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Full Length Research Paper

DNA content of several bermudagrass accessions in Florida

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Bermudagrass (*Cynodon* spp.) is widely used on golf courses and sport fields in Florida. Most cultivars used on golf courses are triploid bermudagrass (*Cynodon dactylon* [L.] Pers. var. *dactylon* × *C. transvaalensis* Burtt-Davy) resulting from hybridizations between tetraploid common bermudagrass and diploid African bermudagrass. In order to breed and develop bermudagrass cultivars with superior characteristics, it is essential to know the ploidy levels of available bermudagrass accessions and select fertile ones. The objective of this study was to determine the DNA content and ploidy level of bermudagrass germplasm accessions in the University of Florida germplasm collection to aid in future cultivar breeding. Flow cytometry was used to determine the nuclear DNA contents of 48 bermudagrass accessions, and one diploid (2%), 19 triploid (40%), 24 tetraploid (50%), one pentaploid (2%) and three hexaploid (6%) accessions were identified. The range of the nuclear DNA contents was 1.17, 1.38 to 1.61, 1.94 to 2.24, 2.47 and 2.64 to 2.75 pg/2C nucleus^{-1} for the respective ploidy levels. As such, tetraploid and hexaploid accessions could be utilized for future breeding efforts. The triploid accessions could be the results of mutations that have occurred in existing commercial bermudagrass cultivars or from natural hybrids between diploid *C. transvaalensis* and tetraploid *C. dactylon*.

Key words: *Cynodon* spp., flow cytometry, nuclear DNA content, ploidy level.

INTRODUCTION

Bermudagrass (*Cynodon* spp.) is widely distributed in China, India, Africa, Australia, South America and the southern region of the United States (Abulaiti and Yang, 1998; Duble, 2010; Wu et al., 2006). In the United States, it is distributed throughout the warmer regions: from Florida northward to Maryland and New Jersey along the east coast, and westward along the southern border to California (Duble, 2010). In Florida, bermudagrass is one of the most common warm-season grasses. Its improved fine-textured cultivars produce a vigorous and dense turf that is widely used on golf courses, sports fields, lawns and parks (Duble, 2010; Trenholm et al., 2003). There are nine species in the genus, *Cynodon*, and the basic chromosome number is nine (Duble, 2010). Tetraploid *C. dactylon*, common bermudagrass (2n = 4x = 36), is the most widespread species (de Silva and Snaydon, 1995; Duble, 2010), while *Cynodon transvaalensis* (2n = 2x = 18), African bermudagrass, is a diploid species (Forbes and Burton, 1963; Wu et al., 2006). Triploid *Cynodon* (2n = 3x = 27) from hybridizations of tetraploid *C. dactylon* and diploid *C. transvaalensis* produce fine-textured, dense bermudagrass cultivars that have become the standards for use on golf courses in Florida and other regions where warm season turfgrasses are utilized. Moreover, pentaploid (2n = 5x = 45) and hexaploid (2n = 6x = 54) plants have been previously reported (Burton et al., 1993; Hanna et al., 1990; Johnston, 1975; Kang, 2007; Wu et al., 2006). 'Tifton 10', released as a hexaploid cultivar, has been used on golf courses, athletic fields and home lawns (Hanna et al., 1990).

Flow cytometry (FCM) provides a rapid and accurate DNA content analysis and ploidy level determination for plant breeding programs (Arumuganathan and Earle, 1991; Dolezel et al., 1989; Schwartz et al., 2010). Genome sizes and ploidy levels of warm-season grass

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species, such as buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], *Paspalum* spp., *Zoysia* spp. and *Cynodon* spp. have been described by using flow cytometry (Jarret et al., 1995; Johnson et al., 1998; Schwartz et al., 2010; Taliaferro et al., 1997; Vaio et al., 2007). Conversely, Arumuganathan et al. (1999) reported the nuclear genome size of diploid, triploid and tetraploid bermudagrass genotypes. Triploid, tetraploid, pentaploid and hexaploid genotypes were identified in Chinese and Korean bermudagrass accessions, respectively, by Kang et al. (2007) and Wu et al. (2006).

However, information about the DNA content or ploidy level of bermudagrass accessions collected in Florida is limited. In order to develop hybrid cultivars with superior turf quality, it is essential to know the ploidy levels of the available bermudagrass genotypes in the University of Florida (UF) germplasm collection. Sterile accessions such as triploids or pentaploids could not be used for future cultivar breeding. The objective of this study was to determine the nuclear DNA content and ploidy level of selected superior UF bermudagrass germplasm accessions, and select fertile accessions for grass breeding.

MATERIALS AND METHODS

Plant materials

Forty-seven *Cynodon* spp. genotypes were selected for having superior turfgrass performance in Gainesville, FL, while three commercial cultivars, 'Tifway', 'Tifgreen' and 'Tifton 10', with known ploidy levels or nuclear DNA contents were included in this test (Table 1) . A known diploid African bermudagrass accession, 'AB33', was also included as a reference. Each genotype was vegetatively propagated into 15 cm diam pots filled with 100% USGA specification green sand and was grown in a glasshouse at the University of Florida. The grass was maintained at a temperature range of 24 to 34°C under natural daylight in May, 2009. Grasses were watered for six minutes a day by an overhead automatic irrigation system and fertilized once every other week, using 24N-8P-16K at a rate of 0.5 kg N / 100 m^2 (1 lb N / 1000 ft^2) per growing month. However, grass leaves were clipped once a week except for aerial stolons.

Flow cytometry

Flow cytometry analyses were conducted in the forage evaluation support laboratory (FESL) at the University of Florida on a Partec PA, one-parameter flow cytometer (Partec GmbH, Otto-Hahn-Str. 32, D-48161 Munter, Germany) with a 100-watt HBO short arc lamp emitting UV light at 420 nm to excite fluorescence. The nuclear DNA content was measured by procedures modified from Arumuganathan and Earle (1991). When, at least, 10 aerial stolons were present in each pot (two months after planting the grass), flow cytometry analysis was started. A terminal node and tip from one stolon was removed from each accession and stored on ice before the flow cytometry analysis. A CyStain® PI Absolute P (05-5002, Partec North America, Inc., Mt. Laurel, NJ) nuclei extraction and DNA staining buffer kit was used to prepare samples. Triploid trout erythrocyte nuclei (BioSure® Inc., Grass Valley, CA) with a nuclear DNA content of 7.2 pg/2C $nucleus^{-1}$ (Hardie and Hebert, 2003; 2004) were used as an internal standard. About 50 mg of fresh nodal or stolon tip tissue from each accession was chopped with a razor blade into tiny pieces on a Petri dish, into which 400 L of nuclear extraction solution was added. After incubation for one minute, the solution was transferred into a 5-mL test tube through a 50-µm Partec CellTrics® monofil nylon filter. After adding 1.6 mL DNA staining to the solution and incubating for another 10 min under room temperature, five drops of triploid trout erythrocyte nuclei were added into the test tube and were mixed well with the solution. The test tube with the solution was put into the flow cytometer and the DNA content of each plant sample was measured based on at least 10,000 scanned nuclei per sample. For each accession, three replications were measured on three different days. The sample's DNA content was calculated by the following formula:

Sample's nuclear DNA content = [(mean position of sample peak) / (mean position of the control peak)] × DNA content of the control (Arumuganathan et al., 1999).

The mean and standard deviation of the genome size were calculated for each genotype, and the ploidy levels of the genotypes were then determined by the genome size ranges of the respective ploidy levels reported previously (Arumuganathan et al., 1999; Kang et al., 2007; Taliaferro et al., 1997; Wu et al., 2006).

RESULTS AND DISCUSSION

Plant leaves were used for nuclear extraction and staining, but the results were not as consistent as those of terminal nodes or stolon tips. The same problems were observed in *Zoysia* spp. (Schwartz et al., 2010). Plant tissues under stress or with disease were also used; however, they yielded variable results. The peaks were not sharp or consistently clear for some samples. The reason could be that the DNA of the diseased plant tissue could be contaminated with that of the pathogen, and an interaction between them could counteract the peaks of the plant samples, which resulted in the unclear peaks. Therefore, healthy, non-stressed nodal plant tissue should be used for flow cytometry studies. Plants have been used as internal controls for grasses in previous FCM studies. Diploid barley (*Hordeum vulgare* L.), hexaploid wheat (*Triticum aestivum* L.) and tobacco (*Nicotiana tabacum* L) were used to test the nuclear DNA contents of 13 turfgrass species (Arumuganathan et al., 1999) . Tetraploid bermudagrass, 'Savannah', was used as an internal standard for bermudagrass in FCM studies because its nuclear content was similar to other genotypes tested (Kang et al., 2007). In this study, we also used triploid bermudagrass cultivar, 'Tifway', with a known DNA nuclear content (Arumuganathan et al., 1999; Wu et al., 2006) as an internal control. However, clear and repeatable peaks were not obtained for all genotypes, especially for those with a nuclear DNA content similar to the control. As such, interactions occur between plant samples and the control, which counteract with the peaks of the samples. Plant control with a DNA content overlapping those of the samples was not a good internal standard in this study. Nonetheless, with a larger nuclear DNA content (7.2 pg/2C $nucleus^{-1}$), no interaction

Table 1. Nuclear DNA content and ploidy level of 48 bermudagrass accessions and three cultivars from the University of Florida.

Accession	DNA content mean ± SD	Inferred ploidy (2n)	Accession	DNA content mean ± SD	Inferred ploidy (2n)
	pg/2C			pg/2C	
102	1.61 ± 0.06	3x	355	2.24 ± 0.12	4x
131	2.02 ± 0.12	4x	445	2.05 ± 0.05	4x
132	2.20 ± 0.12	4x	481	1.94 ± 0.08	4x
157	2.00 ± 0.03	4x	489	1.38 ± 0.16	3x
171	2.09 ± 0.09	4x	490	1.50 ± 0.15	3x
173	1.45 ± 0.08	3x	525	1.54 ± 0.07	3x
227	2.20 ± 0.14	4x	528	2.08 ± 0.16	4x
282	1.49 ± 0.06	3x	PI 289922	2.47 ± 0.07	5x
283	1.54 ± 0.08	3x	PI 290868	2.08 ± 0.06	4x
285	1.48 ± 0.09	3x	PI 290872	2.00 ± 0.03	4x
286	1.49 ± 0.08	3x	PI 290895	1.51 ± 0.02	3x
291	1.47 ± 0.08	3x	PI 291590	1.94 ± 0.09	4x
293	1.52 ± 0.08	3x	UFC03	2.08 ± 0.15	4x
295	2.70 ± 0.01	6x	UFC06	2.19 ± 0.06	4x
296	1.48 ± 0.11	3x	UFC07	2.75 ± 0.09	6x
297	1.63 ± 0.03	3x	UFC11	2.16 ± 0.06	4x
299	1.98 ± 0.05	4x	UFC12	2.06 ± 0.01	4x
301	1.98 ± 0.10	4x	UFC25	1.59 ± 0.05	3x
304	2.16 ± 0.03	4x	UFC26	1.56 ± 0.03	3x
319	1.44 ± 0.02	3x	UFC29	2.00 ± 0.11	4x
320	1.60 ± 0.10	3x	UFC30	1.97 ± 0.06	4x
334	2.03 ± 0.02	4x	Tifway	1.53 ± 0.05	3x
343	2.10 ± 0.06	4x	Tifgreen	1.58 ± 0.05	3x
344	2.64 ± 0.06	6x	Tifton 10	3.06 ± 0.01	6x
347	2.06 ± 0.09	4x	AB33	1.17 ± 0.07	2x
352	1.50 ± 0.01	3x			

was found between trout erythrocyte nuclei and bermudagrass cells in that clear, consistent and repeatable peaks were obtained for all genotypes. Therefore, trout erythrocyte nuclei were a very good internal standard for bermudagrass FCM analysis. Other animal blood cells such as those of Channel catfish (*Ictalurus punctatus*) have also been reported as a good standard for bermudagrass nuclear DNA content measurement (Wu et al., 2006). Using this modified method, the CVs for the peaks of all genotypes were less than 6.0%. The standard deviations of nuclear DNA content ranged from 0.01 to 0.15 pg/2C nucleus^{-1}, which agreed with previous studies by Arumuganathan et al. (1999), Kang et al. (2007) and Wu et al. (2006) in that flow cytometry was a very precise method for bermudagrass genome size measurement.

Mean nuclear DNA contents and ploidy levels for the 48 bermudagrass accessions and three reference cultivars are presented in Table 1. Likewise, the histograms of the flow cytometry peaks are shown in Figure 1. The peaks of the trout erythrocyte nuclei were relatively smaller than those of the plant samples because there were fewer cells in the triploid trout erythrocyte nuclei than the plant samples, but this did not affect the results. Clear and consistent peaks were obtained for all genotypes, and the cells in the G2 phase were also observed. However, the nuclear DNA content of the UF bermudagrass accessions varied from 1.38 to 2.75 pg/2C nucleus^{-1} (Table 2). These values were lower than those of previously reported studies on Korean (Kang et al., 2007) and Chinese accessions (Wu et al., 2006). Using previously reported nuclear DNA content ranges (Arumuganathan et al., 1999; Kang et al., 2007; Taliaferro et al., 1997; Wu et al., 2006) to infer ploidy levels, UF accessions were classified as follows: one (2%) diploid accession with a genome size of 1.17 pg/2C nucleus $^{-1}$, 19 (40%) triploid accessions with a genome size of 1.38 to1.61 pg/2C nucleus^{-1}, 24 (50%) tetraploid accessions with a genome size of 1.94 to 2.24 pg/2C nucleus^{-1}, one (2%) pentaploid accession with a genome size of 2.47 pg/2C nucleus^{-1} and three (6%) hexaploid accessions with a genome size of 2.64 to 2.75 pg/2C nucleus^{-1} (Table 2). Moreover, the

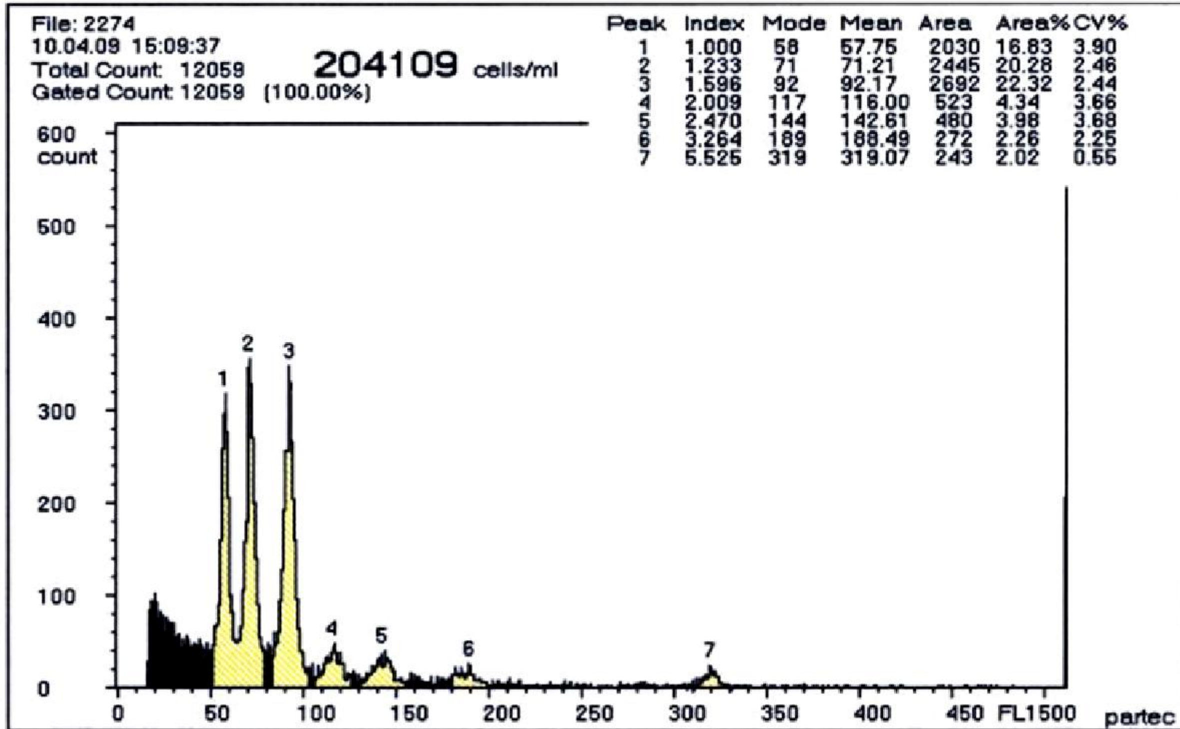

Figure 1. Flow cytometric histogram of diploid, triploid and tetraploid bermudagrass and trout erythrocyte nuclei. Peak 1 = diploid accession; Peak 2 = triploid cultivar (Tifgreen) control; Peak 3 = tetraploid accession; Peak 4 = G2 phase of diploid accession; Peak 5 = G2 phase of triploid cultivar control; Peak 6 = G2 phase of tetraploid accession; Peak 7 = trout erythrocyte nuclei.

Table 2. Nuclear DNA content ranges of 48 bermudagrass accessions and three cultivars from the University of Florida.

Ploidy level (2n)	DNA content (pg/2C)	Genotype
2x	1.17	AB33
3x	1.38 to 1.61	102, 282, 283, 285, 286, 291, 293, 296, 297, 319, 320, 352, 489, 490, 525, 290895, UFC25, UFC26, ‘Tifway’, ‘Tifgreen’, ‘Tifton 10’
4x	1.94 to 2.24	131, 132, 157, 171, 173, 227, 299, 301, 304, 334, 343, 347, 355, 445, 481, 528, PI 290868, PI 290872, PI 291590, UFC03, UFC06, UFC11, UFC12, UFC29, UFC30
5x	2.47	PI 289922
6x	2.64-2.75	295, 344, UFC07

genome sizes of all accessions were previously reported internally by the ploidy ranges.

The nuclear DNA contents of cultivars ‘Tifway’, ‘Tifgreen’ and ‘Tifton 10’ in this study were very close to the values previously reported (Arumuganathan et al., 1999; Wu et al., 2006), which verified the accuracy of this assay. When compared to the Korean and Chinese accessions (Kang et al., 2007; Wu et al., 2006), a lower percentage of tetraploid genotypes were identified among these superior UF accessions. Relatively, more triploid accessions (40%) are identified, which are likely mutants of commercial triploid cultivars. The 48 bermudagrass accessions evaluated were selected based on their multi-year performance, from a larger germplasm collection of 180 accessions. The 180 accessions were collected primarily from managed turf sites in the state of Florida, and many from golf courses that were likely planted with a triploid bermudagrass cultivar. Due to the fact that

triploids are known for having superior turfgrass performance, it is very probable that inadvertently collected triploids would have been selected as part of the group of 48 accessions that represent those genotypes with the best overall multi-year performance. If the entire collection of 180 genotypes had been evaluated, it is the percentage of triploids that would have likely reduced and the percentage of tetraploids would have increased.

Conclusions

All tetraploid and hexaploid accessions could be used in bermudagrass breeding. Triploid accessions with good horticultural characters or disease resistance could be released in the future. This study provided valuable information for future bermudagrass cultivar development in Florida.

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REFERENCES

Abulaiti DSS, Yang G (1998). A preliminary survey of native Xinjiang bermudagrass. J. Xin j. Agric. Univ., 21: 124–127.

Arumuganathan K, Earle ED (1991). Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol. Biol. Rep., 9: 229-233.

Arumuganathan K, Tallury SP, Fraser ML, Bruneau AH, Qu R (1999). Nuclear DNA content of thirteen turfgrass species by flow cytometry. Crop Sci., 39: 1518-1521.

Burton GW, Gates RN, Hill GM (1993). Registration of 'Tifton 85' bermudagrass. Crop Sci., 33:644–645.

de Silva PHAU, Snaydon RW (1995). Chromosome number in *Cynodon dactylon* in relation to ecological conditions. Ann. Bot. (London), 76: 535–537.

Dolezel J, Binarova P, Lucretti S (1989). Analysis of nuclear DNA content in plant cells by flow cytometry. Biol. Plant., 31: 113-120.

Duble RL (2010). Bermudagrass. The Sports Turf of the South. Available at http://plantanswers.tamu.edu/turf/publications/bermuda.html (verified 14 Sep. 2010). Texas Cooperative Extension, Department of Soil and Crop Sciences, Texas A&M University, College Station, TX.

Forbes I, Burton GW (1963). Chromosome numbers and meiosis in some *Cynodon* species and hybrids. Crop Sci., 3: 75–79.

Hanna WW, Burton GW, Johnson AW (1990). Registration of 'Tifton 10' turf bermudagrass. Crop Sci., 30: 1355–1356.

Hardie DC, Hebert PDN (2003). The nucleotypic effects of cellular DNA content in cartilaginous and ray-finned fishes. Genome, 46: 683-706.

Hardie DC, Hebert PDN (2004). Genome-size evolution in fishes. Can. J. Fish. Aquat. Sci., 61: 1636-1646.

Jarret RL, Ozias -Akins P, Phatak S, Nadimpalli R, Duncan R, Hiliard S (1995). DNA contents in *Paspalum* spp. determined by flow cytometry. Genet. Resour. Crop Evol., 42: 237-242.

Johnson PG, Riordan TP, Arumuganathan K (1998). Ploidy level determinations in buffalograss clones and populations. Crop Sci., 38: 478-482.

Johnston RA (1975). Cytogenetics of some hexaploid × tetraploid hybrids in *Cynodon*. M.S. thesis. Oklahoma State Univ., Stillwater.

Kang SY, Lee GJ, Lim KB, Lee HJ, Park IS, Chung SJ, Kim JB, Kim DS, Rhee HK (2007). Genetic Diversity among Korean Bermudagrass (*Cynodon* spp.) Ecotypes Characterized by Morphological, Cytological and Molecular Approaches. Mol. Cells, 25(2): 163-171.

Schwartz BM, Kenworthy KE, Engelkec MC, Genovesic AD, Odomb RM, Quesenberryb KH (2010). Variation in 2c nuclear DNA content of *Zoysia* spp. as determined by flow cytometry. Crop Sci., 50: 1519-1525.

Taliaferro CM, Hopkins AA, Henthorn JC, Murphy CD, Edwards RM (1997). Use of flow cytometry to estimate ploidy level in *Cynodon* species. Int. Turfgrass Soc. R. J., 8: 385–392.

Trenholm LE, Cisar JL, Unruh JB (2003). Bermudagrass for Florida lawns. Florida Lawn Handbook. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.

Vaio M, Mazzella C, Porro V, Speranza P, Lopez-Carro B, Estramil E, Folle GA (2007). Nuclear DNA content in allopolyploid species and synthetic hybrids in the grass genus *Paspalum*. Plant Syst. Evol., 265: 109-121.

Wu YQ, Taliaferro CM, Bai GH, Martin DL, Anderson JA, Anderson MP, Edwards RM (2006). Genetic analyses of Chinese *Cynodon* accessions by flow cytometry and AFLP markers. Crop Sci., 46: 917-926.