leaf blade length on 3rd node (mm)	167.1±48.1**	18.1±18.0	94.2±29.0**	0.0±13.5	2.1±5.6	189.6±25.8**
Stolon growth rate (cm) †	58.48±37.68				0.00±0.00	351.87±47.88**
Ground coverage†	0.014±0.005**				0.000±0.000	0.039±0.005**
Slime mold†	1.08±0.36**				0.08±0.11	2.08±0.29**
Leaf spot†	0.58±0.27*				0.00±0.00	2.13±0.29**
Weed abundance†	0.28±0.15**				0.15±0.17	1.31±0.18**

† Traits from one year data.

*, ** Mean square associated with variance component estimate was significant at the 0.05 and 0.01 probability levels, respectively.

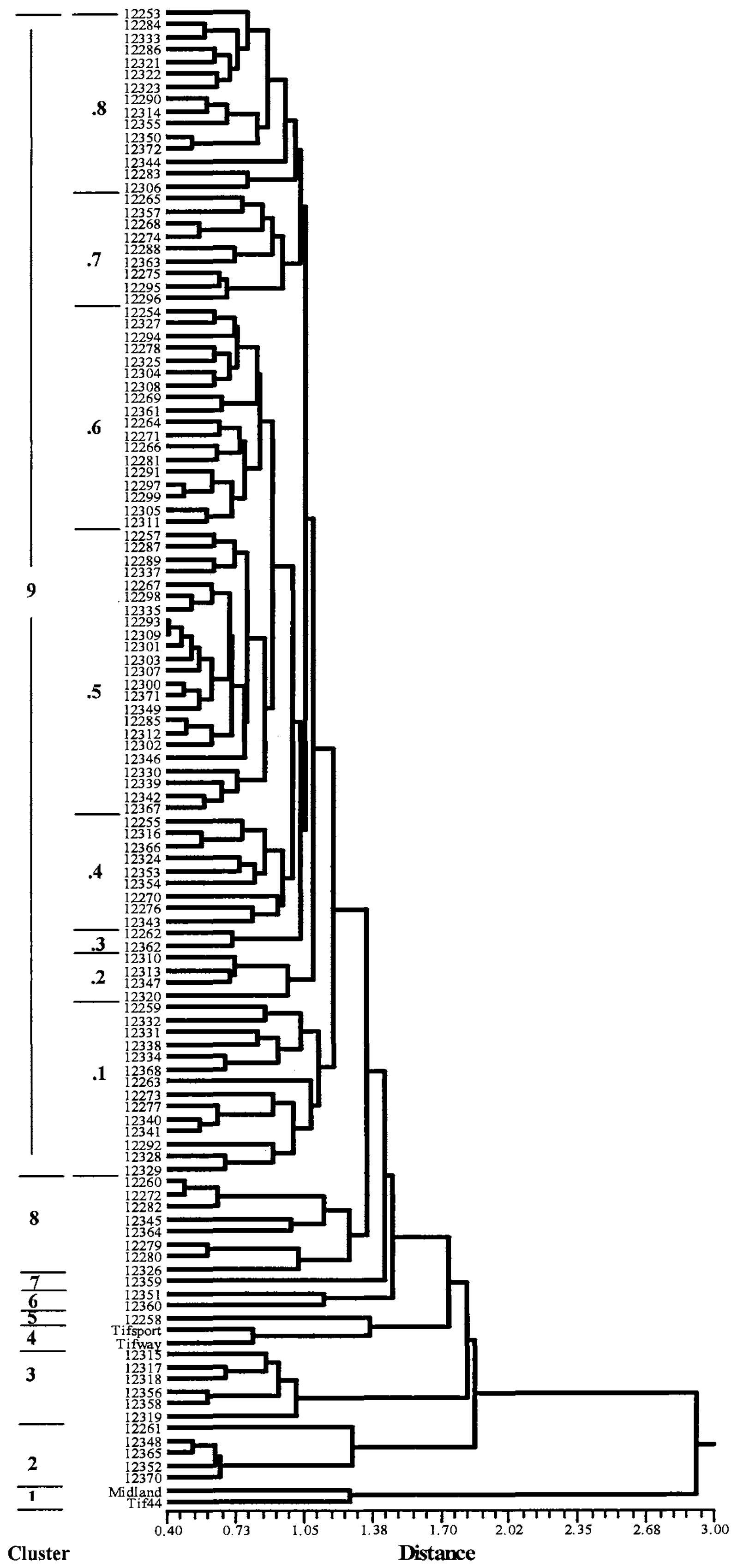
Table 9. Number of Chinese *Cynodon* accessions from 11 provinces and their ploidy levels that Comprising each of the seven phenotypic clusters based on distance coefficient less than 1.28 within a cluster.

	Region†												Ploidy			
Cluster	SC	CQ	YN	SH	HN	GD	ZJ	JS	FJ	SD	BJ	Sum	3x	4x	5x	6x
2	1	0	0	0	3	0	0	0	1	0	0	5	1	1	3	0
3	0	0	0	4	0	0	0	1	1	0	0	6	0	1	0	5
5	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0
6	0	0	0	0	1	0	0	1	0	0	0	2	0	1	0	1
7	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0
8	6	0	1	0	0	0	1	0	0	0	0	8	3	5	0	0
9	68	7	2	4	1	4	3	2	1	1	1	94	0	93	0	1
Sum	75	8	3	8	5	4	4	5	3	1	1	117	4	103	3	7

† SC stands for Sichuan, CQ for Chongqing, YN for Yunnan, SH for Shanghai, HN for Hainan, GD for Guangdong, ZJ for Zhejiang, JS for Jiangsu, FJ for Fujian, SD for Shandong, and BJ for Beijing.

Table 10. Accessions in a core sub-collection of Chinese *C. dactylon.*

Cluster number	Selected accessions for a core subset
2	12261, 12348, 12352, 12370
3	12315, 12317, 12358, 12319
5	12258
6	12351, 12360
7	12359
8	12345,12364, 12280, 12326
9.1	12329, 12292, 12273, 12263, 12368, 12259
9.2	12320, 12310
9.3	12262, 12362
9.4	12343, 12354, 12366, 12255
9.5	12367, 12346, 12371, 12293, 12287
9.6	12311, 12299, 12281, 12361, 12254
9.7	12296, 12363, 12265
9.8	12283, 12350, 12355, 12253
Total	47



12253
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12314
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12350
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12306
12265
12357
12268
12274
12288
12363
12275
12295
12296
12254
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12308
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12260
12272
12282
12345
12364
12279
12280
12326
12359
12351
12360
12258
Tifsport
Tifway
12315
12317
12318
12356
12358
12319
12261
12348
12365
12352
12370
Midland
Tif44
9
.8
.7
.6
.5
.4
.3
.2
.1
8
7
6
5
4
3
2
1
0.40
0.73
1.05
1.38
1.70
2.02
2.35
2.68
3.00
Cluster
Distance

Fig. 1. A phenotypic dendrogram for the Chinese *Cynodon* accessions and four cultivars.

CHAPTER VIII

GENETIC CHARACTERIZATION OF CHINESE *CYNODON* ACCESSIONS BY FIELD EXPERIMENTATIONS:

III. Genetic Variability in Biomass and Trait Relationships

ABSTRACT

Cynodon dactylon is enormously variable and extremely valuable as a forage grass. The taxon is indigenous to and widely distributed from tropical through temperate regions in China. There is no information in the literature on genetic variability for biomass yield in the Chinese *C. dactylon* germplasm. Accordingly, two field experiments were each conducted over 2 years (2002 and 2003) to: 1) characterize the genetic variability for biomass yield in 117 clonal Chinese *Cynodon* accessions (Experiment A) and half-sib families of 54 of the clonal accessions (Experiment B), and 2) characterize the relationships among biomass yield and selected morphological and adaptive characters. A randomized complete block design with three replications was used for both experiments. Differences in biomass yield among the 117 clonal accessions were highly significant ($P<0.01$). Yield differences among half-sib families were significant at $P=0.10$. All quantitatively measured morphological traits were significantly and positively correlated. For traits measured by visual rating, winter kill was positively

correlated with weed prolificacy, but negatively with spring green up. Stepwise selection of regression analyses and path coefficient analyses indicated selection for plant height and adaptive capability in bermudagrass should be the best potential indirect selection traits for increased forage yield.

INTRODUCTION

Cynodon dactylon is enormously variable and extremely valuable as a forage grass for grazing and hay production (Harlan, 1970a). It is widely distributed over all continents and islands between 45° S and 45°N latitudes, where climatic conditions support its survival (Harlan et al., 1970b). Variety *dactylon*, is the cosmopolitan and ubiquitous taxon, and provides the most important forage resources in the species (Harlan, 1970a).

There is enormous genetic variability available in bermudagrass (Kneebone, 1973). The variability within *C. dactylon* var. *dactylon* is thought to have resulted from its interaction with two taxonomic varieties, *C. dactylon* var. *aridus* and var. *afghanicus* (Harlan and de Wet, 1969). Burton (1947) reported data from extensive evaluations of hay yield, sod density, head abundance, frost resistance, disease resistance, vigor, color, percentage weeds, and percentage cover on 147 selections of 5,000 plants derived from intercrosses of three clonal plants identified as 'Tift' and two tall-growing bermudagrass strains from South Africa. He found extensive variation among the 147 selected clonal plants for all variables evaluated (Burton, 1947). A positive correlation was observed between hay yield in first three months post establishment with the total hay yield for 4 years, while there was no association between the total greenhouse yields and field yields (Burton, 1947). In the same study, a correlation coefficient of 0.80 between first year hay yield and the four-year total yield indicated the hay yields for the first year after establishment should give an excellent index of later yield performance (Burton, 1947). De Silva (1991) reported significant amounts of genetic variation for shoot dry weight

among populations collected from five climatic zones (dry, arid, intermediate, wet and hill country) in Sri Lanka. Avis et al. (1980) reported significant genotype × environment interactions in forage yield in bermudagrass indicating the necessity to use multiple environment testing through time (years) and space (locations) to characterize relative genotypic differences. Development of superior cultivars for livestock grazing and hay production started in 1940's in the USA (Burton, 1947). Bermudagrass is grown on an estimated 10 to 12 million hectares in the Southern USA for these purposes (Taliaferro et al., 2004). However, only a tiny fraction of the total germplasm has been studied and even less utilized (Harlan, 1970a).

Cynodon dactylon is indigenous to China and widely distributed from tropical Hainan Island to northern temperate regions. This wide distribution probably suggests large variation in Chinese native *C. dactylon* germplasm. However, genetic variability for biomass yield in Chinese *Cynodon* germplasm has not been documented in the literature. The objectives of the present study were to: 1) characterize genetic variability for biomass yield among 120 clonal *Cynodon dactylon* var. *dactylon* accessions collected from a wide geographic expanse in China and half-sib progenies from 56 of the clonal accessions, and 2) to analyze the relationships among the biomass yield and a number of morphological and agronomic traits of the Chinese *Cynodon* collections.

MATERIALS AND METHODS

The study included two field experiments as described in Chapter VI. Briefly, 120 Chinese clonal accessions and four commercial cultivars were included in experiment A, while half-sib families from 56 of the 120 accessions were included in experiment B. The field plot design for both experiments was a randomized complete block with three replications. Experiments A and B were established June 5 and 8, 2001, respectively, and fertility and cultural management was as described in Chapter VI.

Samples and data were collected using identical methods in both 2002 and 2003. Biomass samples were harvested in every plot by hand clipping a 0.3×0.3 m^2 area. The biomass samples were dehydrated at 55°C (130 °F) in forced air ovens for 72 hr. The biomass yield (ton ha^{-1}) was calculated from the dried sample weight by multiplying a coefficient of 0.1111. Stolon growth rate, height with seedhead, height without seedhead, average internode length, 2nd internode length, 3rd internode length, 2nd internode diameter, 3rd internode diameter, 1st leaf blade width on 3rd node, 1st leaf blade length on 3rd node, 2nd leaf blade width on 3rd node, 2nd leaf blade length on 3rd node were measured on five shoots samples from each plot, while first year coverage, spring greenup I, spring greenup II, spring greenup III, winter kill, sod density, slime mold, leaf spot and weed abundance were visually rated for each plot as described in Table 1 in Chapter VII.

Among the120 Chinese accessions, 56 half-sib families and four commercial cultivars, three accessions and two half-sib families did not survive in at least one plot over 2001-2002 winter. Accordingly, those entries were not included in the data analysis. The PROC Mixed procedure of SAS (SAS Inst., 1999) was used for analysis of variance (ANOVA) and to estimate variance components. Entries were considered as fixed effects,

while year and replication were considered as random effects. The PROC CORR procedure was used to perform phenotypic correlation analyses. All analyses were done on a plot mean basis. Path coefficient analyses were performed using standard methods (Fig.1) (Dewey and Lu, 1959; Das et al., 2004), after stepwise selection procedures using regression analysis.

RESULTS AND DISCUSSION

Variability of biomass yield

Dry biomass yield differences were significant ($P<0.01$) among the 117 clonal accessions in experiment A, but did not differ significantly ($P>0.05$) among the 54 half-sib families in experiment B (Table 1). Effects of year and block for biomass yield were significant ($P<0.01$) for both experiments. The genotype × year interaction for biomass yield was significant in experiment A but not in experiment B. Mean dry biomass weight for the Chinese accessions and 'Midland' and 'Tifton 44' and their relative ranks are presented in Table 2. The different order of rankings among the accessions in experiment A for the 2 years further confirmed the genotype × environment interaction. The mean biomass yield (ton ha^{-1}) for experiment A (Table 2) was 12.9±4.3, ranging from 4.9 to 23.1 in 2002, while the mean in 2003 was 11.8±3.1, ranging from 7.2 to 17.3. The mean and standard deviation of biomass yields in experiment B over the two years was 12.4±3.4, ranging from 3.58 to 21.3. The biomass yields of Midland and Tifton 44 were within the range of the Chinese *Cynodon* accessions for both years. Their intermediate ranking within this range indicated that many of the higher yielding Chinese accessions have potential for use in breeding higher yielding cultivars. Estimates of variance components and associated standard errors for biomass yield for both experiments are given in Table 3. The magnitude of δ^2_G was higher than δ^2_{GxY}, δ^2_Y, δ^2_{GxB} and δ^2_B in experiment A, but lower than δ^2_Y and δ^2_{GxB} in experiment B. The results suggest considerable genetic variability among the 117 accessions, but also indicated that the magnitude of environment effects was large for both experiments. Avis et al. (1980) found considerable genotype by environment (year and location) interactions in

bermudagrass multi-environment tests. The results of the present study strongly support their conclusion that it is necessary to characterize relative genotypic differences in multiple environment testing conducted through time and space (Avis et a., 1980).

Correlations among morphological, adaptive and agronomic traits

Correlation coefficients for 25 morphological, adaptive, and agronomic characters in experiment A are presented in Table 4. Highly positive correlations were found between the two plant heights (HWSH and HNSH), two leaf blade widths (LW I and LW II), two leaf blade lengths (LL I and LL II), two internode diameters (2nd ID and 3rd ID), and among the three greenup variables (I, II and III) and three internode lengths (AIL, 2nd IL and 3rd IL). The results clearly indicate those characters were highly uniform and stable in mature stands, and indicated the predictive capability of the correlated traits, one for the other.

Stolon growth rate (vigor) had significantly positive correlations with first year ground coverage, plant height, stem internode length, internode diameters, leaf blade width, and leaf blade length, and weed prolificacy, but negative correlations with two winter color retentions (WCR I and WCR II) and leaf spot occurrence. The results indicated that large plants grow faster, had better first year ground coverage, and had less leaf spot (*Bipolaris cynodontis*) disease infection. The results also suggested that the faster growing plants had poor winter color retention and more weed infestation. This probably is because most of the faster growing plants were collected from tropical and southern subtropical regions. Winter color retention in November (WCR I) had substantial positive correlation with sod density, internode diameter and leaf blade width. However, color retention in December (WCR II) had substantially greater positive

correlations with winter kill and weed prolificacy, and greater negative correlations with spring green up. The results suggested that more poorly adapted accessions had good color in December, but more winter kill and consequently poorer spring green up. Plant height was positively correlated with other morphological traits such as leaf blade width, leaf blade length, and internode diameter and length. The results indicate that tall plants tend to have larger leaf blades and coarse and long internodes. No significant correlations were found involving slime mold and leaf spot occurrence, indicating that simultaneous selection for resistance may be possible. Biomass dry weight was positively correlated with stolon growth rate, first year ground coverage, genetic color, plant height, spring greenup, internode length, internode diameter, and leaf blade width and length, but negatively correlated with December winter color, leaf spot occurrence, winter kill rate.

Path coefficients analysis

Spring greenup I, 2nd internode diameter, leaf spot occurrence, slime mold occurrence, height without seed head, height with seed head and 2nd leaf blade length on 3rd stem node, were selected and accounted for 48% of the total variation of biomass yield by using selection procedures of multiple regression at the 0.05 probability level (Table 5). Path coefficients for biomass yield and the seven selected characters are given in Table 6. A positive and direct effect of plant height without seedhead on biomass yield was observed (Table 6), indicating that it is a good predictor of biomass yield. The correlation coefficient of plant height without seed head vs. biomass yield was intermediate (0.375) because it was significantly correlated with leaf blade length, leaf blade width, stem internode diameter, internode length, and height with seed head. The latter characters contributed to biomass yield via plant height without seed head; hence

they had either negative or low positive effects (Table 6). Greenup I, leaf spot, and slime mold occurrence were not highly associated with morphological traits, because their direct effects were respectively close to their total effects on biomass yield. The direct path coefficient of Greenup I with biomass yield was substantial (Table 6). It is evident that plants with early green up have a longer growth period resulting in more dry matter accumulation. The results also indicated the plants with early green up had good adaptive capability. It is obvious that if bermudagrass accessions are adapted and tall growing, they will, on the average, be relatively high yielding. The path direct coefficients and correlation coefficients of leaf spot and slime mold occurrences with biomass yield were intermediate, but consistent. This result indicates those two diseases can be important negative agents resulting in reduced forage yields in bermudagrass. *Bipolaris cynodontis* (Marig.) Shoem. can cause serious diseases on leaf, crown and root tissues of bermudagrass, and weaken and even kill stands of susceptible bermudagrass plants (Taliaferro et al., 2004). Slime mold fungi may shade individual grass leaves to the extent that plants may be weakened by inefficient photosynthesis (Smiley et al., 1993).

The enormous amount of genetic variability in the biomass yields of the Chinese *Cynodon* clonal accessions is congruent with the findings from many other studies (Burton, 1947; Avis et al., 1980; Harlan, 1970a, b; Harlan et al., 1969; de Silva, 1991). The genetic variability for biomass in experiment B is significant only at the level of 0.10, probably because our sampling area was small (0.09 m^2 per plot). Biomass yield is also heavily influenced by environment and genotype by environment interactions. The direct positive effect of plant height to biomass yield indicated it to be best trait for

indirect selection for high biomass yield in populations well adapted to a specific environment.

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Table 1. Analyses of variance for dry biomass yield of 117 accessions in experiment A and 54 half-sib families in experiment B.

Source	Experiment A		Experiment B	
	df	Mean square	df	Mean square
Year	1	218.70**	1	396.95**
Block	2	202.63**	2	84.79**
Genotype	116	34.76**	53	16.71
Genotype × Year	116	12.30**	53	8.71
Genotype × Block	232	9.27	106	1.22*
Residual	231	7.66	105	7.35

*, ** Indicate significance at the 0.05 and 0.01 levels of probability, respectively.

Table 2. Mean dry biomass yield (tons ha^{-1}) and relative rank for 117 Chinese *Cynodon* accessions and four cultivars in experiment A.

Identification	2002	Rank	2003	Rank	Avg. 2-yr	Rank
12314	23.1	1	15.7	7	19.4	1
12350	20.7	2	15.5	11	18.1	2
12264	20.5	3	14.1	18	17.3	4
12355	19.1	4	15.8	5	17.5	3
12292	18.3	5	16.1	4	17.2	5
12321	17.8	6	13.7	22	15.7	11
12273	17.4	7	15.6	9	16.5	6
12318	17.3	8	14.9	14	16.1	9
12290	17.2	9	15.6	8	16.4	8
12357	17.1	10	13.9	19	15.5	13
12284	16.9	11	11.3	68	14.1	23
12333	16.8	12	11.9	60	14.3	21
12299	16.7	13	12.1	55	14.4	19
12323	16.7	14	12.6	37	14.6	17
12306	16.3	15	10.5	84	13.4	38
12296	16.2	16	15.8	6	16.0	10
12349	16.0	17	11.1	72	13.6	33
12253	16.0	18	13.5	26	14.7	16
12372	15.9	19	16.9	3	16.4	7
12320	15.9	20	12.4	43	14.1	24
12269	15.8	21	11.2	71	13.5	35
12286	15.7	22	15.2	12	15.4	15
12265	15.4	24	15.6	10	15.5	14
12348	15.3	25	7.7	119	11.5	74
12319	15.3	26	12.8	34	14.1	25
12371	15.3	27	9.4	100	12.3	60
12291	15.1	28	10.1	92	12.6	55
12303	15.1	29	13.6	23	14.4	20
12310	15.1	30	13.9	20	14.5	18
12344	15.0	31	12.5	39	13.8	29
12281	15.0	32	10.8	81	12.9	53
12289	14.9	33	12.2	49	13.5	34
12370	14.7	34	9.7	97	12.2	61
12337	14.6	35	12.6	36	13.6	32
12297	14.6	36	11.3	70	13.0	47
12313	14.5	37	9.2	105	11.8	69
12358	14.4	38	12.5	41	13.5	36
12328	14.4	39	12.5	38	13.4	37
12363	14.3	40	12.0	59	13.2	40
12317	14.3	41	13.3	30	13.8	28
12360	14.2	42	17.2	2	15.7	12
12288	14.2	44	14.1	17	14.2	22
12302	14.2	44	12.3	45	13.3	39
12295	14.2	45	13.2	31	13.7	30
12293	14.1	46	12.2	51	13.1	41
12301	13.8	47	14.2	15	14.0	26
12356	13.8	48	12.3	48	13.0	45
12345	13.7	49	9.1	106	11.4	78
12267	13.7	50	12.1	57	12.9	51
12277	13.7	51	12.1	54	12.9	49
12365	13.6	52	8.8	109	11.2	82
12261	13.6	54	12.1	52	12.9	52

12338	13.6	54	9.3	102	11.4	77
12340	13.6	55	12.2	50	12.9	48
12305	13.5	56	9.6	99	11.5	73
12312	13.5	57	11.3	69	12.4	58
12285	13.4	58	10.2	90	11.8	70
12283	13.3	59	12.5	42	12.9	50
12331	13.2	60	9.6	98	11.4	79
12271	13.1	61	14.2	16	13.6	31
12322	13.0	62	13.1	33	13.1	44
12298	13.0	63	14.9	13	13.9	27
12270	12.7	65	11.6	65	12.1	63
12352	12.6	66	12.3	46	12.5	56
12311	12.6	67	8.0	118	10.3	97
12335	12.6	68	13.3	29	13.0	46
12255	12.5	69	13.7	21	13.1	42
12275	12.4	70	11.9	61	12.1	65
12268	12.2	71	12.1	56	12.1	64
12351	12.1	72	10.9	78	11.5	75
12324	12.1	73	13.5	24	12.8	54
12309	12.0	74	12.1	53	12.1	66
12266	12.0	75	11.3	67	11.7	71
12307	11.8	76	11.0	77	11.4	80
12294	11.7	77	8.6	111	10.1	100
12325	11.6	78	12.3	47	12.0	68
12276	11.6	79	10.4	87	11.0	86
12316	11.2	80	10.6	83	10.9	87
12361	11.2	81	10.9	79	11.0	85
12342	11.1	82	9.7	96	10.4	96
12330	11.1	83	10.2	89	10.7	92
12339	11.1	84	8.6	113	9.8	102
12300	11.0	85	9.8	95	10.4	95
12287	11.0	86	13.1	32	12.0	67
12347	10.9	87	10.5	85	10.7	91
12315	10.9	88	11.8	62	11.3	81
12329	10.9	89	13.4	27	12.2	62
12359	10.9	90	8.3	116	9.6	104
12254	10.8	92	10.8	80	10.8	89
12274	10.8	92	11.4	66	11.1	83
12354	10.8	93	12.3	44	11.6	72
12332	10.5	94	8.7	110	9.6	105
12334	10.3	95	8.0	117	9.1	109
12364	10.3	96	10.7	82	10.5	94
12259	10.2	97	11.1	75	10.7	93
12282	9.8	98	10.3	88	10.0	101
12367	9.8	99	7.2	120	8.5	113
12341	9.6	100	9.9	93	9.8	103
12263	9.6	101	9.2	104	9.4	106
12366	9.5	102	13.5	25	11.5	76
12308	9.4	103	12.7	35	11.1	84
12343	9.3	104	12.1	58	10.7	90
12304	9.3	105	11.1	74	10.2	98
12257	9.2	106	12.5	40	10.9	88
12346	9.2	107	8.6	112	8.9	111
12272	8.8	108	7.2	121	8.0	118
12262	8.7	109	11.6	63	10.2	99
12278	8.4	110	9.2	103	8.8	112

12260	8.0	111	8.4	115	8.2	115
12258	7.6	113	17.3	1	12.5	57
12368	7.6	114	8.5	114	8.1	117
12327	7.4	115	11.0	76	9.2	108
12280	7.1	116	9.9	94	8.5	114
12353	7.0	117	11.6	64	9.3	107
12362	6.0	118	9.1	107	7.6	119
12326	5.1	119	11.1	73	8.1	116
12279	4.9	120	9.0	108	6.9	121
Mean	12.9		11.8		12.4	
Midland	15.4	23	9.3	101	12.4	59
Tif44	12.8	64	13.3	29	13.1	43
Tifsport	3.8	121	10.4	86	7.1	120
Tifway	7.9	112	10.2	91	9.1	110
LSD0.05	5.19		3.96			
LSD0.01	6.84		5.22			

Table 3. Estimates of variance components and their associated standard errors for biomass for 117 accessions in experiment A and 54 half-sib families in experiment B.

	Variance components					
	δ^2_G	δ^2_{GxY}	δ^2_Y	δ^2_{GxB}	δ^2_B	δ^2_{Res}
Experiment A	3.48±0.83**	1.54±0.59**	0.60±0.90	0.80±0.56	0.85±0.89	7.67±0.71**
Experiment B	0.85±0.67	0.45±0.66	2.40±3.47	1.44±0.86*	0.69±0.78	7.35±1.00**

*, ** Indicate significance at the 0.05 and 0.01 levels of probability, respectively.

Table 4. Phenotypic correlation coefficients among 25 morphological, adaptive and agronomic traits for the Chinese *Cynodon* accessions in experiment A.

		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
1	DW†	--																								
2	ASL	.25 **	--																							
3	WCRI	.01	-.42 **	--																						
4	WCRII	-.24 **	-.31 **	.17	--																					
5	CR	.29 **	.78 **	-.51 **	-.39 **	--																				
6	GC	.23 **	.06	.05	-.22 *	.11	--																			
7	SD	.02	-.17	.55 **	-.30 **	-.17	.20 *	--																		
8	HWH	.29 **	.45 **	-.10	-.32 **	.31 **	.13	-.07	--																	
9	HNH	.38 **	.48 **	.00	-.25 **	.30 **	.12	-.07	.94 **	--																
10	SM	-.13	.04	-.16	-.20 *	.10	.17	.03	.36 **	.25 **	--															
11	LS	-.24 **	-.22 *	-.01	.27 **	-.04	-.25 **	-.29 **	-.20 *	-.20 *	-.14	--														
12	WK	-.38 **	.10	-.17	.58 **	-.09	-.18 *	-.37 **	-.26 **	-.20 *	-.22 *	.12	--													
13	GPIII	.35 **	-.01	.13	-.63 **	.17	.16	.37 **	.32 **	.26 **	.26 **	-.13	-.95 **	--												
14	GPII	.37 **	-.02	.13	-.58 **	.14	.15	.36 **	.32 **	.26 **	.20 **	-.16	-.95 **	.98 **	--											
15	GPI	.45 **	.06	-.02	-.52 **	.28 **	.15	.18 *	.33 **	.29 **	.20 *	-.07	-.87 **	.90 **	.90 **	--										
16	AIL	.23 *	.45 **	.10	-.11	.22 *	-.05	-.13	.79 **	.85 **	.09	-.10	-.03	.11	.10	.13	--									
17	2nd IL	.16	.42 **	.06	.02	.11	-.15	-.23 **	.66 **	.73 **	.07	-.15	.16	-.09	-.10	-.08	.89 **	--								
18	3rd IL	.17	.49 **	.08	.00	.15	-.10	-.12	.70 **	.77 **	.09	-.22 **	.15	-.07	-.08	-.06	.91 **	.96 **	--							
19	2nd ID	.33 **	.31 **	-.30 **	-.18 *	.25 **	.22 *	-.51 **	.40 **	.38 **	-.08	-.04	-.05	.05	.05	.16	.34 **	.31 **	.25 **	--						
20	3rd ID	.30 **	.30 **	-.33 **	-.17	.26 **	.20 *	-.54 **	.40 **	.36 **	-.05	-.04	-.03	.04	.03	.15	.33 **	.33 **	.26 **	.98 **	--					
21	LWI	.16	.54 **	-.31 **	-.30 **	.41 **	.06	-.26 **	.66 **	.61 **	.21 *	-.17	-.03	.14	.12	.17	.60 **	.58 **	.58 **	.60 **	.61 **	--				

22	LLI	.17	.48 **	.09	-.15	.23 *	.03	.05	.70 **	.76 **	.16	-.19	-.05	.13	.12	.14	.79 **	.74 **	.80 **	.19 *	.16	.57 **	--			
23	LWII	.14	.58 **	-.32 **	-.28 **	.41 **	.04	-.28 **	.65 **	.61 **	.21 *	-.17	.01	.08	.06	.11	.61 **	.61 **	.61 **	.62 **	.62 **	.95 **	.59 **	--		
24	LLII	.17	.45 **	.09	-.15	.21 *	.04	.03	.69 **	.76 **	.15	-.17	-.06	.15	.13	.17	.79 **	.72 **	.78 **	.20 *	.17	.55 **	.99 **	.56 **	--	
25	WD	-.10	.25 **	-.40 **	.68 **	.15	-.23 *	-.54 **	-.12	-.09	-.19 *	.19 *	.65 **	-.65 **	-.60 **	-.46 **	.02	.13	.14	.09	.10	.04	.08	.09	-.09	--
		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

† DW: dry weight, ASL: average stolon length, WCR I: winter color retention in November, WCR II: winter color retention in December, CR: ground coverage in October 2001, GC: genetic color, SD: sod density, HWH: plant height with seed head, HNH: plant height without seed head, SM: slime mold, LS: leaf spot, WK: winter kill, GP (I, II, III): greenup, AIL: average internode length, 2nd IL: 2nd internode length, 3rd IL: 3rd internode length, 2nd ID: 2nd internode diameter, 3rd ID: 3rd internode diameter, LWI: 1st leaf blade width on third node, LLI: 1st leaf blade length on third node, LWII: 2nd leaf blade width on third node, LLII: 2nd leaf blade length on third node, WD: weed prolificacy.

* and ** indicate significant level at 0.05 and 0.01, respectively.

Table 5. Stepwise selection of dry weight components by a regression model at the significant level of 0.05.

Step	Component	Partial R^2	Accumulative R^2	P value
1	Green up I	0.20	0.20	<0.0001
2	2nd internode diameter	0.07	0.27	0.0012
3	Leaf spot occurrence	0.04	0.31	0.0103
4	Slime mold occurrence	0.05	0.36	0.0036
5	Height without seed head	0.04	0.40	0.0062
6	Height with seed head	0.05	0.45	0.0012
7	2nd leaf blade length on 3rd node	0.03	0.48	0.0150

Table 6. Path coefficients of biomass dry weight components indicating direct and indirect effects on biomass dry weight for Chinese *Cynodon* accessions of experiment A.

	Direct	Indirect via							Total
	effect	GP I	2ID	LS	SM	HNSH	HWSH	LL II	effect
Green up I (GP I)	0.417	----	0.029	0.015	-0.036	0.338	-0.273	-0.044	0.446
2nd internode diameter (2ID)	0.184	0.066	----	0.009	0.014	0.446	-0.338	-0.052	0.328
Leaf spot Occurrence (LS)	-0.213	-0.029	-0.008	----	0.025	-0.231	0.171	0.045	-0.239
Slime mold occurrence (SM)	-0.178	0.083	-0.014	0.029	----	0.289	-0.298	-0.039	-0.127
Height without Seed head (HNSH)	1.178	0.120	0.070	0.042	-0.044	----	-0.789	-0.201	0.375
Height with seed head (HWSH)	-0.836	0.136	0.074	0.044	-0.064	1.112	----	-0.182	0.285
2nd leaf length on 3rd node (LL II)	-0.264	0.070	0.036	0.036	-0.026	0.895	-0.575	----	0.172

#2

VITA

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Doctor of Philosophy

Thesis: GENETIC CHARACTERIZATION OF *CYNODON* ACCESSIONS BY MORPHOLOGY, FLOW CYTOMETRY AND DNA PROFILING

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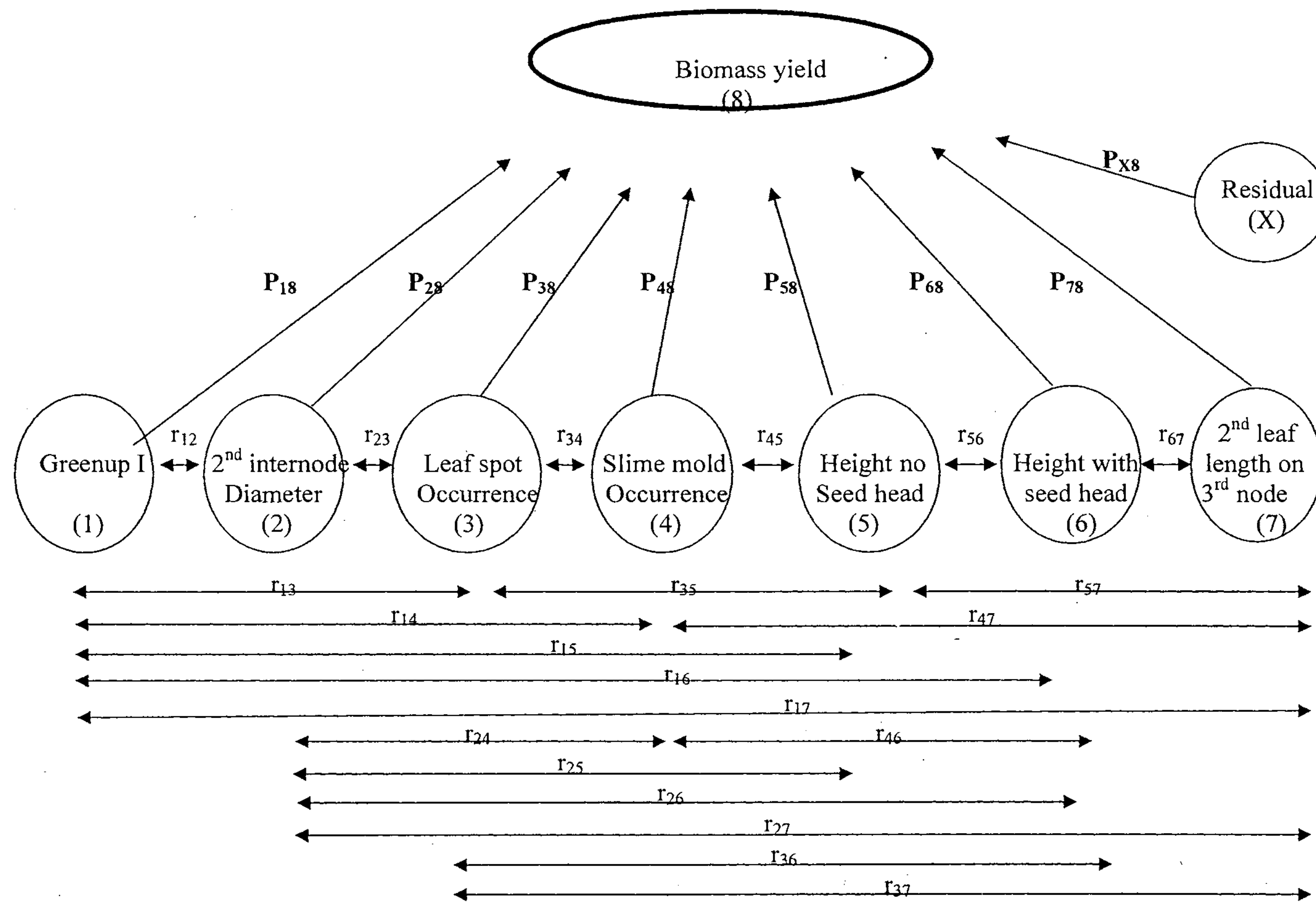

Fig. 1. A path diagram showing cause and effect relationships of Biomass and seven biomass components in bermudagrass. One-directional arrows (⟶) represent direct path (P), and two-directional arrows (⟷) represent mutual correlations.