Identification of Turf Bermudagrasses on the Oklahoma State University Baseball Field and Three Experimental Clones as Revealed with Simple Sequence Repeat Markers

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ADDITIONAL INDEX WORDS. *Cynodon* sp., molecular marker, breeding, genotyping

SUMMARY. Turfgrass varietal identification is critical and allows turfgrass professionals to manage the turf based on the cultural requirements of the variety. On the Oklahoma State University (OSU) Baseball Field (OSUBF) in Stillwater, OK, some bermudagrass (*Cynodon* sp.) plants exhibited desirable traits but their exact identities were unknown due to the installation of multiple varieties over time. Accordingly, the major objective of this study was to identify if the desirable bermudagrass plants were from commercially available known varieties. Recently, the OSU turf bermudagrass breeding program developed and entered three fairway-type clonal bermudagrasses in the 2013 National Turfgrass Evaluation Program (NTEP) bermudagrass trial: OKC 1131, OKC 1163, and OKC 1302. The secondary objective was to create molecular marker profiles for these three experimental lines. Five OSUBF samples were analyzed using simple sequence repeat (SSR) markers, along with 24 clonal, commercially available turf bermudagrass varieties widely used in Oklahoma, the three OSU experimental clones, six randomly selected single plants from 'Riviera', and two controls for common bermudagrass (*Cynodon dactylon*) and african bermudagrass (*Cynodon transvaalensis*). SSR marker genotyping data indicated that the five OSUBF plants were clones of an identical bermudagrass. The OSUBF bermudagrass had the same fingerprint as 'Astro-DLM' bermudagrass for 14 out of 16 SSRs genotyped. Fifteen out of 30 additional SSR markers also showed differences between the OSUBF bermudagrass and 'Astro-DLM'. The three OSU experimental clones were different from each other and had different fingerprints from the other tested varieties based on SSR profiles, indicating they are new breeding lines. These four distinct lines have potential to be released as new varieties if they demonstrate superior turf quality traits and adaptation over time.

In the U.S. transition zone, turf bermudagrasses have been increasingly used on sports fields as they possess excellent traffic and drought tolerance, less damage from diseases and insects, and quicker recovery from damage than many other turfgrasses. Turf bermudagrass has been used on the OSUBF in Stillwater, OK, for decades. However, the identities of bermudagrasses grown on the OSUBF have been lost due

to the use of multiple varieties over several years. Seeded bermudagrasses ('Guymon'), variety not specified common bermudagrass ('Cheyenne', 'JackPot', 'NuMex Sahara', and 'Riviera'), as well as vegetatively propagated bermudagrass ('Patriot') have all been planted on the OSUBF at various times from 1987 to date (D. L. Martin, unpublished data). 'Patriot' and 'Riviera' were developed and released by the Oklahoma Agricultural Experiment Station. 'Patriot'

is a clonally propagated F₁ hybrid from a cross of common bermudagrass 'Tifton 10' and an OSU african bermudagrass selection, whereas Riviera bermudagrass is a synthetic variety derived from the intercrossing of three clonal parent plants selected on the basis of turf quality and transition zone adaptation (Taliaferro et al., 2004a, 2004b).

To have suitable bermudagrass sod for repair of damaged spots on the OSUBF, a mini sod farm has been established and maintained on site. Bermudagrass plants in one area of the mini sod farm have been observed to show favored traits, including allowing for successful establishment of overseeded perennial ryegrass (*Lolium perenne*) in the fall and excellent transition back to bermudagrass in the early summer. However, the identity of the specific bermudagrass(es) of this area is unknown.

In 2013, three promising vegetatively propagated experimental bermudagrass lines developed by the OSU turf bermudagrass breeding program were entered in the 2013 NTEP bermudagrass trial to allow for testing over a wide geographic area. DNA fingerprints to identify these bermudagrass entries have not been established.

Identification of bermudagrass varieties was challenging until DNA molecular markers were developed, providing powerful identification tools over the last several years. For example, using 11 SSR markers, 32 commercially released clonal varieties and OSU experimental lines in bermudagrass were identified by Wang et al. (2010). SSR markers differentiated 'Tifgreen' bermudagrass and its mutation derived varieties (Harris-Shultz et al., 2011). In this study, the objectives were to use SSR markers to 1) determine if the OSUBF selections were the same plant; 2) identify if the grass was one of the U.S. commercially released,

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	μL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
2.54	inch(es)	cm	0.3937
28.3495	oz	g	0.0353
2.8350×10^{10}	oz	ng	3.5274×10^{-11}
1	ppm	ng·μL ⁻¹	1
$(^{\circ}\text{F} - 32) \div 1.8$	$^{\circ}\text{F}$	$^{\circ}\text{C}$	$(^{\circ}\text{C} \times 1.8) + 32$

clonally propagated varieties; and 3) verify if the three recent OSU experimental NTEP entries were new, distinct lines.

Materials and methods

PLANT MATERIALS. Thirty-two turf bermudagrass plants constituted the first set of plant materials used in identification of the OSUBF bermudagrass plants. Four plugs were taken from sod on the OSUBF mini sod farm on 12 June 2013 and grown in

four respective flats for morphological observation in a greenhouse on the OSU Agronomy Research Farm. Five samples were taken from the four flats based on morphological observations and then grown in separate 6-inch pots [designated as OSUBF 1 to 5 (Table 1)]. The first set of plant materials also comprised 16 clonal turf bermudagrass varieties widely used in Oklahoma, three OSU experimental lines (OKC 1131, OKC 1163, and OKC 1302),

and six randomly selected single plants from 'Riviera' bermudagrass (Table 1). 'Michigan' and 'Uganda' were included as controls for common bermudagrass and african bermudagrass, respectively (Table 1). To obtain the single plants from 'Riviera', 1 g of seeds were germinated in a pot and 64 individual plants were transplanted into respective containers after growing to the two-leaf stage, from which six plants ['Riviera 1' to 'Riviera 6' (Table 1)]

Table 1. Selected turf bermudagrass varieties and unknown bermudagrasses on the Oklahoma State University Baseball Field (OSUBF, Stillwater, OK) sod farm that were genotyped using simple sequence repeat markers.

No.	Name	Species	Chromosomes (2n)	Original source/reference ^z
1	OSUBF 1	<i>Cynodon</i> sp.	Unknown	OSUBF sod farm
2	OSUBF 2	<i>Cynodon</i> sp.	Unknown	OSUBF sod farm
3	OSUBF 3	<i>Cynodon</i> sp.	Unknown	OSUBF sod farm
4	OSUBF 4	<i>Cynodon</i> sp.	Unknown	OSUBF sod farm
5	OSUBF 5	<i>Cynodon</i> sp.	Unknown	OSUBF sod farm
6	'Astro'	<i>Cynodon dactylon</i> × <i>Cynodon transvaalensis</i>	27	Tulsa Grass and Sod Co. (Tulsa, OK)
7	'U3-NY'	<i>C. dactylon</i>	36	Anderson et al. (2001)
8	'U3-NC'	<i>C. dactylon</i>	36	Anderson et al. (2001)
9	'U3-SIU'	<i>C. dactylon</i>	36	Anderson et al. (2001)
10	'U3-TGS'	<i>C. dactylon</i>	36	Anderson et al. (2001)
11	'Premier'	<i>C. dactylon</i>	36	Parsons and Lehman (2007)
12	'OKC 70-18'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	27	OAES
13	'Patriot'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	36	Taliaferro et al. (2004b)
14	'Latitude 36'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	27	OAES
15	'NorthBridge'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	27	OAES
16	'OKC 1131'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	Unknown	OAES
17	'OKC 1163'	<i>C. transvaalensis</i>	Unknown	OAES
18	'OKC 1302'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	Unknown	OAES
19	'Midfield'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	27	Pair et al. (1994a)
20	'Quickstand'	<i>C. dactylon</i>	36	Phillips et al. (1997)
21	'Midlawn'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	27	Pair et al. (1994b)
22	'Midiron'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	27	Alderson and Sharp (1994)
23	'Tifway'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	27	Burton (1966)
24	'Cardinal'	<i>C. transvaalensis</i>	18	unknown
25	'Riviera 1'	<i>C. dactylon</i>	36	OAES
26	'Riviera 2'	<i>C. dactylon</i>	36	OAES
27	'Riviera 3'	<i>C. dactylon</i>	36	OAES
28	'Riviera 4'	<i>C. dactylon</i>	36	OAES
29	'Riviera 5'	<i>C. dactylon</i>	36	OAES
30	'Riviera 6'	<i>C. dactylon</i>	36	OAES
31	'Michigan'	<i>C. dactylon</i>	36	de los Reyes et al. (2001)
32	'Uganda'	<i>C. transvaalensis</i>	18	Juska and Hanson (1964)
33	'Baby'	<i>C. dactylon</i>	unknown	Porter (1997)
34	'Midway'	<i>C. transvaalensis</i> × <i>C. dactylon</i>	27	Alderson and Sharp (1994)
35	'Vamont'	<i>C. dactylon</i>	36	VAES
36	'GN-1'	<i>C. transvaalensis</i> × <i>C. dactylon</i>	27	Brosnan and Deputy (2008)
37	'Aussie Green'	<i>C. transvaalensis</i> × <i>C. dactylon</i>	27	Baldwin et al. (2006)
38	'Greenfield'	<i>C. dactylon</i>	36	OAES
39	'Tifton 10'	<i>C. transvaalensis</i> × <i>C. dactylon</i>	54	Hanna et al. (1990)
40	'Astro-DLM'	<i>C. dactylon</i> × <i>C. transvaalensis</i>	27	Tulsa Grass and Sod Co.
41	'Celebration'	<i>C. dactylon</i>	36	Sod Solutions (Awendaw, SC), Riley (2000)

^zOAES = Oklahoma Agricultural Experiment Station, Stillwater, OK; VAES = Virginia Agricultural Experiment Station, Blacksburg, VA.

Table 2. Forty-six simple sequence repeat (SSR) primer pairs (PP) used to genotype the bermudagrass samples and varieties in identifying Oklahoma State University Baseball Field unknown bermudagrass and three experimental varieties.

No.	SSR PP identity	Repeat motif	Forward primer sequences (5'-3')	Reverse primer sequences (5'-3')
1	CDCA 31/32	(CA)23-(GA)12	TCCCTGATTCTCTGAAAGGA	CGGTGACATTGAATACCGAG
2	CDCA 55/56	(GT)9	CGAGTCCATGCCTAACTCAA	ACGGAAGGGTCAGTGGTAAC
3	CDCA 77/78	(GT)25	GAAGATGTCATCACGATGGG	CGTACGACCGAGTTCTCTGA
4	CDCA 133/134	(AC)14-(AAG)5	CACCCATTACAGTGAGCACC	TGCTTGGAACAACCTTCTG
5	CDCA 155/156	(TC)16-(AC)15	CTCCCTCGTCCATTTTCATTT	CGTTGGCACTCACTACCACT
6	CDCA 379/380	(AC)7-(GT)37	AGCACAGGCTTCTTATGCAA	TTATGAAGATAGCCCGGTCC
7	CDCA 747/748	(TG)14	GAATCCTACCCAAACGGAA	ATGCAGCTAACAAATGGTGC
8	CDE 89/90	(TA)5	CGCGCTTCTCTACTTCTG	AACAGACGCCATCATCATC
9	CDE 127/128	(GAG)5	GGAAAGAATCAAGGACCGAC	GAGCAACTGAGCAAGCAAAG
10	CDE 215/216	(CCG)6	ACCACAAGCCAAACCCCTACT	AAGTCGAGGTTGAGGTGCTT
11	CDE 375/376	(GGAC)5	AGACGTTTCAGCGTCGTCTAC	TGCACACATCCAGCTAGTGA
12	CDCA 491/492	(GT)12	CTTGTTCTTGGGTCTTGT	AGCTCAAGCACCATTGTCAG
13	CDGA 1795/1796	(AC)5-(AG)36	TTCGTGGACTCTGGCTATTG	GCCCAGGTAACGTGTTCTTT
14	CDATG 1889/1890	(GCT)7-(GAT)7	AAACGTGAGAGGCTCTTGCT	GTATGACACACGGAAGGACG
15	CDATG 1999/2000	(ATG)7	CCAGGTTTCGCATCAGATA	TGCATATCATGAACACGACG
16	CDAAC 2523/2524	(TGT)9	AAGGCCTAACCCAATTTGC	ACAATGCTTTTCATCCTCCC
17	CDCA 7/8	(CA)10	GAATCCCCAAAGACGGATGT	TCGAGATGACTTGGAAAGGA
18	CDCA 21/22	(AC)13-(AG)14	GGGCCTCCCCTTTTATACAT	GGTAACCAATCAAGGCCACT
19	CDCA 81/82	(TG)38	CCATATAACGGGTTTCAGCCT	TCAAGAGTTCAGCCTCATGC
20	CDCA 117/118	(CT)9-(GT)12	CCCTGCCTCTTCAAGGTAAG	ACAAAGTTCCTCCGATCACC
21	CDCA 125/126	(TG)20	AAATTGACCCTCCACAAAGC	AGTCAGGGGTTTCCATTTTG
22	CDCA 139/140	(CA)42	TATATGCTCAGACGATGGGC	GACCCGAAGGTGTGATTCTA
23	CDCA 247/248	(GT)8	TACCTCGCTGGACTGAAAGTG	GCAGTAGTCCCACCAACCTT
24	CDCA 313/314	(CA)23	GGGTCATGAGTCAAATGTGC	CTTTTGTGAGCCAGAAGCAA
25	CDCA 319/320	(AC)22	CATGTTCCAGACAAGGATGG	GCAACAAACAGCCACAGAAT
26	CDCA 401/402	(TC)7-(AG)9-(TG)21	ACAATCCAGAACGGGAGAAG	GCTCGTTTTGAGCGTTGTAA
27	CDCA 441/442	(CA)14-(GA)8	ATCAGGAACTTTTGGTTGCC	GTGTGATTGCCAGTACCAA
28	CDCA 463/464	(CA)12-(GA)31	CAGGAAAACGAGACGAGAGA	CCGGGACGTCAGATATTTTT
29	CDCA 529/530	(AC)12	GTTTGAAGTGCATCAGCACC	GAAGTGGAAACATCCTGCCT
30	CDCA 623/624	(AC)17-(CA)5	CGAGACCTAGTGAACAGCGA	GGCCGTGCTTAAAGGAATAG
31	CDGA 783/784	(GA)14	CACTGTTTACCCATCCAACG	TTTTTCGTACACACCCAGAA
32	CDGA 791/792	(CT)20	CAAATCCTCGACATTTCCCT	GTGAACGAATGAAGTGGGTG
33	CDGA 805/806	(GA)22	ACCGGTAAGCCATGCTATC	GCATTCAAACAGAACGCAAC
34	CDGA 807/808	(CA)12-(GA)32	CAGGAAAACGAGACGAGAGA	CCGGGACGTCAGATATTTTT
35	CDGA 915/916	(GA)13	AAGACGAGCAGAGAAGAGCC	CGTTCTGGATAGGTGGGAGT
36	CDGA 1195/1196	(CT)15	ACCACCAATAGCACACCAGA	CGGAACAAGGAGTGAGACAC
37	CDGA 1583/1584	(AG)25	GTATCGTCATCGTCTGGTG	TCGGCCAGAAAACCTCTATT
38	CDGA 1601/1602	(GA)13	CCTGCTGGTCAGAACTCAAC	TATTGGTTGCACCTTCCAGA
39	CDGA 1697/1698	(CT)7-(TC)10-(GC)5	AGTGGTCGGTCATCTTCCTC	AACAGCTGGAGCCTAGGAAA
40	CDGA 1783/1784	(CT)24	CCTCCTCCCAACATCTTCTG	GAAATGCATGTTCTTGCAC
41	CDATG 1829/1830	(TCA)15	GAATCTGTGGGCGAATAAT	AAGCTGATGCAGGAGAGGAT
42	CDATG 1891/1892	(TCA)6	AGACAAAGGGATCACTCGCT	CACCAAGTCCATCAAGGCTA
43	CDATG 1905/1906	(TGA)6	GACTGCTTCACCGAGTACCA	TCCTGAGCGACCTCTTCC
44	CDATG 2059/2060	(TGA)6	ATTAGTGGTTTTTGGGCAGG	TCCAGTCATTGAGGAATTG
45	CDAAC 2675/2676	(AAC)6	TAGCCTACCCCAACTTGCTT	GTATACTGGCTTCATGGGCA
46	CDAAC 2693/2694	(GTT)6-(TGT)9	TTGCCTACCAAACACGAAAG	TCCAACTCGTGTAAATTGCC

were randomly selected and used in the experiment.

Eight additional clonal varieties [no. 33–40 (Table 1)] and the OSUBF no. 1 sample formed the second set of plant materials and were genotyped with 16 SSR markers. ‘Astro-DLM’ was another source of ‘Astro’ (D.L. Martin, unpublished data). The third set included OKC 1131, OKC 1163, and OKC 1302;

‘NorthBridge’ and the current four NTEP clonal bermudagrass standards; ‘Patriot’, ‘Latitude 36’, ‘Tifway’, and ‘Celebration’. The plants of this third set were genotyped with 16 SSR markers to establish SSR marker profiles for the three OSU experimental clones. All the plants were grown in 6-inch pots and maintained for healthy growth with daily watering and regular fertilization.

DNA ISOLATION. Healthy leaf tissue was sampled from each of the 41 plants (Table 1) grown in the greenhouse. DNA was isolated from fresh leaf tissue following a modified phenol–chloroform extraction method (Nalini and Jawali, 2004). About 1 g of leaf tissue was cut and frozen in a –80 °C freezer, grinded in a tissue homogenizer (Geno/Grinder; SPEX SamplePrep,

Table 3. Band sizes (in bps) of eight simple sequence repeat (SSR) primer pairs (PP) in five Oklahoma State University Baseball Field (OSUBF) bermudagrass samples, 16 commercial bermudagrass varieties, three experimental lines, six 'Riviera' plants, 'Michigan', and 'Uganda'. Unclear bands are underlined.

SSR PP	OSUBF					Band size (bp)										'Latitude 36'	'North- Bridge'	'OKC 1131'
	1	2	3	4	5	'Astro'	'U3-NY'	'U3-NC'	'U3-SIU'	'U3-TGS'	'Premier'	'OKC 70-18'	'Patriot'					
CDCA 31/32	181	181	181	181	181	190	190	181	190	181	185	181	181	181	185	185		
	173	173	173	173	173	<u>160</u>	173	142	173	142	177	149	160	142	160	160		
CDCA 55/56	297	297	297	297	297	297	306	306	306	306	306	297	297	297	297	297		
	288	288	288	288	288	288	297	288	297	288	288	288	288	288	288	288		
CDCA 133/134	242	242	242	242	242	242	223	223	215	223	242	242	242	242	202	233		
	223	223	223	223	223	207	202	202	202	202	223	223	223	202	202	223		
CDCA 379/380	259	259	259	259	259	270	259	254	— ^z	254	254	280	267	270	270	292		
						235					252	275	252	259	247	275		
CDCA 747/748	193	193	193	193	193	193	193	193	193	193	193	193	193	193	189	189		
	170	170	170	170	170	175	170	166	170	166	170	170	170	170	170			
CDE 89/90	156	156	156	156	156		156	156	156	156	160	156	156	156				
	194	194	194	194	194	191	191	191	191	191	200	191	191	191	191	194		
CDE 127/128	191	191	191	191	191	184	184	179	184	179	191	184	184	184	184	191		
	184	184	184	184	184					184						177		
CDE 491/492	212	212	212	212	212	212	212	212	212	212	212	212	212	212	212	212		
	207	207	207	207	207		208	208	208	208	205	205	205	205	205	205		
CDCA 491/492	289	289	289	289	289	276	289	289	289	289	283	276	283	283	283	283		
	276	276	276	276	276	268	276	276	276	276	276	257	276	276	276	276		
									268	268		268	268	268	268	268		
												257	257	257	257	257		

(Continued on next page)

Table 3. (*Continued*) Band sizes (in bps) of eight simple sequence repeat (SSR) primer pairs (PP) in five Oklahoma State University Baseball Field (OSUBF) bermudagrass samples, 16 commercial bermudagrass varieties, three experimental lines, six 'Riviera' plants, 'Michigan', and 'Uganda'. Unclear bands are underlined.

SSR PP	‘OKC 1163’			‘Midfield’			‘Quickstand’			‘Midlawn’			‘Midiron’			‘Tifway’			‘Cardinal’			‘Riviera’ ‘Riviera					
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^zFailed reaction.

Metuchen, NJ), and vortexed in extraction buffer (pH = 8.5) consisting of 1% (w/v) N-lauroylsarcosine, 100 mM Tris, 100 mM sodium chloride, and 10 mM ethylenediaminetetraacetic acid disodium salt (Na₂EDTA). The homogenate was emulsified with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1 v/v/v) and centrifuged for 10 min at 14,000 *g*_n. The supernatant was then transferred to a new tube and mixed with an equal volume of chloroform:isoamyl alcohol (24:1 v/v), centrifuged for 5 min at 14,000 *g*_n. The supernatant was then transferred to a new tube containing an equal volume of isopropanol alcohol and 1/10 volume of 3 M sodium acetate (pH = 5.2). The DNA was precipitated by centrifugation for 5 min at 14,000 *g*_n and then was dissolved in 50–100 μ L Tris-EDTA buffer after the pellet was washed with 1 mL 70% ethanol and dried completely. Finally, the DNA was quantified using a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific, Waltham, MA) and diluted to 10 ng- μ L⁻¹ for polymerase chain reaction (PCR).

SSR MARKERS AND PCR AMPLIFICATION. The 11 SSR primer

pairs (PPs) used for varietal identification by Wang et al. (2010) and additional 35 SSR PPs [no. 12–46 (Table 2)] were selected based on the amplification efficiency and polymorphism from initial screening. PCR was conducted following the procedure described by Wang et al. (2010) with minor modifications. Briefly, PCR amplification was performed in a 10 μ L volume reaction, which consisted of 15 ng DNA, 1 μ L 10 $\times$ PCR buffer (New England BioLab, Ipswich, MA), 200 μ M deoxynucleotide, 0.2 μ M forward primer tailed with M13 forward primer sequence (5'-CACGACGTTGTAAAACGACG-3'), 0.2 μ M reverse primer, 0.02 μ M infrared-M13 forward primer labeled with either 700 or 800 nm florescent dye (LI-COR, Lincoln, NE), 0.025 U Taq DNA polymerase (New England BioLab), and water to bring the total volume up to 10 μ L. PCR was performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA) following the program: 5 min at 95 °C for denaturation, followed by 14 cycles of 20 s at 94 °C, 60 s at 58 °C, 30 s at 72 °C, and another 28 cycles of 20 s at 94 °C, 60 s at 55 °C,

30 s at 72 °C, and a 10 min final extension at 72 °C (Wang et al., 2010).

GEL ELECTROPHORESIS AND DATA ANALYSIS. Two plates of PCR products labeled with 700 and 800 nm were pooled and loaded into a 6.5% KB Plus gel (LI-COR), and separated by running in a LI-COR 4300 DNA Analyzer. Bands from the gel were visually scored and the band size determined using Saga Generation 2 Lite software (LI-COR). In visual scoring of gel images, the bands detected by each SSR PP were recorded for presence (1) and absence (0) for each sample. Jaccard's similarity coefficients were calculated to construct dendrograms using the unweighted pair group method with arithmetic average with the SAHN module of NTSYS-pc (Rohlf, 2000) version 2.0 software (Exeter Software, Setauket, NY).

Results and discussion

In the initial identification, the first set of 32 samples [no. 1–32 (Table 1)] was genotyped with eight SSR markers [no. 1, 2, 4, 6–9, 12 (Table 2)] with two replications of

Fig. 1. Dendrogram depicting similarity coefficients of five Oklahoma State University Baseball Field bermudagrass plants (OSUBF 1 to 5), 21 clonal bermudagrass varieties, and six plants from 'Riviera' assessed with eight simple sequence repeat primer pairs.

Table 4. Band sizes (in bps) in Oklahoma State University Baseball Field (OSUBF) bermudagrass samples and eight commercial bermudagrass varieties amplified with 16 simple sequence repeat (SSR) primer pairs (PP). Unclear bands are underlined.

SSR PP	OSUBF 1	'Baby'	'Midway'	'Vamont'	'GN-1'	'Aussie Green'	'Green-field'	'Tifton' 10	'Astro-DLM'
Band size (bp)									
CDCA 31/32	162	155	175	158	152	175	169	155	162
	147	140	162	140	140	145	143	145	147
			153	129		137	138	140	142
CDCA 55/56			137				128		
	363	355	355	363	355	355	375	363	363
	355		330	337	337		355	340	355
CDCA 77/78	340						350		340
	235	235	232	232	244	235	238	235	235
	208	<u>208</u>	208	208	221	215	235	221	235
CDCA 133/134							225	208	208
	205	210	210	205	210	210	202	223	205
	187	192	192	187	192	192	184		187
CDCA 155/156	297	287	262	287	266	277	283	277	297
	277	266	241	252	259	262	277	266	277
	266	237	215		231	237	252		266
CDCA 379/380	268	263	271	271	280	285	268	280	263
	244	256	263		256	263	263	268	244
		240	240				240		240
CDCA 747/748	202	202	196	195	203	195	198	198	202
	180	180	188	189	195	181	175	175	198
	161	161	171	173	187	175			180
CDE 89/90					140	165			161
	190	212	187	187	212	187	187	235	190
	182	187	174	174	195	182	182	187	182
CDE 127/128	162	164	140	164	185	172	172	174	164
						162	164	164	
	270	270	266	270	270	270	270	270	270
CDE 215/216	266	264	264	266	260	266	266	266	266
	260	257	260	264			264	264	
	225	225	225	225	225	225	225	225	225
CDE 375/376		214	220	220	220	220	210	210	222
							196	196	220
	304	304	304	304	286	304	304	304	304
CDCA 491/492	286	286	286	286	282	286	286	294	286
			282	282			282	286	
	298	280	280	288	280	298	298	288	298
CDGA 1795/1796	288	272	261	272	272	280	280	272	288
		261			261	261	272		280
							256		261
CDATG 1889/1890	351	357	351	343	351	327	351	327	351
	332	327	327	318	327	302	343		332
	296	296			318		336		296
CDATG 1999/2000					296		327		
	311	304	292	307	301	316	301	298	310
	301	301			292	301		292	301
CDAAC 2523/2524	292	298						288	292
	271	271	265	271	265	275	271	278	271
	269	269	261	269	261	271	269	275	269
CDAAC 2523/2524	265	265				265		271	
		261				261			
	205	— ^z	— ^z	— ^z	211	205	205	211	211
	183				194	183	183	205	205
								194	198
								183	183

^zFailed reaction.

PCR reactions. Using the eight SSR markers, no differences were detected among the five OSUBF bermudagrass samples (Table 3; Fig. 1). None of the clonal varieties, experimental entries, or single 'Riviera' plants had the same banding patterns as the five OSUBF plants. This indicated that the samples from the OSUBF were the same plant and they were genetically different from the other varieties tested. Cluster analysis results on the SSR data are given in Fig. 1, indicating genetic similarity coefficients between the OSUBF plant and each of the other tested varieties varied from 0.60 to 0.86. From the genetic profiles of the commercial varieties, the U3 grasses were divided into two groups, U3-NY and U3-SIU were in one group while U3-NC, U3-TGS, and 'Quickstand' were in the other. The U3 grouping results were basically consistent with that of Anderson et al. (2001), but the sample used to represent 'Quickstand' in this study could be a contaminant in our 'Quickstand' plot based on previous report by Wang et al. (2010). Similarly, the grouping of 'Latitude 36' and 'NorthBridge' was in agreement with the findings of Wang et al. (2010).

In the second experiment, the OSUBF plant and eight additional clonal varieties (Baby, Midway, Vamont, GN-1, Aussie Green, Greenfield, Tifton 10, and Astro-DLM) were genotyped with 16 SSRs, again with two replications (PCR products' band sizes were shown in Table 4). The OSUBF plant was genetically more distant from the other seven clonal varieties tested than it was from 'Astro-DLM' (Fig. 2). The 'Astro-DLM' plant had the same banding patterns as the OSUBF sample for 14 SSRs, but exhibited different banding patterns for the other two SSR PP—CDCA 31/32 and CDAAC 2523/2524 (see sample's labeled band sizes in Table 4). The similarity coefficient between OSUBF and 'Astro-DLM' was 0.87 based on the 16 SSRs (Fig. 2). To further discern genetic differentiation between the OSUBF grass and 'Astro-DLM', 30 additional SSR PPs [no. 17–46 (Table 2)] were used to genotype the two grasses. Of the 30 SSRs, 15 showed polymorphic banding patterns between the two grasses. Six of them plus CDCA 31/32 and CDAAC 2523/2524 are shown in Fig. 3. The SSR marker

profiles further indicated that the bermudagrass from the OSUBF was different from 'Astro-DLM'. Although the OSUBF bermudagrass is different from the tested varieties, its origin is unknown. 'Astro-DLM' is a different variety than 'Astro' used in the first experiment with the latter being believed to be a contaminant.

The three OSU experimental clones (OKC 1131, OKC 1163, and OKC 1302) showed different banding patterns from each other. They also showed different banding patterns as compared with the other 24 commercially available varieties, including vegetatively propagated varieties and single plants selected from 'Riviera' (Fig. 1). The three OSU experimental bermudagrasses, four standard clonal varieties (Celebration, Latitude 36, Patriot, and Tifway) in the 2013 NTEP bermudagrass test, and 'NorthBridge' were genotyped with 16 SSR markers (PCR products' band sizes were shown in Table 5). The similarity coefficients between the three experimental clones and the other five clonal varieties ranged from 0.53 to 0.78 based on these 16 SSRs (Fig. 4). The results provided strong evidence that these three experimental plants were new bermudagrasses.

Fig. 2. Dendrogram of the Oklahoma State University Baseball Field (OSUBF) bermudagrass plants and eight additional clonal bermudagrass varieties amplified with 16 simple sequence repeat markers.

Fig. 3. Polymorphic fingerprints between Oklahoma State University Baseball Field bermudagrass sample (1) and 'Astro-DLM' (40) genotyped with 15 simple sequence repeat (SSR) primer pairs (8 were showed here). Each reaction was replicated four times. SSR markers were given on the top of the gel and under each SSR marker two samples 1 and 40 were labeled. DNA ladder was labeled by base pairs (bp).

Fig. 4. Dendrogram of three Oklahoma State University bermudagrass experimental varieties and five commercial standard bermudagrass varieties as assessed with 16 simple sequence repeat primer pairs.

Table 5. Band sizes (in bps) for three Oklahoma State University bermudagrass experimental clones ('OKC 1131', 'OKC 1163', and 'OKC 1302') and five commercial bermudagrass varieties with 16 simple sequence repeat (SSR) primer pairs (PP).

SSR PP	'OKC 1131'	'OKC 1163'	'OKC 1302'	'Patriot'	'OKC 1119'	'OKC 1134'	'Tifway'	'Celebration'
Band size (bp)								
CDCA 31/32	184	181	201	174	201	201	184	201
	150	174	174	155	174	181	145	202
			152	145	150	154		158
CDCA 55/56	371	350	356	363	350	356	350	371
	356	346	330	346	346	346	346	356
	346	330		330	330	330		346
	330							330
CDCA 77/78	294	272	286	294	294	272	263	270
	246	263	198	251	263	263	222	263
	239			246	209	209	209	222
	199			199				209
CDCA 133/134	234	208	234	234	251	251	226	234
	216		216	216	216	216	216	216
	208		208	208	208	208	208	208
								204
CDCA 155/156	286	269	330	312	330	286	315	330
	269	256	261	286	261	269	302	312
	261		248	275	248	261		304
								275
CDCA 379/380	304	260	273	267	271	273	287	281
	278	255	260	258	260	249	278	271
	255						255	
CDCA 747/748	193	193	193	193	193	193	193	193
	182			170	170		182	182
	175			164	164			175
CDE 89/90								167
	184	186	184	221	184	186	184	184
		184	166	184	166	184	174	166
				166		166	166	154
CDE 127/128	267	267	267	267	267	267	267	267
	264	259	264	264	264	264	259	264
	256		259	256	256	256		
CDE 215/216	223	220	217	223	223	223	233	215
	215	217	215	215	215	215	220	210
							215	
CDE 375/376	291	278	293	293	291	291	308	301
	283		283	283	283	283	301	291
	278		278	278	278	278	278	283
CDCA 491/492	286	282	286	286	286	286	282	296
	282	275	282	282	282	282	275	290
	275		275	266	266	275		275
								271
CDGA 1795/1796	365	395	341	341	341	365	341	341
	316		316	325	316	316		325
CDATG 1889/1890	317	302	317	302	317	308	294	302
	308	294	308	294	308	294	290	290
	302		302		302			
	294		294		294			
CDATG 1999/2000	280	270	280	280	280	280	285	285
	270		274	274		270	270	
CDAAC 2523/2524	216	207	207	207	207	207	216	— ^z

^zFailed reaction.

In summary, SSR genotyping data in this investigation indicated the OSUBF bermudagrass plants were identical. The SSR data further indicated the OSUBF plant and three

OSU experimental clones were different from each other and from all tested varieties. Each of the four clonal bermudagrasses has the potential to be released as a new variety if

they are clearly superior to existing varieties in one or more important characteristics or a combination of characteristics for turf performance and adaptation.

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