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BREEDING SEED- AND VEGETATIVELY-PROPAGATED TURF BERMUDAGRASSES FOR GOLF COURSES

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Executive Summary

The principal objective of the turf bermudagrass breeding program at Oklahoma State University is to develop improved seed- and vegetatively-propagated cultivars for the transition zone. Research supporting the breeding effort includes: 1) the procurement and evaluation of new turf bermudagrass germplasm, 2) development of laboratory and field methods to measure plant response to low temperature and disease stress, and 3) identification of genes involved in plant response to low temperatures and disease stress.

The development of seeded turf bermudagrass cultivars for the transition zone requires combining into breeding populations cold hardiness, economic seed yield potential and acceptable turf quality. Phenotypic recurrent selection (PRS) for these traits in broad genetic base *C. dactylon* populations has resulted in incremental improvement with each cycle of selection. A first product of this breeding effort, OKS 91-11, was released in January 1997. OKS 91-11, initially synthesized in 1991, was included in the 1992-96 NTEP bermudagrass test. Breeding improvement in the broad base populations has now reached threshold levels that will allow more rapid progress in seeded turf bermudagrass cultivar development.

African bermudagrass, *C. transvaalensis*, is important because of its role as a parent in crossing with *C. dactyon* plants to produce the sterile triploid ($2n=3x=27$ chromosome) hybrids like 'Tifgreen' and 'Tifway'. African bermudagrass has not been extensively studied and only a few plant introductions have been available for breeding research in the US. Research with African bermudagrass over the past 7 years has demonstrated significant genetic variation for adaptation and turf performance characteristics. African progeny plants with superior performance characteristics were identified and are being used in breeding and other research.

Intra- and inter-specific crossing has been employed over the past 5 years to develop vegetatively-propagated hybrid turf bermudagrass cultivars. Selected parental plants of *C. dactylon* and *C. transvaalensis* were crossed to produce large progeny populations which were screened for turf performance. Approximately 50 select hybrid plants are now in advanced stages of evaluation. Potentially valuable fertile hybrid plants from $2n=6x=54$ chromosome *C. dactylon* x $2n=2x=18$ chromosome *C. transvaalensis* crosses have been obtained. These tetraploid ($2n=4x=36$ chromosome) plants have one full genome (9 chromosomes) from *C. transvaalensis* and 3 full genomes (27 chromosomes) from *C. dactylon*. Open-pollinated and hybrid progeny from these plants have shown desirable turf characteristics.

A laboratory procedure was developed to quantify relative cold hardiness of bermudagrass plants. The freeze tolerance of bermudagrass plants has

traditionally been assessed by observing survival following test winters. Because freeze injury under field conditions is strongly influenced by many environmental factors, multiple observations through time and space are required to elucidate differences in freeze tolerance and geographic adaptation of cultivars. This laboratory procedure may be used in combination with field studies to more quickly and accurately assess freeze tolerance of bermudagrass plants. The procedure also enables and facilitates other cold hardiness research with turf bermudagrass.

The survival of bermudagrass cultivars exposed to freezing temperatures is determined by their ability to cold acclimate. A cold regulated protein from 'Midiron ' and 'Tifgreen' bermudagrass was identified as chitinase. More recent research used Differential Display-Reverse Transcription PCR procedures to study changes in translatable mRNA populations during cold acclimation of freezing tolerant 'Midiron' bermudagrass. At least 30 differentially expressed cDNA species were identified using 40 arbitrary and anchored primer combinations. Nine up-regulated and two down-regulated cDNA species have been confirmed. These cDNA's range from 170 to 470 base pairs in length. These partial cDNAs were sequenced and aligned with other known genes in genome databases. Homologies were found with *Iti*65 (low temperature-induced) from *Arabidopsis thaliana*, a transcription factor (*nus*G) from *Thermus thermophilus*, a non-dormancy cDNA clone from *Avena fatua*, and a secretory/excretory heat shock protein cDNA from *Brugia malayi*. Efforts are underway to isolate the full length sequences corresponding to the partial cDNAs in order to characterize the cDNAs and confirm identity of these genes.

Sixty-two accessions representing 11 *Cynodon* taxa (species and taxonomic varieties) were used in a molecular study of genetic relatedness. Phylogenetic analysis of accessions was performed using the DAF (DNA Amplification Fingerprinting) procedure. Parsimony and bootstrap analysis was performed to produce the consensus phylogenetic tree using the PAUP 3.0 program. The results demonstrate interesting genetic relationships among and within *Cynodon* taxa. For instance, much diversity was found within the cosmopolitan species *C. dactylon*. Accessions within taxonomic varieties generally were grouped together, except for *C. dactylon var. dactylon*, in which wide differences were evident. Small, but definite, variations were found in *C. arcuatus, C. transvaalensis, and C. plectostachyus,* all endemic species to small geographic regions of southern Africa.

Introduction

The turf bermudagrass-breeding program at Oklahoma State University was initiated in 1986 under the joint sponsorship of the United States Golf Association and the Oklahoma Agricultural Experiment Station. The initial broad objective was to develop fine-textured, cold-tolerant, seed-propagated varieties for the transition zone. The program was expanded in 1990 to include the development of superior vegetatively propagated varieties. Associated research supporting the breeding effort includes: 1) the procurement and evaluation of new turf bermudagrass germplasm, 2) development and refinement of techniques to measure plant response to abiotic and biotic induced stresses, 3) characterization of breeding lines and new cultivars for cold tolerance and spring dead spot disease reaction, and 4) identification of genes involved in plant response to low temperatures and organisms causing spring dead spot disease.

BREEDING AND EVALUATION

Breeding Seed-Propagated Varieties

Over the past 10 years, phenotypic recurrent selection (PRS) for finer texture and increased seed yield has been practiced in two broad-genetic base *Cynodon dactylon* populations. One population was developed from cold-hardy germplasm subjected to PRS for increased fertility (% of florets setting seed) and finer texture. The second population was developed from cold sensitive germplasm with high seed production potential. This population was developed by initially selecting for seed yield and turf quality among spaced plants growing at Yuma, Arizona. Each of these populations has undergone an additional cycle of selection within the past year and are presently designated as $C_{5fer\text{-}5tex}$ and C_{4ct}. A new breeding population was developed in 1994 from *C. dactylon* germplasm collected from the Peoples Republic of China in 1993.

Experimental varieties have been synthesized from these populations at various stages of cyclic development and evaluated for turf performance and adaptation characteristics. The cold-hardy, seed-propagated, experimental strain OKS 91-11, evaluated in the 1992-96 NTEP trial, was officially released in December 1996. The variety was licensed with the Johnstons Seed Company of Enid in partnership with Seed Research of Oregon. OKS 91-11 consistently ranked high in turf quality and cold hardiness in the 1992 NTEP test. It is more cold hardy than Mirage or Jackpot enabling it to better survive the first winter following seeding (Fig. 1). Seeded bermudagrasses are most susceptible to freeze injury during the winter following seeding. OKS (BERPC) 91-3, the best of eight seed-

propagated bermudas tested by Bob Carrow at Griffin, Georgia, is being increased for potential release. OKS 91-3 is cold-tolerant and well adapted to the transition zone of the USA. OKS 95-1, a three-clone synthetic, was entered into the 1997 NTEP bermuda trial. These parental clonal plants were selected from germplasm collected in the Peoples Republic of China in 1993. This synthetic potentially offers higher seed yields, better adaptation to the southeastern US, and higher turf quality than other cold hardy seeded bermudagrasses. Preliminary results indicate it to be somewhat less cold-hardy than OKS 91-11, but still more cold hardy than Arizona Common and similar types.

Twenty-eight plants were selected from breeding nurseries in 1997 for use as parents in narrow-base synthetic varieties. The plants were selected on the basis of seed yield potential, turf quality, and stand persistence. The plants were selected after 3 years observation. Each year the breeding nurseries containing the plants were allowed to set seed, then for the duration of the growing season they were mowed at three-fourth inch in order to assess turf quality. This management and duration of time facilitates selection of plants that develop and maintain the best turf quality while allowing simultaneous selection for seed production characteristics. Assessment of plants for a minimum of 2 years following the year of establishment is necessary to identify plants with performance stability. Differences among plants in performance characteristics, particularly stand retention, are often not expressed for 2-3 years following establishment.

Overall, recurrent selection within broad genetic-base, seeded bermudagrass populations has, over the past 10 years, refined them to the point of acceptable turf quality. Attainment of this threshold level of performance in turf quality and adaptation in these populations will permit new varieties to be developed at an accelerated rate. Continued PRS will incrementally improve the populations and the varieties developed from them.

Breeding Vegetatively-Propagated Varieties

African bermudagrasses, *C. transvaalensis*, selected for adaptation and turf quality features, have been used extensively in crosses with *C. dactylon* tetraploid plants over the past 5 years. About 3,000 progeny plants per year have been produced. Two F_1 triploid hybrids, OKC 18-4 and OKC 19-9 that performed well in preliminary tests at OSU (Tables 1,7-10) were entered in the 1997 NTEP bermudagrass test.

Thirty-two hybrid plants were selected from initial screening nurseries in 1997, increased vegetatively in the greenhouse, and planted in a replicated evaluation nursery at the Turf Research Center. These plants were selected from nurseries planted in 1995 and maintained under mowing at three-fourth inch. It is noteworthy that many of the 36 selected plants had common parents. Fourteen of the 36 plants had as one parent 3200W 41-8. The 3200W 41-8 plant is an F_1

hybrid from the cross of an African (*C. transvaalensis,* 2n = 2x = 18 chromosomes) plant with 'Tifton 10' (*C. dactylon*, 2n = 6x = 54 chromosomes). The 3200W 41-8 plant has 2n = 4x = 36 chromosomes, presumably comprised of one genome (9 chromosomes) from the African parent and 3 genomes (27 chromosomes) from the Tifton 10 parent. Several F_1 progeny were produced from the African x Tifton 10 cross, but only two of the hybrids were fertile. The 14 selected plants from the 3200W 41-8 plant resulted from OP seed, or from backcrossing 3200W 41-8 to African or a *C. dactylon* parent. *C. dactylon* plants producing superior F_1 hybrids when crossed with the African parents were Q27774 from Australia, PRC-7 from China, and open-pollinated offspring of Texturf 10. These results are noteworthy because they point to the importance of the parents in producing good F_1 progeny plants. Parents having high genetic combining ability for turf quality and adaptation features are essential in breeding good vegetatively-propagated hybrid plants. The breeding value of the fertile interspecific hybrids like 3200W 41-8 will be further pursued by using them in crosses with both *C. transvaalensis* and *C. dactylon*. Crossing and/or backcrossing them with *C. dactylon* plants provides a means of incorporating into hybrid derivatives less than the full genomic measure of *C. transvaalensis* chromosomes.

Evaluation of Breeding Materials – Turf Research Center

African Bermudagrass Field Testing. A field screening trial of two of the most promising African bermudagrass selections begun in 1995 was continued in 1997. Entries in this trial were maintained under cutting heights of 0.25 or 0.375 inches, simulating low teebox and fairway heights of cut. Nitrogen fertility was 5 lbs of N per 1,000 ft^2 yr. Both Ct 2747 and Ct 2567 African bermudagrasses demonstrated earlier spring greenup (Table 2) but were less dark in color than Tifway bermudagrass (Table 2). Overall, Tifway had greater or equal visual quality (Table 3) on all rating dates at both cutting heights in 1997. No significant differences were present for percent ground cover among Tifway and the African bermudagrasses (Table 4). Tifway had a significantly greater percent of area covered with seed heads at the higher height of cut on 21 July 1997 (Table 4). No significant difference in resistance to Spring Dead Spot Disease caused by *Ophiosphaerella herpotricha* were found in Spring of 1997 (Table 5). Additional growing seasons will be needed to properly test for resistance of these selections to this disease.

The amount of force (ft lb) required to tear apart an 18 in long by 12 inch wide by 0.6 in thick slab of sod of each entry was determined on 4 August 1997 (Fig. 2). Although Tifway had significantly great sod strength than either African bermudagrass selection (Table 5), both African bermudagrasses maintained integrity following cutting and handling during the sod strength test. No differences were present in sod strength between the two African

bermudagrasses. Both African bermudagrass selections appeared to have suitable sod strength for harvest and handling.

Hybrid Bermudagrass Field Testing. Field testing of experimental hybrid bermudagrasses planted in summer of 1995 was continued in 1997. Tifway and Midlawn bermudagrasses were included as standards. Field plots were fertilized with 5 lbs of N per 1,000 ft^2 yr and cut at 0.375 and 0.75 inches. Several experimental selections had visual quality equal to or greater than Tifway bermudagrass (Table 6). Tifway had visual color ratings numerically darker green though not significantly greater than most of the experimental grasses tested (Table 7), with the exception of OKC 19-9. Substantial variation in the percentage of plot area covered in seedheads (Table 7) was noted for a rating date in 1997. No significant differences in the size of Spring Dead Spot Disease infection areas were noted among trial entries in this first year of disease screening (Table 8). No significant differences were noted among Tifway and the experimental hybrids for shoot survival to the disease on a per unit area basis (Table 8), however, Midlawn had a statistically higher shoot survival index than any other entry in the trial. A natural infestation of Bermudagrass Stunt Mites (*Eriophyes cynodoniensis*) allowed for mite damage ratings to occur (Table 9). Only selection OKC 3-1 appeared particularly damaged by the infestation. Sod shearing strength was measured using the same method described in the African bermudagrass section of this report. Tifway required significantly more force to shear than all other entries in the trial (Table 9). Substantial differences were present among experimental lines. No problems were noted in cutting or handling of any of the entries. As the material tested had been planted over 2 years earlier, sod strength may have been lower than that of a younger (normally 70 day to 1 year old) bermudagrass crop.

Some of the most promising vegetatively propagated OSU bermudagrass lines from the 1995 field trial and from field space plantings were placed in a trial at Oklahoma City Golf and Country Club in Oklahoma City. The trial contains five experimental OSU lines plus Midlawn and Tifway as standards. The experimental design is a randomized complete block with 3 replications of treatment. The trial was planted on 30 May 1997 via 2 inch diameter plugs on approximately 18 inch centers. The trial is under the care of Superintendent Craig Elms, CGCS (Fig. 3). The trial receives regular irrigation, cutting 3 times per week and will receive a maintenance nitrogen regime of 5 lbs of N per 1,000 ft^2 yr in 1998. Test cutting heights are 0.25 and 0.5 inches.

The rate at which the entries established varied significantly (Table 10). All entries covered by the end of the 1997 growing season. Although some entries are slower growing than others, slower growing entries should not be viewed as problematic, since sprigging rate can be increased to more quickly establish a slow growing variety in a commercial setting. Our trials require establishment via plugs to help reduced the potential of mechanical contamination among the plots which more readily occurs when small plot research is established via sprigging.

A sod strength measurement test and recovery from divoting is planned for the Oklahoma City Golf and Country Club trial in 1998. Plugs were cut for shoot density counts from this trial in October 1997, however, shoot count data were unavailable at the time of printing of this report.

An additional USGA funded vegetative bermudagrass trial was established at the OSU Turfgrass Research Station at Stillwater in August of 1997. Additionally, the 1997 NTEP Bermudagrass Trial was planted in September 1997 at the same site. Both trials will be cut at 0.5 inches and fertilized with 5 lbs of N per 1,000 ft^2 yr. Ratings will include the standard rating for color, quality, texture and density. Additionally, divot recovery ratings will be conducted on these studies. Also, the USGA funded trial was inoculated with the Spring Dead Spot causal agent *Ophiosphaerella herpotricha* (O.h.) while the NTEP bermudagrass trial was inoculated with O.h. as well as SDS causal agents *O. korrae* and *Leptosphaeria narmari* from isolations at the Shangri-La Golf Course in Grove Oklahoma by Dr. Ned Tisserat of Kansas State University (KSU). Ph.D. candidate Henry Wetzel of KSU assisted the OSU turfgrass team in the inoculations (Fig. 4).

Seeded Bermudagrass Evaluations. A field evaluation of the seeded OSU release OKS 91-11 against commercial standards of Mirage and Jackpot was continued in 1997. This study is watered regularly to prevent wilting and fertilized with 5 lbs of N per 1,000 ft^2 per yr and maintained under mowing treatments of 0.5 and 1.5 inch heights of cut.

OKS 91-11 provided equal to or greater visual quality to the two standard seeded bermudagrasses on all rating dates (Table 11). No statistical differences were present among the three grasses for percent living cover or color ratings (Table 12). Visual density ratings suggested OKS 91-11 was less dense than the two commercial lines at the lower cutting height but no differences were present at the higher height of cut (Table 12). No difference in shoot count density among the three entries were found at either height of cut (Table 13). Additionally, no statistical differences were present in percent area affected by Spring Dead Spot Disease or the number of shoots surviving in the affected areas or in the shoots per unit infected area (Table 13). At least two seasons of data are required to have at least a basic level of understanding of the resistance of bermudagrass selections to Spring Dead Spot Disease. This study will be continued in 1998.

LABORATORY EVALUATION OF FREEZE TOLERANCE

A study to determine the relative freeze tolerance of several fertile bermudagrass plants (Table 14) was completed. Sterile cultivars were also included as standards. Plants were clonally propagated and established in conetainers for about 10 weeks. Acclimation took place in a controlled environment chamber at 8/2 °C (day/night) temperatures with a 10 h photoperiod for 4 wks. Conetainers were placed into a freeze chamber (Fig. 5) and cooled

rapidly to −2°C. Plants and soil were induced to freeze with ice chips then held overnight at −2 °C. The freeze chamber was then programmed to cool linearly at 1 °C per hour after ≈ 15 h at −2 °C. For each cultivar, three conetainers were removed at each test temperature. Target temperatures (1 °C intervals) spanned a range anticipated to kill some, but not all, of the plants. Conetainers were held overnight at ≈ 4 °C after removal from the freeze chamber. Following thawing, plant response to freezing stress was visually evaluated as regrowth in a greenhouse (Fig. 6). Weak shoots that died after emergence were not counted as viable. The experiment was replicated on three dates and regrowth data were pooled to generate survival vs. temperature curves. Responses of the different plants to freezing temperatures are given in Fig. 6a-b. Midiron and A12195 were the most freeze resistant, with T_{50} values of about −10.5 °C. Morrill, Quickstand, Guymon, and Beijing had T_{50} values between −9 and −10 °C. The 9959 accession had a T_{50} value of approximately −8.6, while Tifgreen and PRC-7 had similar $T_{50's}$ near −7.5 °C. The T_{50} of Arizona Common was approximately -6.6 °C while Zebra was the most susceptible to freeze injury. The ability of most of these plants to serve as parents in controlled crosses will facilitate genetic studies of cold hardiness at the molecular and organism levels.

IDENTIFICATION OF GENES INVOLVED IN COLD ACCLIMATION

One of the most persistent problems associated with the use of turf bermudagrass is its susceptibility to damage caused by freezing. However, some cultivars exhibit tolerance to freezing following periods of acclimation at temperatures slightly above 0°C, a process known as cold acclimation or hardening.

Studies have shown that the ability to cold acclimate is primarily controlled at the gene level, resulting in a number of biochemical and physiological changes that enable plants to adapt to freezing stress. These changes are associated with the expression of genes that code for antifreeze proteins, which are similar to pathogenesis-related proteins such as chitinase and glucanase (Antikainen et al., 1996; Gatschet et al., 1996; Hon et al., 1995), and/or genes that code for a family of hydrophilic proteins (COR, LTI) that may have potential roles in protecting cells against freeze-induced dehydration damage (Hajela et al., 1990; Nordin et al., 1991; Nordin et al., 1993; Gilmour et al., 1992).

The bermudagrass cultivar 'Midiron' (*C. dactylon* X *C. transvaalensis*) exhibits a high level of tolerance to freezing (LT_{50}=-11°C) after cold acclimation for 28 days at 8°C/2°C (Anderson et al., 1988). Previous results in our laboratory using two dimensional protein electrophoresis indicate differential expression of a chitinase in 'Midiron' crowns following 2 and 28 days of cold acclimation at 8°C/2°C (Gatschet et al., 1994; Gatschet et al., 1996). We therefore continued our efforts to study differential gene expression in crowns of cold acclimated 'Midiron' to be able to isolate and characterize genes that may have potential roles

in regulating the ability of this cultivar to withstand freezing. Ph.D. candidate Benildo de los Reyes has been a major contributor to this research.

Plant Materials and Cold Acclimation. Plants from vegetatively propagated stocks of 'Midiron' were planted in 'conetainers' and were grown at normal temperature and light conditions (28°C/24°C, 12 hours photoperiod) for one month. The plants were transferred to a controlled environment chamber for cold acclimation (CA) at 8°C/2°C, 10 hours photoperiod, for a period of 28 days. Crown tissues were harvested from both control and CA plants after 2 and 28 days in the chamber. Following acclimation, a portion of the 28 days CA plants were transferred to a normal temperature chamber for deacclimation (DAC). Crown tissues were harvested from these plants after 2 days of deacclimation.

Total RNA Isolation from Crown Tissues. The crown tissues isolated from individual plants were pooled within each treatment (Control, 2 days CA, 28 days CA and 2 days DAC). The crowns were washed with phosphate buffered saline and then immediately frozen and ground in liquid nitrogen. Total RNA was isolated by Guanidine-HCl/Phenol-chloroform following the procedure of Logemann et al. (1987). Total RNA samples were treated with DNaseI to remove contaminating genomic DNA using Message Clean Kit (GenHunter Corp., Nashville, TN). The quantity and quality of the isolated total RNA were analyzed by formaldehyde gel electrophoresis and standard A_{260}/A_{280} absorbance measurements.

Results/Discussion. Two types of differential expression patterns are observed in the differential display analysis of cold acclimated and deacclimated 'Midiron' crown tissue mRNA (Figure 7). The first type consists of upregulated bands which are those that are highly expressed during CA and not in the controls. This type also exhibits a decrease in the level of expression following 2 days of deacclimation, suggesting a rapid turnover of the COR mRNAs. The second type consists of downregulated bands which are those that are expressed in the controls and not during CA. This type represents genes that may be silenced by the low temperature treatments during cold acclimation.

The reverse prime analysis was able to confirm nine upregulated and two downregulated bands from a total of 19 bands that were cloned and studied (Table 15, Figure 8). The length of the partial cDNA sequences are consistent with the size of the original bands observed in the differential display autoradiograms. All sequences are flanked by a polyA tail (anchored oligo-dT primer sequence) and the arbitrary primer sequence indicating that none of the confirmed COR cDNAs are products of end to end priming by the arbitrary primer from cDNA or contaminating genomic DNA template.

The results of the database search suggest that five of the COR cDNAs may be homologous to genes of known function. Among these, the 330 bp upregulated

cDNA Cyn330 is the most promising candidate for full length cloning because of its sequence similarity with the *lti65* gene from *Arabidopsis thaliana* (Table 15, Fig. 7). The expression of the *lti65* gene in *Arabidopsis thaliana* is induced by both low temperature and desiccation stress (Nordin et al., 1991; Gilmour et al., 1992, Nordin et al., 1993). Based on this information, the possible function of this gene may be interpreted as that which may be involved with a mechanism of adaptation to both low temperature and drought stress conditions, although further experiments are needed to prove this. Northern blot analysis of total RNA from control, CA and DAC plants detected two bands corresponding to Cyn330 (Fig. 9) which are approximately 0.8 and 1.1 kb. The expression of both bands are highly induced during cold acclimation and are reduced to a level significantly lower than the CA samples after the 2-day deacclimation period. This result is parallel to the expression pattern observed in differential display results and further confirms upregulation of this gene in response to CA.

Based on the information discussed above and the tropical origin of bermudagrass, this gene (Cyn330) may have potential contribution in freezing tolerance of bermudagrass possibly by preventing freeze-induced dehydration damage to cells. However, isolation of the full length cDNA is necessary to be able to align Cyn330 with the *Arabidopsis thaliana lti65* gene sequence and to be able to design further experiments to prove this hypothesis. Similarly, the full length cDNAs corresponding to the other upregulated bands cloned in *pCR2.1* (Table 15) is yet to be isolated for further characterization and verification of the partial sequence homology observed. Other interesting cDNAs are Cyn370, Cyn380a and Cyn380b. Like Cyn370, these cDNAs may be potentially involved with cold acclimation because of their similarity with other known genes that are expressed under a temperature-stressed environment.

PHYLOGENETIC STUDIES OF *CYNODON* TAXA

The genus *Cynodon* is comprised of nine species and ten varieties (Harlan et al., 1970a & c, Table 16). *Cynodon* taxa range from narrow endemics to the cosmopolitan *C. dactylon* var. *dactylon* (Harlan et al. 1970a & b, Taliaferro, 1995). Classical cytotaxonomic studies have provided information on the genetic relatedness of *Cynodon* taxa and races as indicated by hybridization potential and cytogenetic characteristics of hybrids (Harlan and de Wet, 1969, Harlan et al. 1969, 1970a). Interspecific hybridization has been demonstrated among many of the *Cynodon* species, the exceptions being *C. arcuatus*, *C. barberi*, and C. *plectostachyus* (Harlan et al. 1969). The use of DNA markers to quantify genetic variation within and among plant taxa has gained widespread acceptance in recent years (Caetano-Anolles et al., 1995; Cerny et al., 1996; Kazan et al., 1992; Paterson et al., 1991; Weaver et al., 1995; Williams et al., 1990). Such markers provide relatively unbiased quantitative estimates of genetic diversity among plants (Clegg, 1990). Additional information on genetic relatedness of *Cynodon* germplasm will facilitate decision making regarding its use in breeding,

preservation, and fundamental investigation. Accordingly, this study assessed genetic diversity of *Cynodon* accessions and taxa by means of DNA amplification fingerprinting (DAF). Postdoctoral Assistant Senayet Assefa led in conducting this research.

Plant Material: Sixty-two *Cynodon* germplasm accessions representing eight species and six varieties were used in the study (Table 17). Accessions were part of the *Cynodon* germplasm collection maintained at Oklahoma State University. Individual accessions were grown in the green house in 15 cm diameter pots under uniform conditions.

DNA isolation and Amplification: Total genomic DNA was isolated from young leaf tissues (4 g) as described by Sastry et al. (1995). Amplification was accomplished using the procedures of Caetano-Anolles and Bassam (1993). The reaction mixture (25 µL) was overlaid with mineral oil and amplified in a PTC- 200 thermocycler programed to 35 cycles of 30 s at 96°C, 30°C, and 72°C, respectively. Amplification processes were repeated twice to generate the DNA profiles. DNA amplification products were size fractionated in a 5% polyacrylamide-7M urea gel. The amplified DNA samples (5 µL) were applied to the gels in 5 µL aliquots containing 5 M urea and 0.02% xylene cyanol FF (BIO-RAD). Size calibration used a 100 bp DNA ladder (Gibco BRL., Gaithersberg, MD).

DNA Staining: The DNA fraction was silver stained as described by Bassam and Caetano-Anolles (1993). The 8 x 10 cm, 1 mm thick, mini gels were fixed for 20 min in 7.5% glacial acetic acid, washed twice with deionized distilled water, impregnated with silver nitrate for 30 min, briefly rinsed with deionized distilled water, and developed in sodium carbonate for about 4 to 6 min at 8 to 12°C. Image development was stopped in 7.5% glacial acetic acid. Gels were dried between two cellulose acetate plastic sheets following soaking in deionized distilled water and drying solution, respectively.

Data Analysis: The *Cynodon* accessions were evaluated using 12 arbitrary octamer primers (Table 18). Bands (≤ 2000 bp in length) generated by each primer in DAF gels were visually scored as present (1) or absent (0), and entered into the PAUP analysis as unordered, nondirected, and unweighted Wagner binary characters (16). Ambiguous bands were recorded as missing data. The data were analyzed by heuristic search using tree-bisection-reconnection (TBR) branch swapping to identify minimal tree. The analysis incorporated 100 bootstrap replications of TBR, with simple addition sequence, MULPRARS option, and 50% consensus calculation. Minimum trees were rooted by the midpoint rooting mode and the phylogenies were constructed. The data were also used to generate distance matrices based on matching (Puterika et al., 1993) and similarity (Nei and Li, 1979). The fraction of bands matching in two accessions was calculated using the formulae: Matching (M) = N'_{AB}/N_T and Similarity (S) = $2N_{AB}/(N_A + N_B)$, where

N_{AB} is the number of bands shared by individual A and B, N_A and N_B are the number of bands in individual A and B respectively, N'_{AB} is the total number of matches in individual A and B (both bands present or both bands absent), and N_T is the total number of fragments scored in the over all study. Maximum, minimum, and average value of the similarity-matching coefficients were calculated.

RESULTS/DISCUSSION

DNA Fingerprinting of Bermudagrass Accessions. Ten of 12 primers produced polymorphic banding patterns (Table 18). DAF profiles were generated from replicate samples and amplifications. Fig. 10 and 11 demonstrate the DAF fingerprinting produced from *C. aethiopicus, C.arcuatus C. plectostachyus, C. transvaalensis,* and *C. dactylon* leaves using primers 2273 and 2252. An average of 53.9 ± 7.3 amplification products (≤ 2000 bp in length) were obtained from the 10 primers producing polymorphic bands (Table 18). Of 539 loci (bands) scored, 496 (92%) were polymorphic (i.e. designated bands were missing in at least one of the accessions) and separated all species and accessions.

Genetic Relatedness. PAUP resulted in a single minimal phylogenetic tree (Fig. 12). Pairwise matching and similarity coefficients among and within the *Cynodon* taxa are given in Table 19. In general, the three methods provided information leading to similar conclusions on genetic relatedness. The combined information indicates:

1) accessions generally clustered by taxonomic division (species or variety) except for *Cynodon dactylon* var. *dactylon.*
2) *C. dactylon* var. *dactylon* accessions tended to group by geographic origin as indicated by clustering of accessions from Philippines, Australia, and Afghanistan.
3) intraspecific variation among accessions was least for *C. arcuatus*, and *C. transvaalensis* and greatest for *C. dactylon*.
4) the most distantly related taxa to be *C. arcuatus* and *C. nlemfuensis* followed by *C. aethiopicus* and *C. nlemfuensis.*
5) the most closely related taxa to be *C. transvaalensis* and *C. plectostachyus* followed by *C. arcuatus* and *C. aethiopicus*.

The relative magnitudes of variation for relatedness among accessions within *C. arcuatus*, *C. transvaalensis*, and *C. dactylon* were expected. *C. arcuatus* and *C. transvaalensis* are narrow endemic species (Harlan et al., 1970b). Classical cytotaxonomic studies have documented enormous variability in *C. dactylon*, with the greatest amount occurring in the cosmopolitan *C. dactylon* var. *dactylon* (Harlan and de Wet, 1969). These studies also indicated *C. arcuatus*, *C. plectostachyus*, and *C. barberi* to be genetically isolated from each other and from

the rest of the genus (Harlan et al., 1969). The relatively close relatedness indicated in this study between *C. arcuatus* and *C. aethiopicus*, and between *C. plectostachyus* and C. *transvaalensis* is not supported by estimates of relatedness based on hybridization potential. For example, C. *transvaalensis* crosses rather easily with most varieties of *C. dactylon* and with *C. nlemfuensis*, but not with *C. plectostachyus. C. aethiopicus* will cross with *C. dactylon* and *C. nlemfuensis,* but not with *C. arcuatus.*

SPRING DEAD SPOT DISEASE MOLECULAR STUDIES

Bermudagrass varieties and experimental strains in replicated tests at the Turf Research Center have been evaluated for response to *Ophiosphaerella herpotricha* and/or *Leptosphaeria korrae* over the past two years. Disease incidence caused by *O. herpotricha* was strongly negatively correlated with cold tolerance. The identification in our lab of a bermudagrass chitinase protein associated with cold acclimation suggests the possibility of such proteins having dual roles in protecting against both low temperatures and disease. Additional field and molecular research was recently initiated to explore the biochemical and genetic bases of the disease.

Table 1. Mean performance ratings for vegetatively propagated turf bermudagrasses in test 94-1, Stillwater Agronomy Research Station. Stillwater, OK.

Entry No.	Strain	1995-97 (N = 36)			4/9/97
		Quality[1]	Color[1]	Heading[1]	Greenup[2]
1	3200W 1-6	6.7	6.9	8.7	50
2	3200W 3-3	6.6	6.8	8.8	75
3	3200W 12-2	6.8	6.9	8.3	68
4	3200W 12-4	6.9	7.2	8.9	75
5	3200W 12-7	5.7	6.0	7.6	10
6	3200W 18-4	7.3	7.3	8.7	62
7	3200W 19-9	6.1	6.4	7.8	60
8	3200W 23-8	6.9	6.9	8.8	95
9	3200W 25-6	5.7	6.0	8.6	25
10	3200W 26-8	4.9	5.3	7.1	25
11	3200W 31-8	4.7	5.6	6.2	15
12	3200W 35-3	6.7	6.9	8.9	45
13	3200W 39-3	6.3	6.4	8.8	50
14	3200W 39-7	6.0	6.0	7.9	35
15	3200W 39-8	4.4	5.6	6.7	35
16	3200W 41-5	6.5	6.7	8.5	30
17	3200W 41-8	5.9	6.2	8.4	30
18	3200W 47-3	6.7	7.1	8.3	55
19	3200W 47-3	6.1	6.3	8.9	65
20	3200W 50-1	6.0	6.1	8.0	20
21	3200W 55-3	5.9	6.1	8.1	45
22	3200W 55-7	6.1	6.2	8.5	30
23	PRC-7	5.9	6.1	7.8	40
24	PRC-55	6.5	6.4	8.3	70
25	Beijing	5.7	5.9	7.5	55
26	Tifgreen	6.4	6.3	8.0	72
27	Midfield	6.6	6.4	8.4	77
28	Midlawn	6.4	6.6	8.5	75
29	U-3	5.5	5.8	7.3	45
30	Tifway	6.5	6.6	8.0	30
CV (%)		9.3	8.6	6.5	28
5% LSD		0.3	0.3	0.2	38

[1]Turf quality, color, and seedhead density rated on a scale of 1 to 9, with 9 being best i.e. highest quality and color and fewest seedheads.
[2]% greenup

Table 2. Spring greenup, color and density of African and Tifway bermudagrasses in the African bermudagrass tee/fairway study. Turfgrass Research Center, Stillwater, OK. (1)

	Spring Greenup				Color		Density	
	Low (2)	High	Low	High	Low	High	Low	High
Genotype	28 March 97		10 April 97		22 May 97		22 August 97	
2567	6.7	6.7	7.7	7.7	4.0	5.0	5.0	7.0
2747	5.0	5.0	7.3	7.3	4.0	5.0	5.0	6.7
Tifway	3.0	3.0	7.3	7.3	6.3	7.3	5.0	7.3
LSD (0.05)	**1.5**	**1.5**	**NS**	**NS**	**0.8**	**0.8**	**NS**	**NS**

(1) Ratings were on a scale of 1-9, with 9 being best.
(2) The low cutting height = 0.25 inches and the high cutting height = 0.375 inches.

Table 3. Visual quality of African and Tifway bermudagrasses in the African bermudagrass tee/fairway study. Turfgrass Research Center, Stillwater, OK.(1)

	Visual Quality									
	Low (2)	High	Low	High	Low	High	Low	High	Low	High
Genotype	21 May 97		30 June 97		16 July 97		21 Aug 97		16 Sept 97	
2567	4.0	5.0	6.3	7.0	5.0	8.0	4.7	6.0	5.0	6.7
2747	4.0	5.0	7.0	6.7	5.3	7.0	5.0	6.7	5.0	6.0
Tifway	6.3	8.0	7.0	6.3	8.0	9.0	4.7	7.3	5.3	7.7
LSD (0.05)	**0.8**	**0.1**	**NS**	**NS**	**0.8**	**0.1**	**NS**	**0.9**	**NS**	**0.8**

(1) Ratings were on a scale of 1-9, with 9 being best.
(2) The low cutting height = 0.25 inches and the high cutting height = 0.375 inches.

Table 4. Percent cover and seedhead ratings for African and Tifway bermudagrasses in the African bermudagrass tee/fairway study, Turfgrass Research Center, Stillwater, OK.

	Percent Cover (1)				Percent Seedheads (1)	
	Low (2)	High	Low	High	Low	High
Genotype	22 May 97		22 Aug 97		21 July 97	
2567	99.0	99.0	71.7	91.7	0.0	0.0
2747	99.0	99.0	75.0	92.3	0.0	0.0
Tifway	99.0	99.0	80.0	97.0	1.0	5.0
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**1.0**

(1) Ratings for percent cover and seedheads were on a scale of 0-100.
(2) The low cutting height = 0.25 inches and the high cutting height = 0.375 inches.

Table 5. Evaluation of African and Tifway bermudagrasses for resistance to Spring Dead Spot Disease and sod shear strength (1).

Genotype	Area of Infection (cm2) 1 May 97	Shoots in Infected Area (2) 1 May 97	Sod shearing strength (3) (ft lbs of force)
2567	17.0	9.1	73
2747	31.1	9.8	72
Tifway	38.9	5.5	173
LSD (0.05)	**NS**	**NS**	**34**

(1) Cutting height = 0.375 inches.
(2) Study inoculated with *Ophiosphaerella herpotrica* fungus in Sept. 1996 .
(3) Foot pounds of force required to shear apart an 18 inch long by 12 inch wide by 0.6 inch deep slab of sod. Mean of 9 samples.

Table 6. Visual quality of vegetatively-propagated bermudagrasses planted on July 21, 1995. Turfgrass Research Center, Stillwater, OK.

	Visual Quality (1)									
	Low (2)	High (2)	Low	High	Low	High	Low	High	Low	High
Genotype	21 May 97		30 Jun 97		16 Jul 97		21 Aug 97		16 Sept 97	
OKC 46-8	7.7	7.0	7.3	5.7	7.0	7.7	6.0	8.0	6.3	7.3
OKC 47-3	7.7	7.7	7.7	6.0	7.3	7.7	5.0	7.3	6.3	7.0
OKC 47-10	6.3	5.0	6.0	5.0	7.3	7.3	6.7	6.3	6.7	6.7
OKC 19-9	8.3	8.7	8.7	8.0	7.7	9.0	8.3	8.7	7.7	8.0
OKC 3-3	7.0	7.3	8.0	7.0	6.0	7.3	6.7	7.7	6.7	7.3
Midlawn	7.3	7.7	7.3	5.7	7.0	7.3	5.3	6.7	6.7	7.7
Tifway	7.3	8.0	7.3	5.7	7.7	8.7	5.3	7.7	6.0	7.7
OKC 39-3	8.0	7.7	6.0	5.0	8.0	7.7	7.7	7.3	7.0	6.7
OKC 7-2	7.0	7.3	7.0	5.7	6.0	7.7	7.0	7.7	5.3	6.0
OKC 3-1	5.7	6.7	7.0	6.3	6.3	7.3	5.3	6.3	6.3	6.7
LSD (0.05)	**1.1**	**0.9**	**1.1**	**1.5**	**0.9**	**NS**	**1.4**	**1.3**	**NS**	**1.0**

(1) Ratings were on a scale of 1-9, with 9 being best.
(2) The low mowing height = 0.375 inches and the high mowing height = 0.75 inches.

Table 7. Percent cover, color, density and percent seedheads of vegetatively-propagated bermudagrasses planted on July 21, 1995. Turfgrass Research Center, Stillwater, OK.

	Percent Cover (1)				Color (2)		Density (2)		Percent Seedheads (1)	
	Low (3)	High (3)	Low	High	Low	High	Low	High	Low	High
Genotype	22 May 97		21 Aug 97		22 May 97		21 Aug 97		21 Jul 97	
OKC 46-8	97.3	96.3	93.0	99.0	7.7	7.7	6.7	9.0	1.3	13.3
OKC 47-3	97.7	97.3	80.0	92.0	7.0	7.3	5.7	8.7	0.7	17.3
OKC 47-10	95.3	89.3	93.3	96.0	7.3	6.7	6.7	9.0	5.3	18.3
OKC 19-9	99.0	99.0	98.3	98.3	9.0	9.0	8.3	8.7	0.7	4.0
OKC 3-3	98.7	99.0	96.3	98.3	7.0	7.0	6.7	7.7	0.3	1.0
Midlawn	97.3	98.3	88.3	97.3	7.3	7.3	6.0	8.0	1.3	11.7
Tifway	99.0	99.0	86.7	99.0	8.0	8.0	6.3	9.0	3.0	18.3
OKC 39-3	97.7	97.7	97.0	99.0	8.3	8.7	8.3	9.0	11.7	36.7
OKC 7-2	98.7	98.0	96.7	98.3	7.3	7.3	7.0	8.3	3.0	15.7
OKC 3-1	97.3	98.3	88.3	94.0	6.7	7.0	5.7	7.0	0.0	0.7
LSD (0.05)	**NS**	**2.6**	**6.5**	**NS**	**1.1**	**1.1**	**1.2**	**0.8**	**5.1**	**16.6**

(1) Percent cover and percent seedhead ratings were on a 0-100 scale.
(2) Color and density ratings were on a scale of 1-9, with 9 being best.
(3) The low mowing height = 0.375 inches and the high mowing height = 0.75 inches.

Table 8. Evaluation of vegetatively-propagated bermudagrasses (1) for resistance to Spring Dead Spot Disease (2) and shoot density.

Genotype	Infected Area 1 May 97 (cm2)	No. of Live Shoots in Infected Area 1 May 97	Live Shoots Per Unit Infected Area 1 May 97	Shoot Density 5 May 97 (shoots per cm2)
OKC 46-8	77.4	1.7	0.033	4.5
OKC 47-3	161.7	4.1	0.028	6.2
OKC 47-10	139.1	2.2	0.016	5.4
OKC 19-9	107.6	1.3	0.014	5.3
OKC 3-3	82.3	2.1	0.038	4.5
Midlawn	58.4	3.7	0.096	4.1
Tifway	141.6	2.3	0.019	3.8
OKC 39-3	179.7	2.7	0.019	6.9
OKC 7-2	159.1	2.8	0.029	5.1
OKC 3-1	97.1	3.9	0.040	3.7
LSD (0.05)	**NS**	**NS**	**0.036**	**1.9**

(1) Study is mowed at 0.375 inches.
(2) All plots were inoculated in Sept. 1996 with a virulent strain of *Ophiosphaerella herpotricha*.

Table 9. Stunt Mite damage, seedhead branching and sod strength of vegetatively-propagated bermudagrasses planted on July 21, 1995.

	Mite Damage (1)		No. of Branches on Seedhead Spikes(2)	Sod shearing strength
	Low (3)	High (3)		
Genotype	11 Jun 97		21 Jul 97	(ft lbs of force)
OKC 46-8	9.0	9.0	2.4	84
OKC 47-3	9.0	9.0	2.1	59
OKC 47-10	9.0	9.0	2.8	69
OKC 19-9	9.0	9.0	2.2	79
OKC 3-3	9.0	9.0	3.0	90
Midlawn	9.0	9.0	2.7	76
Tifway	9.0	9.0	2.9	144
OKC 39-3	9.0	9.0	2.0	41
OKC 7-2	9.0	9.0	2.9	56
OKC 3-1	6.7	6.7	2.0	57
LSD (0.05)	**0.3**	**0.3**	**0.3**	**29**

(1) Stunt mite damage was rated on a 1-9 scale, where 9 is no damage and 1 = entire plot damaged.
(2) Average number of branches to the floral spike of 10 samples per replicate.
(3) The low mowing height = 0.375 inches and the high mowing height = 0.75 inches.

Table 10. Establishment ratings for bermudagrass at Oklahoma City Golf and Country Club (1,2).

	Percent Cover (3)	
Genotype	30 Jun 97	8 Aug 97
OKC 18-4	26.7	100.0
OKC 41-8	26.7	99.7
Midlawn	25.0	99.0
Tifway	20.0	99.3
OKC 39-3	18.3	99.0
OKC 19-9	15.0	98.0
OKC 47-3	10.0	91.7
LSD (0.05)	**8.1**	**2.0**

(1) Study established from plugs on 30 May 1997.
(2) Maintenance cutting heights will be 0.25 and 0.5 inches.
(3 Percent cover ratings were made on a scale of 0-100 percent.

Table 11. Visual quality ratings of seeded bermudagrass varieties at Stillwater, OK.

	Visual Quality (1)									
	Low (2)	High (2)	Low	High	Low	High	Low	High	Low	High
Genotype	22 May 97		30 Jun 97		16 Jul 97		21 Aug 97		16 Sep 97	
OKS 91-11	6.7	5.7	7.3	5.7	8.0	7.3	7.0	5.7	7.0	7.0
Jackpot	5.7	6.3	5.3	6.3	7.7	6.7	7.0	5.7	7.0	6.7
Mirage	5.3	6.0	6.0	5.0	7.7	6.3	6.7	5.3	7.0	7.0
LSD (0.05)	0.8	NS	NS	NS	NS	0.8	NS	NS	NS	NS

(1) Quality ratings were on a scale of 1-9, with 9 being best.

(2) The low mowing height = 0.5 inches and the high mowing height = 1.5 inches.

Table 12. Percent cover, density and color ratings of seeded bermudagrass varieties at Stillwater, OK.

	Percent Cover (1)				Density (2)		Color (2)	
	Low (3)	High (3)	Low	High	Low	High	Low	High
Genotype	22 May 97		21 Aug 97		21 Aug 97		22 May 97	
OKS 91-11	97.3	96.7	95.7	97.3	8.0	9.0	7.7	7.3
Jackpot	96.7	97.3	96.3	97.0	8.3	9.0	7.0	7.0
Mirage	94.0	96.3	98.3	96.0	9.0	9.0	7.0	7.0
LSD (0.05)	NS	NS	NS	NS	0.8	NS	NS	NS

(1) Percent cover ratings were on a 0-100 scale.

(2) Density and Color ratings were on a scale of 1-9, with 9 being best.

(3) The low mowing height = 0.5 inches and the high mowing height = 1.5 inches.

Table 13. Evaluation of seeded bermudagrasses for resistance to Spring Dead Spot Disease. Turfgrass Research Center, Stillwater, OK. (1)

	Infected Area (cm2)		Total No. of Shoots in Infected Area		Shoots per Unit Infected Area		Shoots Density of Healthy Area	
	Low (2)	High (2)	Low	High	Low	High	Low	High
Genotype	5 May 97		5 May 97		5 May 97			
							(shoots per cm^2)	
OKS 91-11	42.9	56.8	1.3	1.2	0.050	0.0900	2.2	2.0
Jackpot	90.6	161.6	2.0	0.9	0.040	0.0050	2.6	1.6
Mirage	122.5	140.0	0.8	0.1	0.007	0.0009	2.3	1.6
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS

(1) Plots were infected with Ophiosphaerella herpotricha fungus in Sept. of 1996.

(2) The low mowing height = 0.5 inches and the high mowing height = 1.5 inches.

Table 14. Origins of bermudagrass clonal plants characterized for relative freeze tolerance.

Plant ID	Ploidy	Origin
A12195	4x	Michigan State Univ. Campus, E. Lansing, MI
Morrill	4x	Salt Lake City, Utah
Quickstand	4x	Quicksand, Kentucky
Guymon	4x	Guymon, Oklahoma (clonal parent of 'Guymon')
A9959	4x	Yugoslavia
Beijing	4x	Beijing, Peoples Republic of China
PRC-7	4x	Guangzhou, Peoples Republic of China
Arizona Common	4x	Yuma Co., Arizona
Zebra	4x	Oklahoma State University, Stillwater, OK
Tifgreen	3x	Tifton, Georgia
Midiron	3x	Hays, Kansas

Table 15. List of cold regulated partial cDNA clones isolated from 'Midiron' crown tissues.

CDNA	Primer Combination	Expression Pattern	Size (bp)	Sequence Similarity
Cyn180a	T_{12}MC/AP4	upregulated	180	
Cyn180b	T_{12}MA/AP7	upregulated	180	
Cyn180c	T_{12}MG/AP8	upregulated	180	
Cyn180d	T_{12}MC/AP2	downregulated	180	
Cyn210	T_{12}MG/AP3	upregulated	210	
Cyn270	T_{12}MG/AP3	upregulated	270	Human hormone-like protein
Cyn330	T_{12}MA/AP4	upregulated	330	Low Temperature-Induced Gene *Iti65* *(Arabidopsis thaliana)*
Cyn370	T_{12}MA/AP3	upregulated	370	Transcription Factor *NusG* *(Thermus thermophilus)*
Cyn380a	T_{12}MA/AP3	upregulated	380	Transcription Factor *NusG* *(Thermus thermophilus)*
Cyn380b	T_{12}MC/AP1	upregulated	380	Non-dormancy cDNA *(Avena fatua)*
Cyn470	T_{12}MA/AP1	downregulated	470	Heat Shock Protein *(Brugia malayi)*

Table 16. Classification (taxonomic) of the genus *Cynodon*.[a]

Species	Variety	Distribution
C. aethiopicus Clayton et Halan		East Africa rift valleys.
C. arcuatus J.S. Presl. ex C.B. Presl.		Madagascar and southern India to northern Australia.
C. barberi Rang. et Tad.		Southern India.
C. dactylon (L.) Pers.	*dactylon*	Cosmopolitan
	afghanicus	Afghanistan steppes.
	aridus	Southern Africa northward to Plestine; east to south India.
	coursii	Madagascar
	elegans	Southern Africa, south of lat. 13° S.
	polevansii	Near Barberspan, South Africa
C. incompletus Nees	*incompletus*	South Africa; Transvaal to Cape.
	hirsutus	South Africa; Transvaal to Cape.
C. nlemfuensis Vanderyst	*nlemfuensis*	East Africa
	robustus	East Tropical Africa.
C. Plectostachyus (K. Schum.) Pilger		East Tropical Africa
C. transvaalensis Burtt-Davy		South Africa.
C. x magennissii Hurcombe		South Africa.

[a]After Harlan et al. (9).

Table 17. Classification (taxonomic) of the genus *Cynodon.*

Species	Accessions		Origin
	Okla. No.	PI	
C. aethiopicus	A10414	292057	Zambia
	A10416	292059	Tanzania
	A10417	292060	Tanzania
C. arcuatus	A10103	289610	India
	A10105	289612	Madagascar
	A10106	289613	Madagascar
	A10107	289614	Madagascar
	A10108		Madagascar
	A12201		Madagascar
C. barberi	A10609		India
C. dactylon *var.* dacatylon	A10459	292571	Philippines
	A10460	292572	Philippines
	A10681		Australia
	A10981		Israel
	A9958		Italy
	A9959		Yugoslavia
	A9945		Turkey
	Q27766		Australia
	Q27768		Australia
	Q27769		Australia
	Q27770		Australia
	Q27771		Australia
	Q27772		Australia
	Q27773		Australia
	Q27775		Australia
	Q27778		Australia
	A12191		China
	A12203		United States
C. dactylon *var.* afghanicus	A8800	289370	Afghanistan
	A8152		Afghanistan
	A8153		Afghanistan
C. dactylon *var.* coursii	A10124	289715	Madagascar
	A10125		Madagascar
	A10126		Madagascar
	A10127		Madagascar
	A10128		Madagascar
	A10129		Madagascar
	A10235	291146	South Africa
	A10236	291147	South Africa
	A10237	291148	South Africa
	A10238	291149	South Africa
	A10239	291150	South Africa
	A10254	291165	South Africa
	A10333	191610	South Africa
	A10369	291731	South Africa
	A10385	291747	South Africa

Table 17. Classification (taxonomic) of the genus *Cynodon.* (cont.)

Species	Accessions		Origin
	Okla. No.	PI	
C. dactylon var. *incompletus*	A10273		South Africa
	A10278	291189	South Africa
	A10342	291619	South Africa
	A10344	291621	South Africa
C. nlemfuensis var. *robustus*	A10565	293661	Kenya
	A102202		Zimbabwe
	Q28315		Zimbabwe
	Q28316		Zimbabwe
	Q28317		Zimbabwe
C. plectostachyus	A10412	292055	Rhodesia
	A10549	293645	Kenya
	A20554	293650	Kenya
C. transvaalensis		290905	South Africa
		291591	South Africa
		289922	South Africa
		290874	South Africa

Table 18. List of octamer primers used to generate DNA Amplification Fingerprinting from 62 germplasms.

Code[a]	Sequence[b]	Reference[c]	No. of bands[d]	Polymorphic bands
1923	GACGTAGG	1	0	
1924	GTAACCCC	1	64	0
1985	GTTACGCC	1	64	60
1986	GTAACGCC	1,2	55	60
2249	GAAACGCC	1,2	53	49
2250	CCTCGTGG	2	0	47
2273	GATCGCAG	2	54	0
2252	GTCCATTC	1	59	47
2286	GTATCGCC	1	43	53
2287	GAGCCTGT	1	45	40
2288	GAGGGTGG	1	55	43
2289	GTCCAATC	1	47	53
				44

[a] Group of primers synthesized at the Core Facility, Department of Biochemistry and Molecular Biology, OSU.
[b] The fraction of GC content ranges from 50 to 75%.
[c] References: 1, Caetano-Anolles et al. (3); 2, Weaver et al. (18).
[d] Bands scored were ≤ 2000 bp in length.

Table 19. Similarity and Matching (italic type) indices averaged among and within *Cynodon* taxa created using the method of Nei and Li (1979) and Puterka et al. (1993). Ranks shown in parenthesis.

Within taxa			Among taxa										
Similarity	Matching	Taxa	Cae	Car	Cdd	Cda	Cdc	Cde	Cii	Cnr	Cpl	Ctr	Cba
0.812 (5)	0.850 (5)	Cae	-	0.702	0.452	0.551	0.489	0.456	0.411	0.422	0.440	0.409	0.357
0.921 (1)	0.920 (2)	Car	*0.759*	-	0.492	0.539	0.529	0.459	0.447	0.469	0.504	0.446	0.404
0.588 (10)	0.680 (10)	Cdd	*0.569*	*0.572*	-	0.548	0.468	0.441	0.476	0.480	0.473	0.443	0.530
0.875 (3)	0.887 (4)	Cda	*0.618*	*0.608*	*0.648*	-	0.557	0.470	0.484	0.491	0.468	0.550	0.413
0.862 (4)	0.894 (3)	Cdc	*0.614*	*0.593*	*0.595*	*0.669*	-	0.553	0.446	0.463	0.458	0.425	0.432
0.690 (9)	0.782 (9)	Cde	*0.604*	*0.582*	*0.594*	*0.616*	*0.676*	-	0.499	0.492	0.474	0.439	0.380
0.792 (7)	0.831 (7)	Cii	*0.538*	*0.532*	*0.592*	*0.573*	*0.565*	*0.630*	-	0.737	0.502	0.509	0.412
0.758 (8)	0.787 (8)	Cnr	*0.528*	*0.525*	*0.574*	*0.572*	*0.571*	*0.608*	*0.777*	-	0.548	0.540	0.407
0.797 (6)	0.834 (6)	Cpl	*0.565*	*0.574*	*0.592*	*0.591*	*0.584*	*0.616*	*0.637*	*0.631*	-	0.734	0.406
0.878 (2)	0.923 (1)	Ctr	*0.561*	*0.560*	*0.595*	*0.602*	*0.583*	*0.615*	*0.646*	*0.646*	*0.811*	-	0.370
*	*	Cba	*0.551*	*0.574*	*0.648*	*0.581*	*0.572*	*0.603*	*0.587*	*0.557*	*0.455*	*0.593*	-

* only one accession included

Cae = *Cynodon aethiopicus;* Car = *Cynodon arcuatus;* Cdd = *Cynodon dactylon,* dactylon; Cda = *Cynodon dactylon, afghanicus;* Cdc = *Cynodon dactylon, coursii;* Cde = *Cynodon dactylon, elegans;* Cii = *Cynodon incompletus, incompletus;* Cnr = *Cynodon nlemfunesis, robustus;* Cpl = *Cynodon pletostastachyus;* Ctr = *Cynodon transvaalensis;* Cba = *Cynodon barberi.*

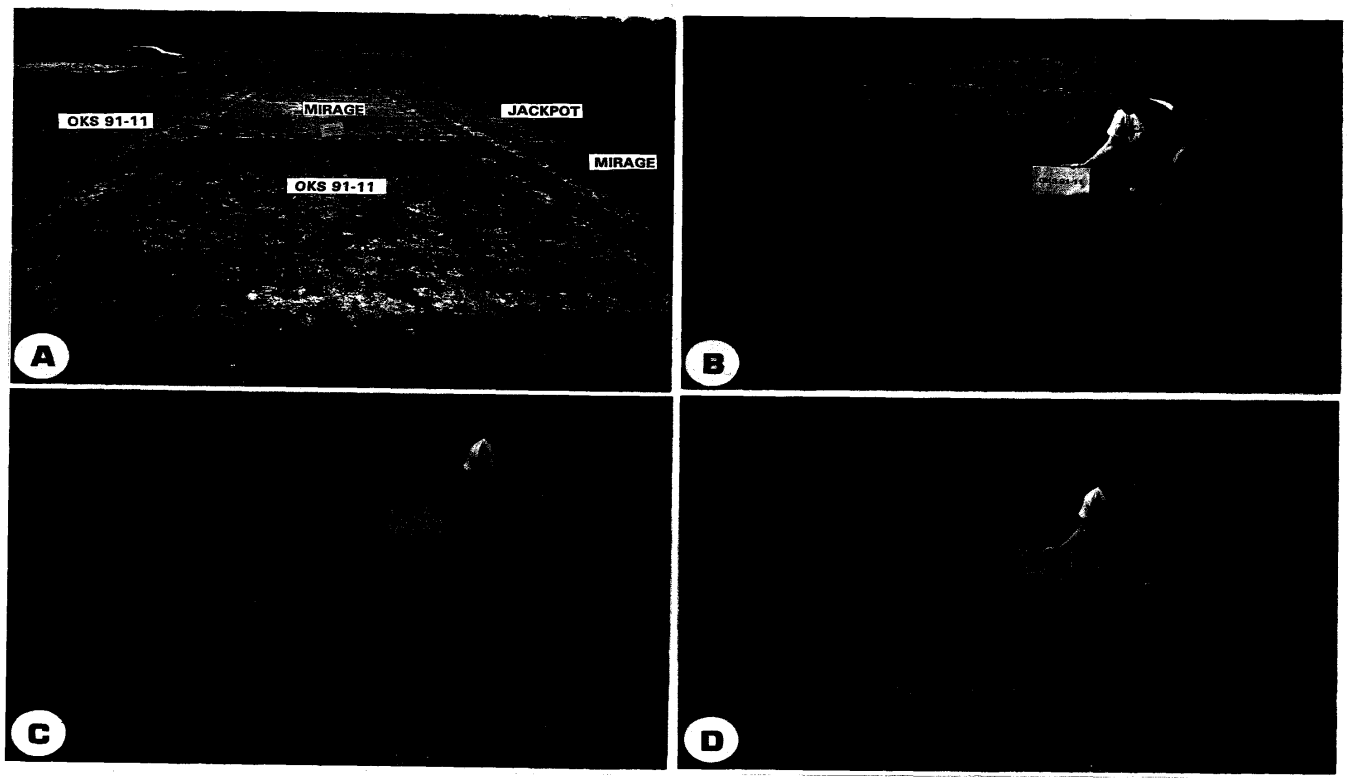


Fig. 1. Relative greenup of OKS 91-11, Jackpot and Mirage seed-propagated bermudagrasses in plots on the OSU Turf Center. Plots were seeded July 1995. (A) Overview of plots on 5/15/96. (B-D). Photographs taken 5/20/96 showing relative greenup of OKS 91-11 (B), Jackpot (C), and Mirage (D).

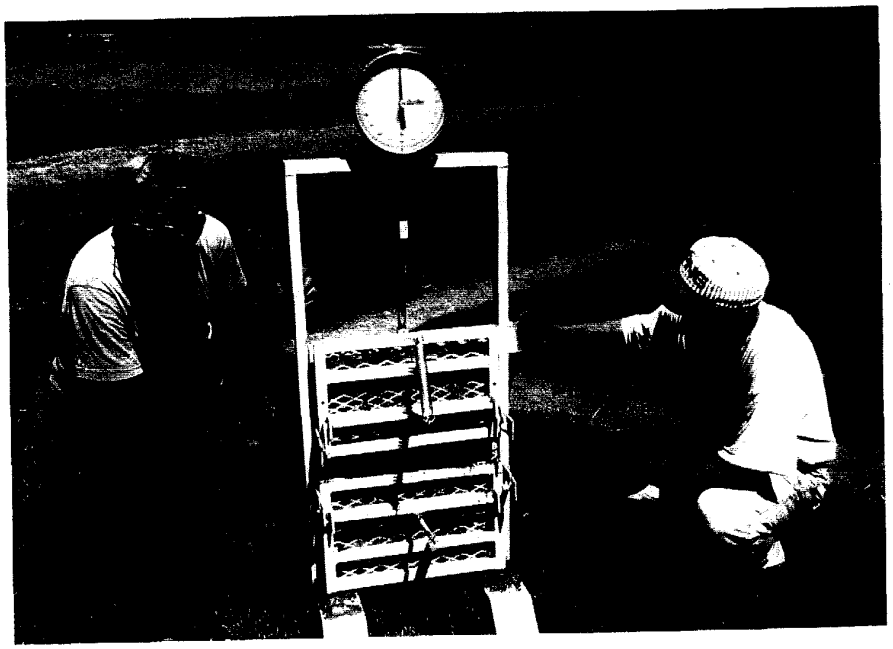


Fig. 2. Machine to measure sod shear strength.

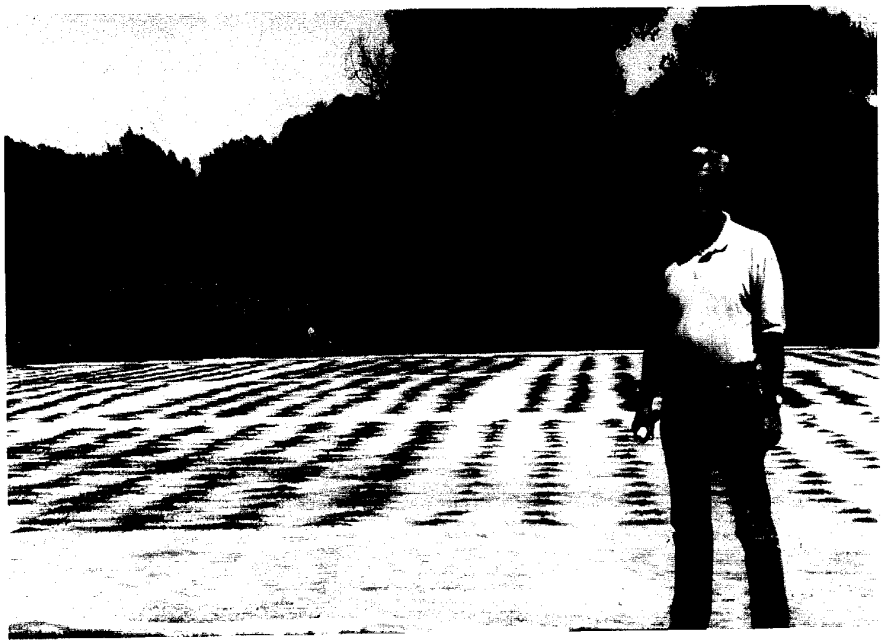


Fig. 3. Oklahoma City Golf and Country Club Superintendent Craig Elms at site on newly planted bermudagrass test.

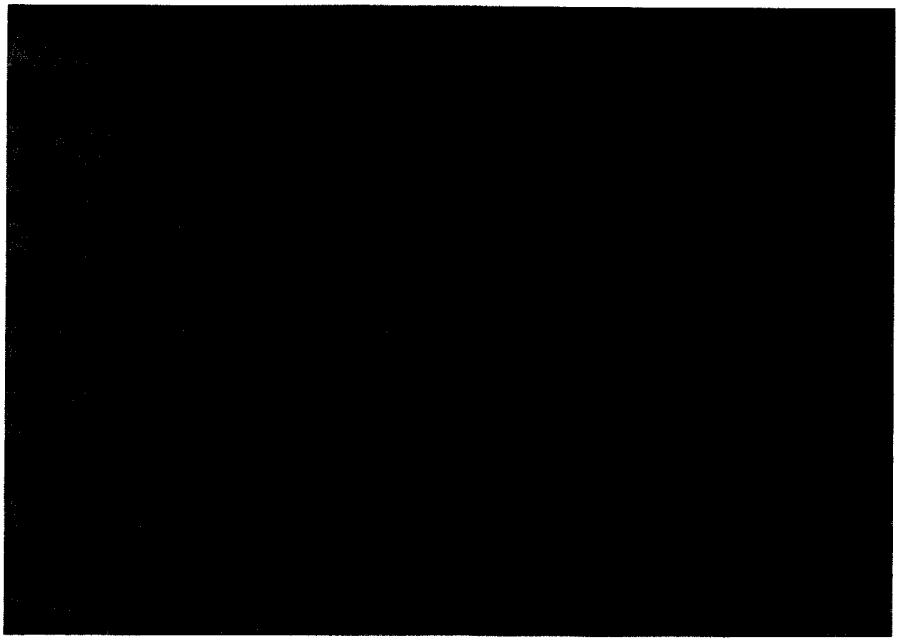
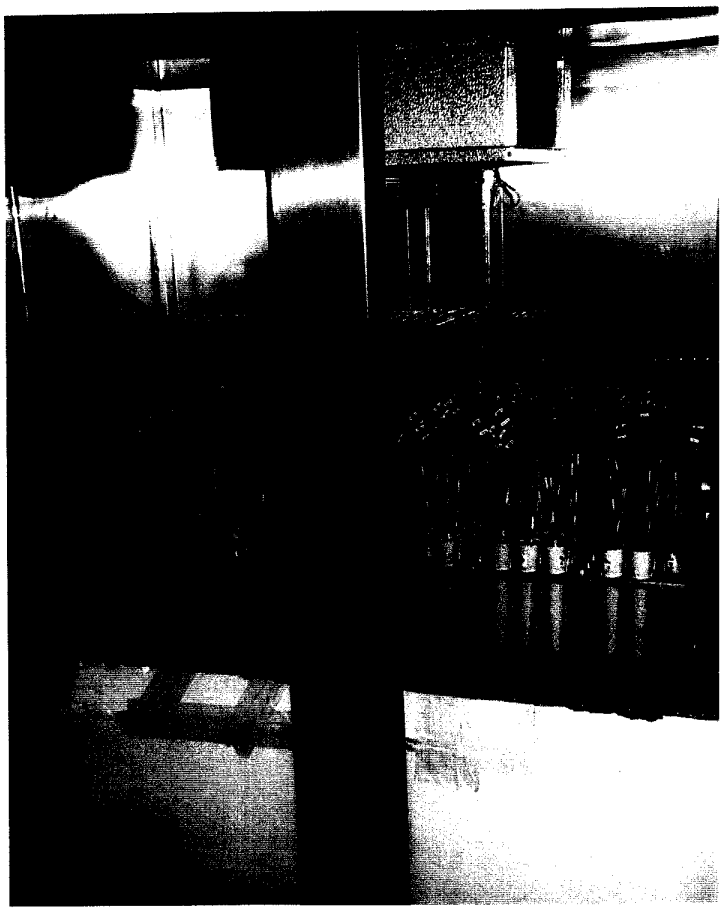


Fig. 4. Henry Wetzel, Kansas State University Ph.D. student inoculates bermudagrass plots with spring dead spot causal organism.



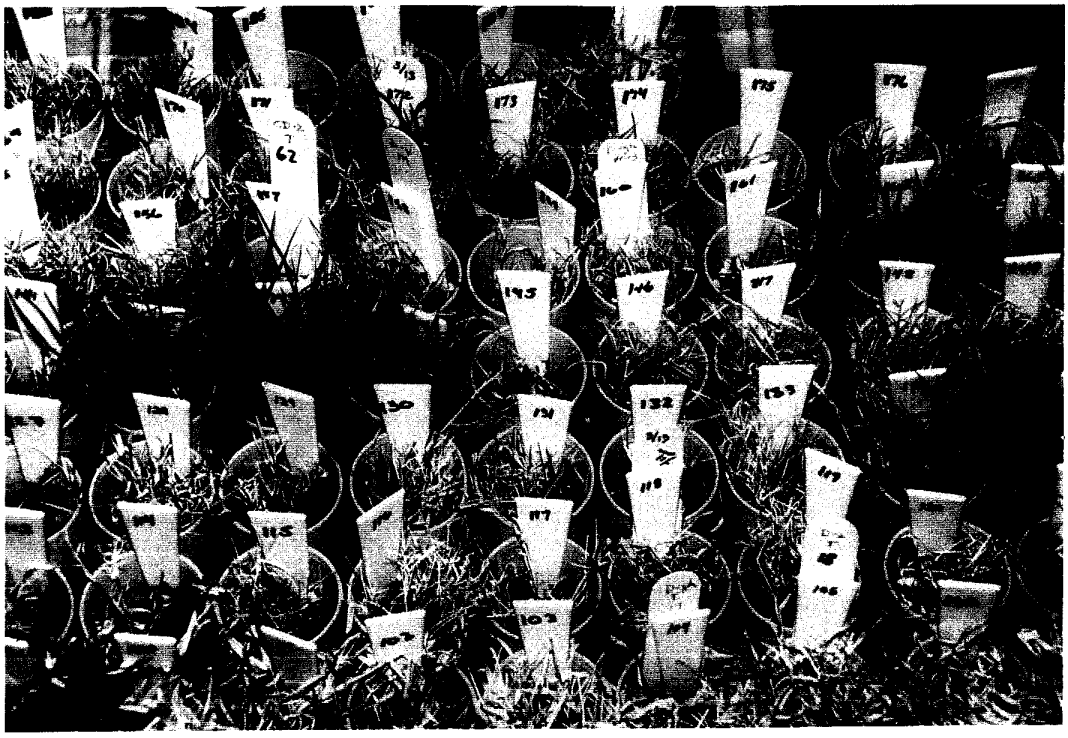
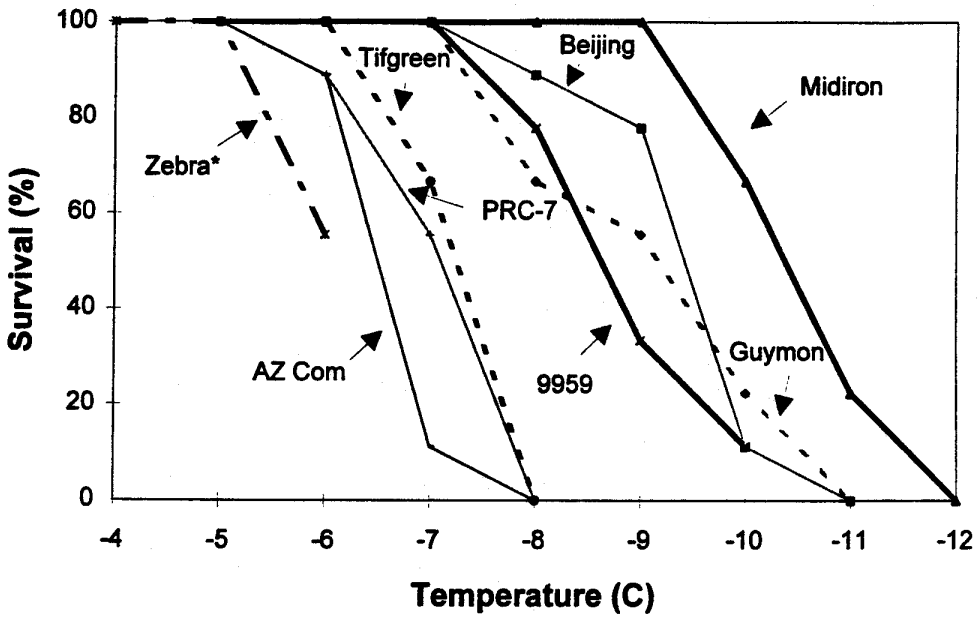


Fig. 5. (Top) Bermudagrass plants in freeze chamber.

Fig. 6. (Bottom) Regrowth evaluation of bermudagrass plants following exposure to freezing temperatures.



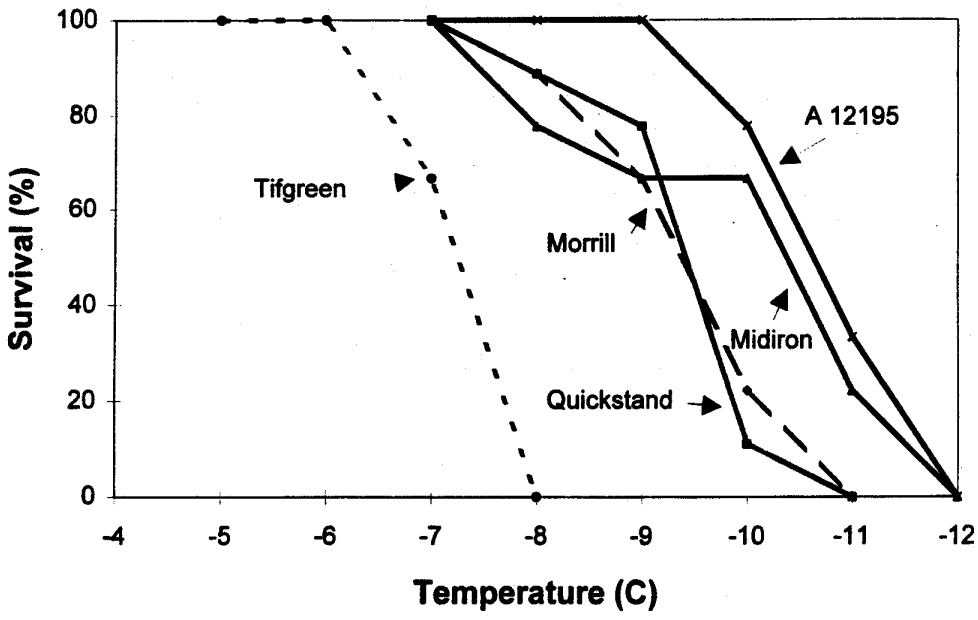


Fig. 6a-b. **Response of cold acclimated bermudagrass cultivars to freezing temperatures.**

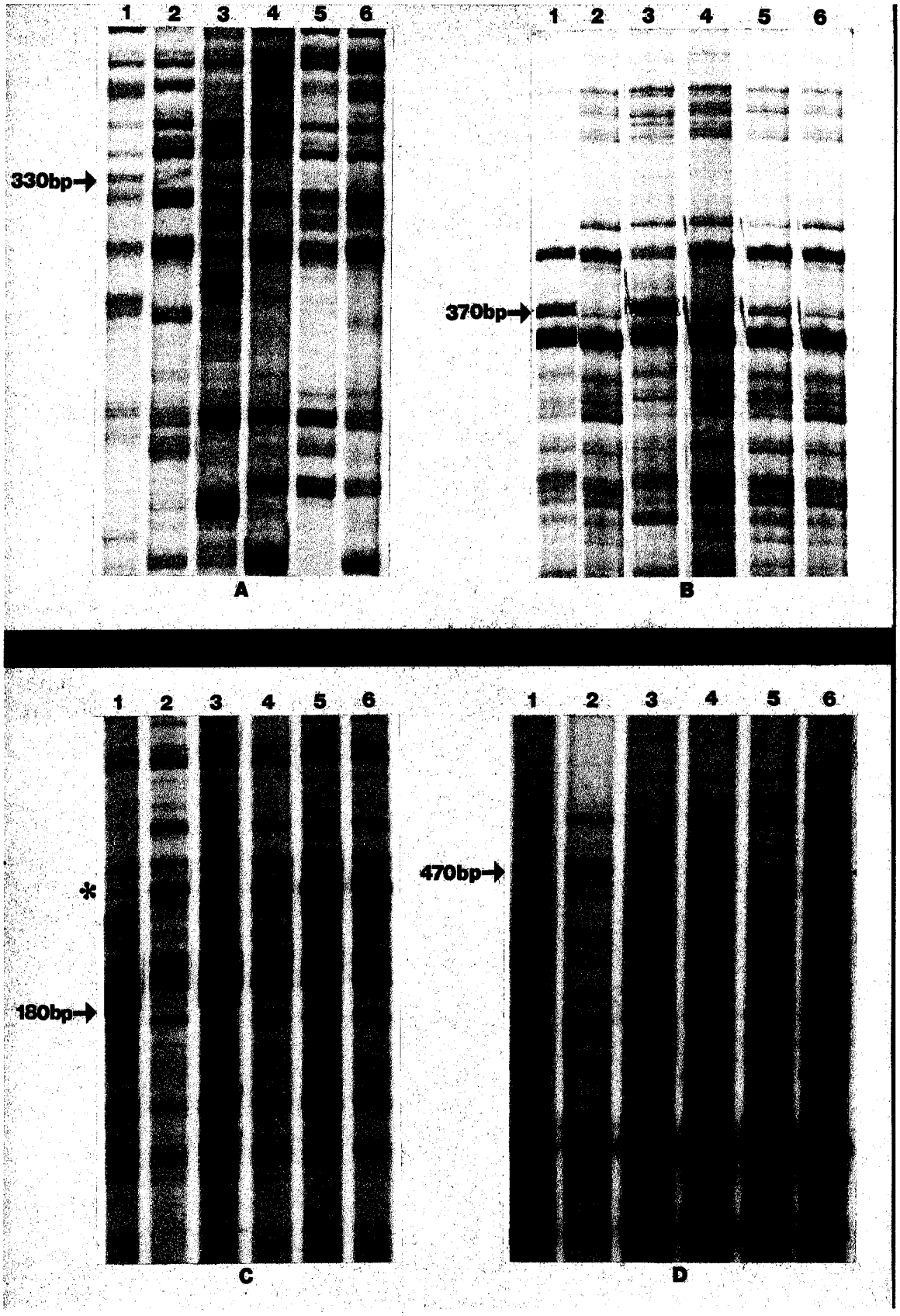


Fig. 7. Differential display of crown tissue mRNA showing upregulated cDNA Cyn330 (A), Cyn370 (B), Cyn180 (C) and down regulated cDNA Cyn470 (D). Lanes 1, 3, 5 = control, lane 2 = 2 days cold acclimated, lane 4 = 28 days cold acclimated, lane 6 = 2 days deacclimation. *no data on reverse prime analysis.

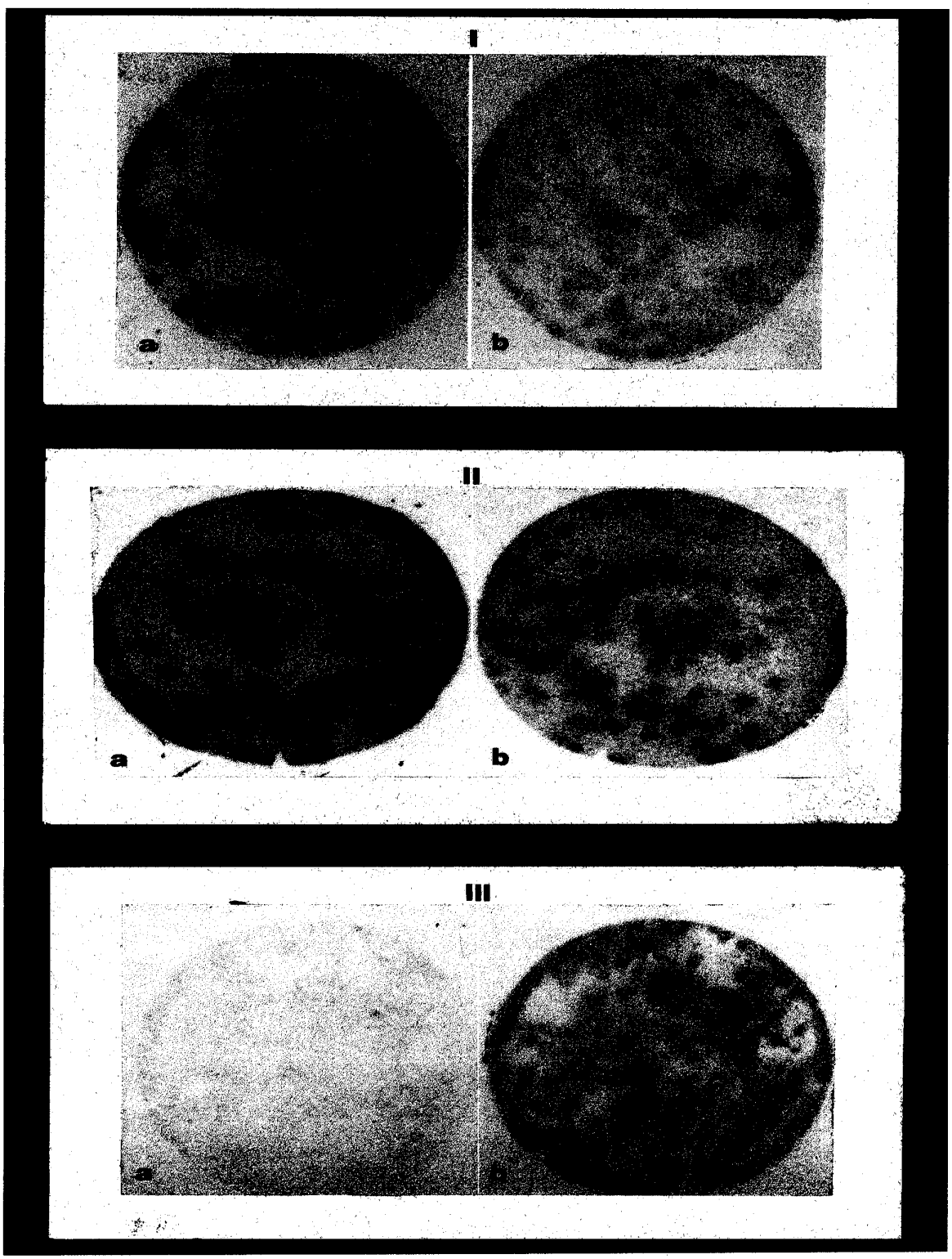


Fig. 8. Reverse prime analysis on duplicate colony lifts from Cyn330 (I), Cyn370 (II) and Cyn470 (III). a = colony lift hybridized with labeled cDNA reverse transcribed from 28 days cold acclimation total RNA; b = colony lift hybridized with labeled cDNA reverse transcribed from control total RNA.

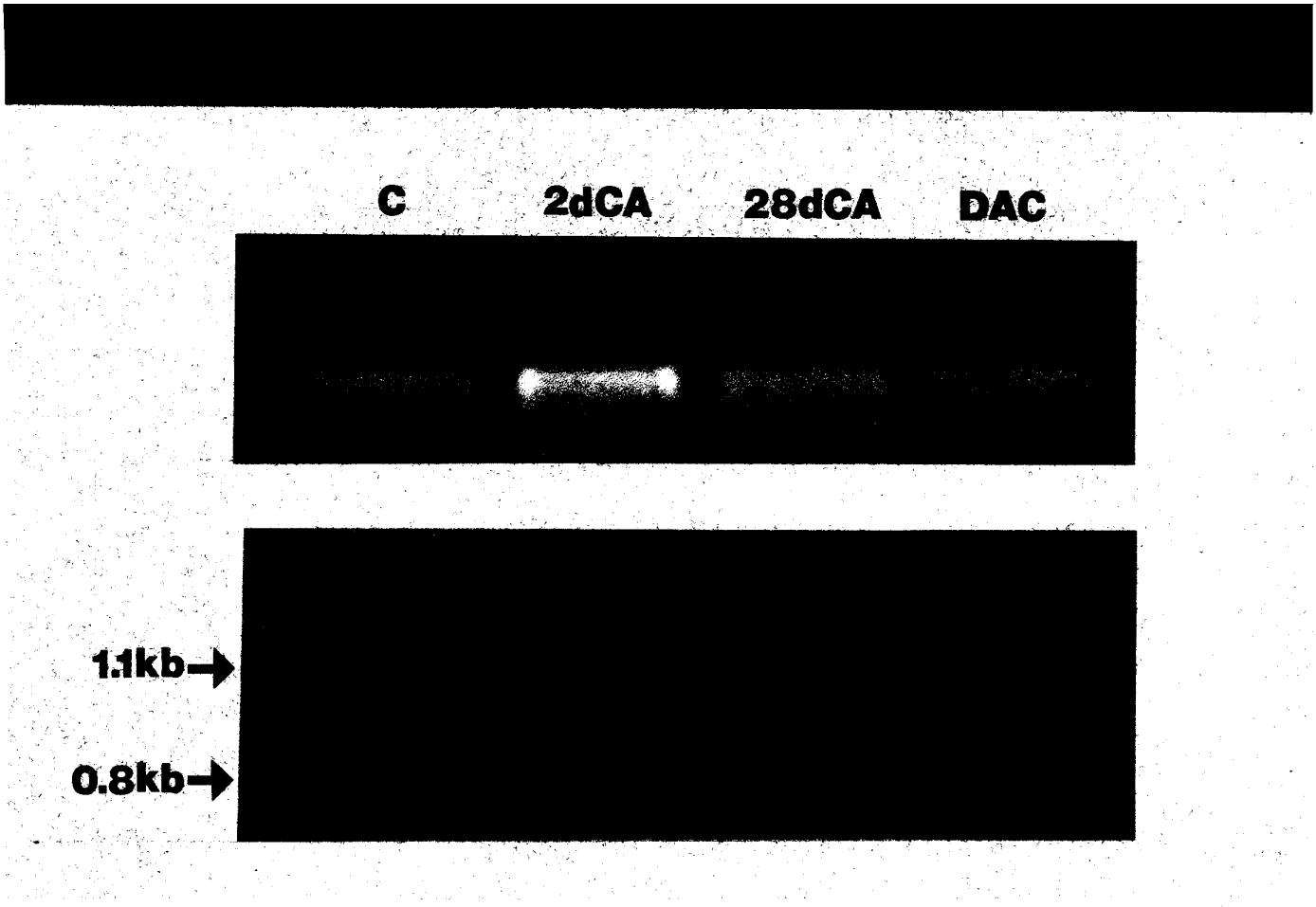


Fig. 9. Northern blot showing expression pattern of Cyn330 during cold acclimation and deacclimation of 'Midiron'. The approximate sizes of mRNA hybridizing to the probe are shown with arrows.

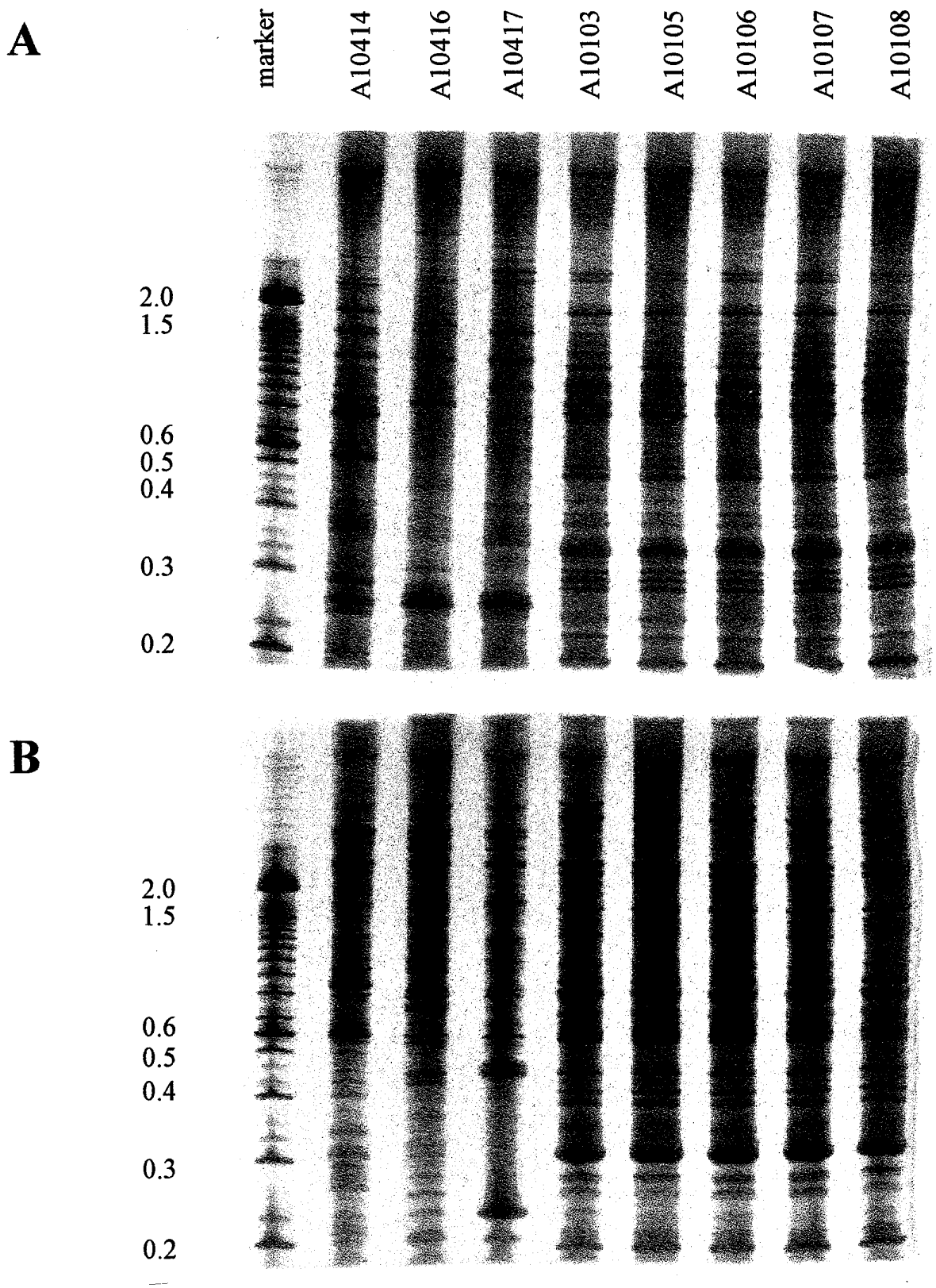


Fig. 10. DNA amplification profiles of bermudagrass accessions. A and B reproducible DNA patterns of *C. aethiopicus* and *C. arcuatus* amplified with arbitrary primers GATCGCAG and GTCCATTC. Molecular weights are given in Kb.

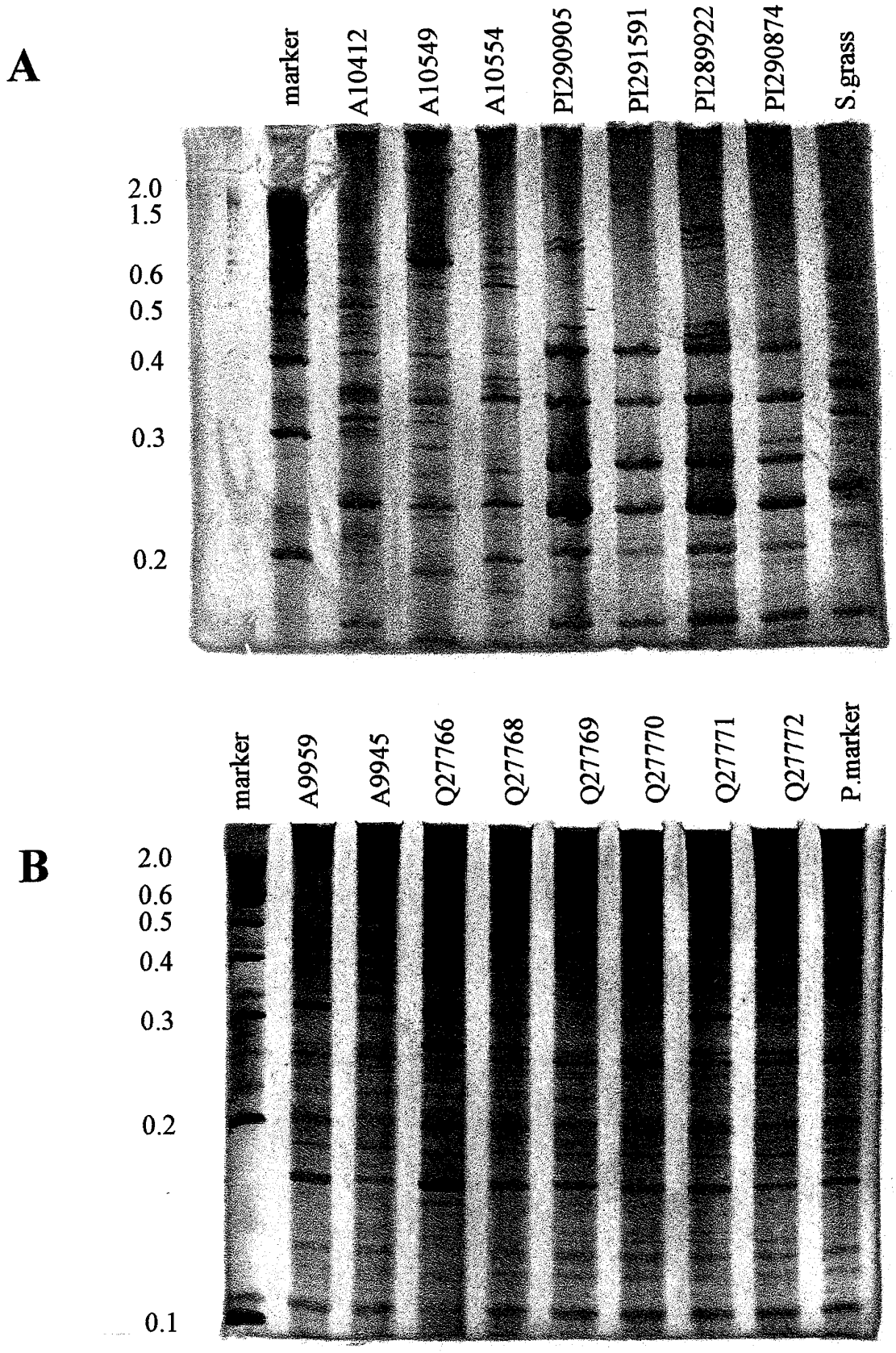


Fig. 11. DNA amplification profiles of bermudagrass accessions. A and B reproducible DNA amplifications of *C. plectostachyus, C. transvaalensis,* and *C. dactylon* generated using primer GATCGCAG. Molecular weights are given in Kb.

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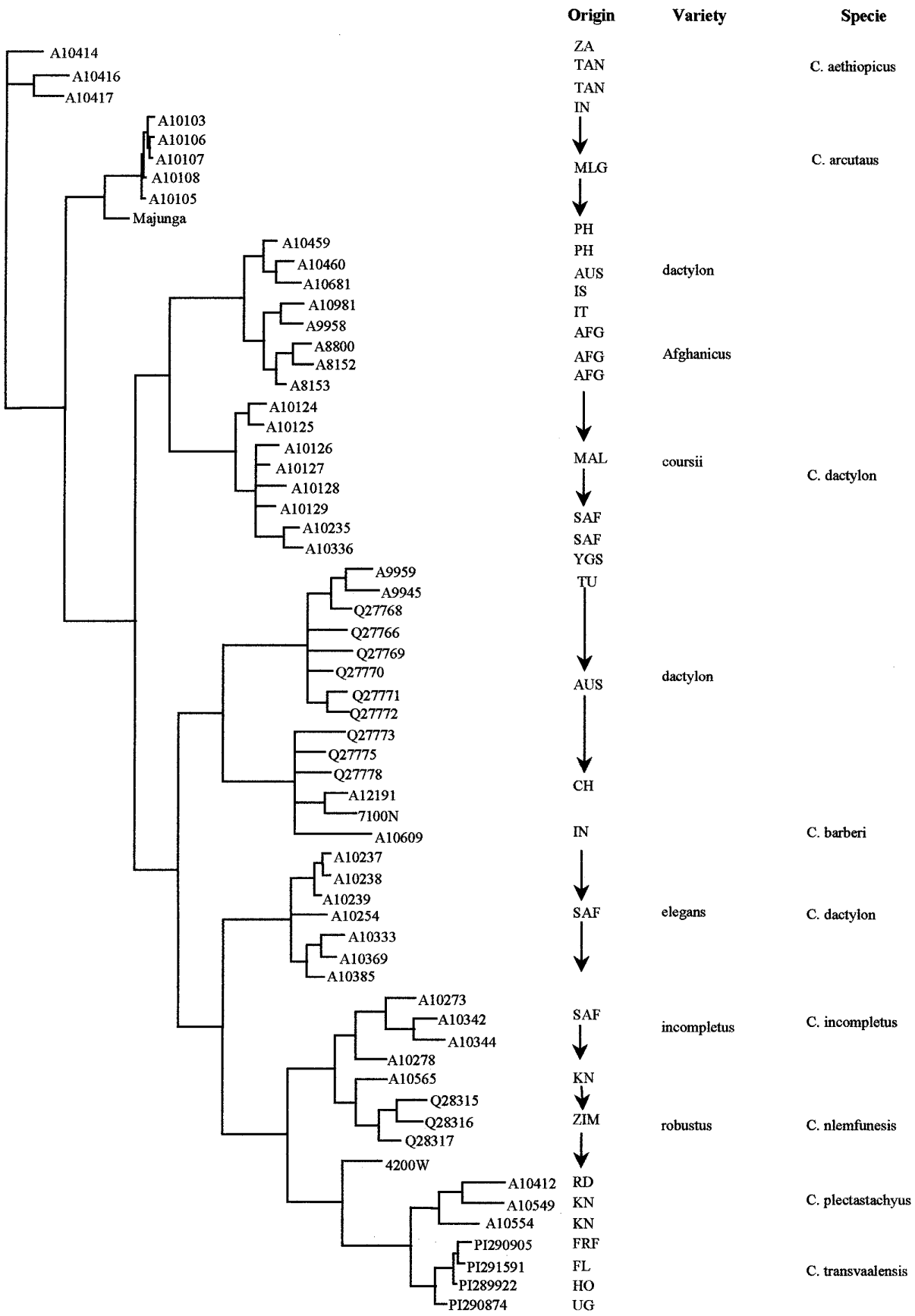

Fig. 12. Minimal phylogenetic tree for the 62 *Cynodon* accessions.