



Molecular characterization of some turfgrass cultivars using randomly amplified polymorphic DNA (RAPD) markers

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Abstract

Seven turfgrass cultivars (hybrid bermudagrass “Tifdwarf, Tifway, and Tifgreen”, local bermudagrass, common bermudagrass, Paspalum, and Saint-Augustinegrass) were investigated using RAPD fingerprints as genetic marker to establish genetic identities and assess genetic diversity. From 30 primers tested, only 16 were selected as reproducible, giving 172 bands. The RAPD profiles obtained were successfully used to differentiate the genotypes. The bermudagrass hybrid (Tifdwarf) was distinguished by a 1500-bp amplification fragment produced by primer OPA-16. The 1000-bp amplification fragment produced by primer OPA-15 was absent in all cultivars except the Tifgreen. Based on the pairwise comparison of amplification products, the genetic similarity was estimated. The seven tested turfgrass cultivars showed variation at the DNA level. Common bermudagrass was more related to other bermudagrass hybrids than local bermudagrass. Saint-Augustinegrass was quite distinct from other turfgrasses. The UPGMA cluster analysis separated seven turfgrasses into five distinct groups comprising: (i) the bermudagrass hybrids (Tifdwarf, Tifway, and Tifgreen), (ii) common bermudagrass, (iii) paspalum grass, (iv) Saint-Augustinegrass, and (v) local bermudagrass. The consensus fingerprint profile using the genetically defined RAPD markers is a useful and reliable method for establishing the genetic identities of the turfgrass cultivars. Also, the polymorphism detected and its reproducibility suggest that RAPD markers can be used successfully in turfgrass for studying the genetic diversity of cultivars.

Key words: Turfgrass, RAPD markers, PCR methods, DNA extraction, cultivars, cluster.

Introduction

Bermudagrass species such as *Cynodon dactylon* and *C. transvaalensis* and their derived hybrids (Tifway, Tifgreen, and Tifdwarf) are most commonly used in turfgrass areas for various purposes. Bermudagrasses are propagated vegetatively and are well known for their vigor and heat and drought tolerance¹⁷. However, different problems have been noticed such as new planted springs lack of mature roots¹⁷, exhibition of a mosaic of patches of different kinds of Bermudas (off-type grasses), and a variation of plants caused by repeated growing on the same area, which may be due to mutations or contaminations by seeds from other places. In Kingdom of Saudi Arabia, turf grasses are spread commercially depending on public markets. Some of these turfgrasses such as bermudagrass cultivars are difficult to be distinguished morphologically.

Therefore, a reliable technique to detect genetic diversity among plants is required. DNA profiling techniques provide an accurate and efficient tool to identify turfgrass cultivars or species. Williams et al.²⁷ have demonstrated the use of single arbitrary primers to identify DNA. The amplification is similar to the polymerase chain reaction (PCR) and generates an almost infinite number of polymorphisms useful in cultivar identification³.

Several advantages and applications have been recorded for the molecular genetic approaches such as random amplification polymorphic DNA (RAPD)¹⁰. Two different approaches using RAPDs can be taken to identify synthetic turfgrass cultivars. The first involves assaying within a heterogeneous population and basing identification on the proportions of polymorphisms

or shared markers. For example, based on the presence or absence of bands, a genetic distance matrix between individuals can be calculated. A dendrogram is then created by means of Analysis of Molecular Variance to partition variation among individuals within the population and variation between populations. This approach identifies the genetic relatedness of an unknown to a cultivar already present on the dendrogram and could be useful to identify closely related populations and in studying diversity among and within cultivars¹².

A second approach involves assaying a genetically mixed sample (bulk sample) of many individuals with the intention of obtaining a representation of the heterogeneous population and basing identification on the presence of distinguishing polymorphisms. Assaying bulk samples is preferred for cultivar identification because it is more economical and efficient than assaying many individual samples¹⁰.

A major advantage of RAPD markers over some other DNA-based markers is that they require no prior sequence information, and no prior knowledge about any particular gene in a target taxon¹⁶. Therefore, RAPD markers can be used in the systematic study of wild plants and new crops. PCR methods require lower amounts of genomic DNA, are nonradioactive, relatively low cost, and can be developed rapidly.

Several studies have highlighted the benefits of RAPD markers to assess genetic diversity in crops, including blueberry (*Vaccinium* L. spp.)¹⁴, *Brassica* L. spp.^{6, 8} *Chrysanthemum* L. spp.²⁰, cranberry (*Vaccinium macrocarpon* (Ait.) Pursh¹⁵, grain amaranth (*Amaranthus* spp., Adams)²², palms (*Elaeis*

guineensis A. Cheval)²¹, and basil (*Ocimum spp.*)²⁴.

RAPD procedure has been extensively applied in turfgrasses¹³, creeping bentgrass²⁵, bermudagrass¹ and bentgrass¹⁹. DNA amplification fingerprinting (DAF) technique has been used successfully in centipedegrass²⁶, sweet potato²³ and petunia⁵. This later procedure has also been used to distinguish between interspecific bermudagrass hybrids and between original and mutant genotypes such as Tifway and its irradiated mutant (Tifway II)⁹.

The objectives of this study were to (I) determine the usefulness of RAPD markers to distinguish between seven different turfgrasses cultivars through fingerprinting, and (ii) estimate the genetic relationship between turfgrasses based on RAPD markers.

Materials and Methods

DNA extraction: Genomic DNA of seven turfgrass cultivars (Table 1), hybrid bermudagrass “Tifdwarf, Tifway, Tifgreen”, local bermudagrass, common bermudagrass, Paspalum and Saint-Augustinegrass, was extracted from 0.5 g of bulk sample of leaf tissues for each cultivar. The bulk sample of leaves was first ground into fine powder with liquid nitrogen. DNA was extracted in 15 ml of CTAB buffer consisting of: 1.5 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1% CTAB. The homogenate was incubated for 2 hours at 65°C with occasional mixing. Following incubation, 5 ml of chloroform/isoamylalcohol (24:1) was added to the tubes, mixed, and centrifuged at 2600 g for 10 min. The aqueous phase was removed to a fresh tube and an equal volume of ice-cold isopropanol was added followed by centrifugation as above to precipitate the DNA. The pellet was washed in 70% ethanol and dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 0.1mM EDTA).

RAPD-PCR amplification: Thirty random primers were obtained from Operon Technologies, Alameda, USA. RAPD analysis was conducted in a thermal cycler (Thermolyne Amplitron). The reaction mixture (25µL) contained 1x PCR buffer with MgCl₂ (50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2 mM MgCl₂ and Triton X-100), 200 µM each of dATP, dCTP, dGTP, and dTTP, 30 ng template DNA, 50 pM of oligonucleotide primer and 1.5 unit of Taq polymerase. The mixtures were subjected to the following conditions: hold at 94°C for 2 min for starting, followed by 40 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min, and a final hold at 72°C for 5 min. PCR products were visualized along with a DNA marker on 2% agarose gel with 1X TAE buffer and

detected by staining with an ethidium bromide. Gels were photographed on Polaroid films under UV light.

Data handling and cluster analysis: Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each random primer. If a product was present in a genotype, it was designated “1”, if absent it was designated “0” after excluding unreproducible bands. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to generate similarity coefficients based on SIMQUAL module. The similarity coefficients were then used to construct a dendrogram by UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) using NTSYS-PC¹⁸.

Results and Discussion

DNA amplification fingerprinting: Thirty primers of arbitrary nucleotide sequence were used to amplify DNA segments from seven turfgrass cultivars (hybrid bermudagrass “Tifdwarf, Tifway, Tifgreen”, local bermudagrass, common bermudagrass, Paspalum, and Saint-Augustinegrass). The number of amplification bands per primers varied between 3 and 20. Of the 30 primers tested, 16 primers were selected for further analysis based on the intensity, size and number of amplified products (Table 2). To ensure reproducibility and genetic pertinence of RAPD marker data, the primers generating no, weak or complex patterns were discarded.

Analysis of the 16 selected primers among the seven turfgrass cultivars included in this study generated 172 bands, 165 (96%) of which were polymorphic. There were 10.3 polymorphic bands per primer in average. Primers were evaluated for their ability to identify any one cultivar as illustrated by the example of Primers OPA-12, 15, and 16 shown in Fig. 1. The bermudagrass hybrids (Tifdwarf, Tifway, and Tifgreen) were distinguished by a 1500-bp amplification fragment produced by primer OPA-16. This amplification fragment was present in Tifdwarf, but not in cultivars Tifway and Tifgreen (Fig. 1a). The 1000-bp amplification fragment produced by primer OPA-15, which distinguished cultivar Tifgreen in Fig. 1b, was absent in all cultivars except the Tifgreen cultivar. The common bermudagrass cultivar was distinguishable by a 700-bp amplification fragment produced by primer OPA-15. Also, local bermudagrass cultivar was distinguishable by a 1400-bp amplification fragment produced by primer OPA-12, which was absent in common bermudagrass and bermudagrass hybrids (Fig. 1c). Paspalum, and Saint-Augustine grasses showed differences in their banding patterns using the tested primers.

Table 1. List of turfgrass genera and cultivars used in this study.

Turfgrass	Scientific name
Hybrid bermudagrass including: *Tifdwarf (TD) *Tifway (TW) *Tifgreen (TG)	<i>Cynodon dactylon</i> x <i>C. transvaalensis</i>
Common bermudagrass (CB)	<i>Cynodon dactylon</i> (L) Pers.
Local bermudagrass (LB)	<i>Paspalum vagantum</i>
Paspalum (Pa)	<i>Stenotaphrum secundatum</i> (Walt.) Kuntze
St. Augustinegrass (St)	

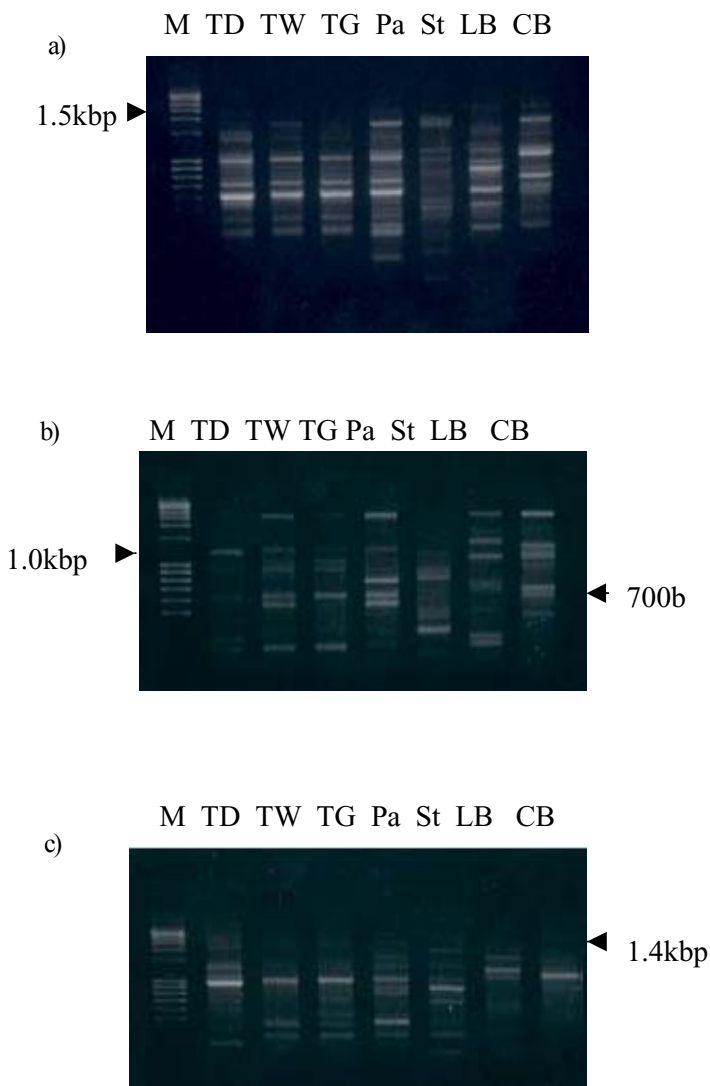


Figure 1. Comparison of RAPD banding patterns generated with Operon primers A16(a), A15(b), and A12(c) and seven turfgrasses DNAs. "M" indicates 100-bp size marker ladder.

Polymorphism between various genera and cultivars can arise through: nucleotide changes that prevent amplification by introducing a mismatch at one priming site; deletion of a priming site; insertions that render priming sites too distant to support amplification; and insertions or deletions that change the size of the amplified product²⁷. Therefore, using RAPD technique, a DNA fingerprints were performed based on the varying sizes of DNA fragments copied from different turf grasses, determined by 10-mer primers and DNA differences (presence, absence, and location of recognition sites) were determined. Golembiewski et al.¹⁰ demonstrated that RAPD markers have the potential to be a valuable tool for the identification of synthetic turfgrass cultivars.

Cluster analysis: The RAPD markers produced by 16 primers were tested to construct a similarity coefficients matrix (Table 3). The genetic similarity coefficients among all turfgrasses ranged from 0.40 to 0.92. Tifgreen cultivar was more closely related (0.92) to Tifway cultivar than Tifdwarf cultivar (0.75). Possible explanation for this result could be that Tifdwarf cultivar is believed to have arisen naturally by mutation from Tifgreen cultivar^{1,2}. Indeed, Tifdwarf has been considered a probable somatic mutation of Tifgreen¹¹. Common bermudagrass was more related to other bermudagrass hybrids than local bermudagrass. Saint-Augustinegrass was quite distinct from other turfgrasses. The UPGMA cluster analysis separated seven turfgrasses into five distinct groups (Fig. 2) comprising: (i) the bermudagrass hybrids (Tifdwarf, Tifway and Tifgreen), (ii) common bermudagrass, (iii) paspalum grass, (iv) Saint-Augustinegrass, and (v) local bermudagrass. Collectively, the cluster analysis definitively points to wide genetic differences between the bermudagrass hybrids and other turfgrasses genotypes. This result may be due to the fact that these genome constitutions are diverse enough to create distinct RAPD banding patterns which allow genotypes to be differentiated¹⁹. However, the bermudagrass hybrids were very closely related. Thus, RAPD banding patterns of the bermudagrass hybrids are expected to be more similar than other

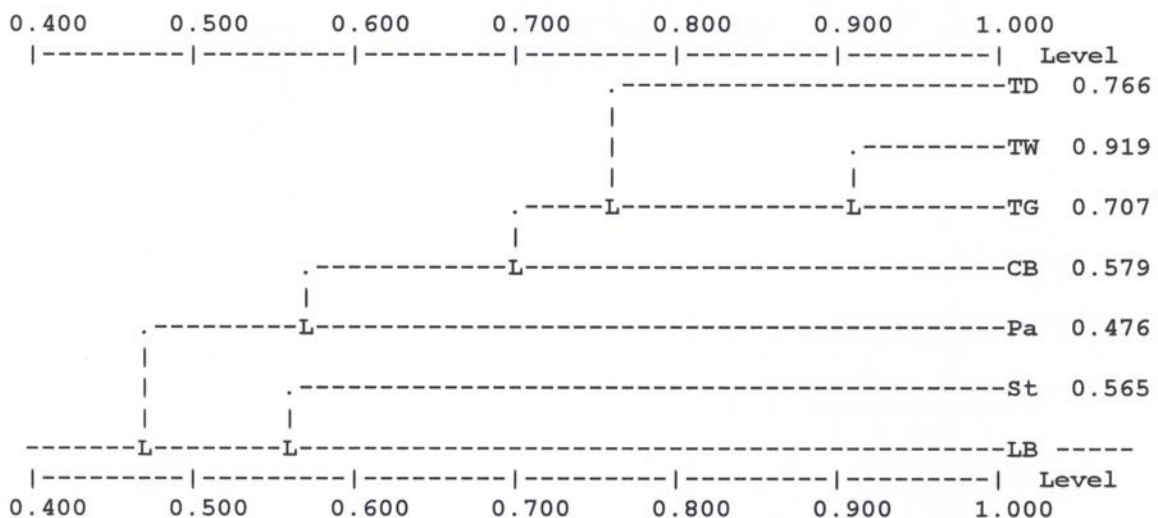


Figure 2. Dendrogram constructed from similarity coefficients and showing the clustering of seven turfgrass cultivars.

Table 2. Selected Operon primers with the number of amplified products and polymorphic fragments.

Primers	Sequence 5' to 3'	Amplified products	Polymorphic fragments
OP-A04	ATCGGGCTG	13	13
OP-A06	GGTCCCTGAC	10	10
OP-A08	GTGACGTAGG	10	10
OP-A09	GGGTAACGCC	12	11
OP-A11	CAATCGCCGT	9	9
OP-A12	TCGGCGATAG	14	13
OP-A13	CAGCACCCAC	11	11
OP-A14	TCTGTGCTGG	10	9
OP-A15	TTCCGAACCC	15	14
OP-A16	AGCCAGCGAA	20	20
OP-F02	GAGGATCCCT	13	13
OP-F03	CCTGATCACC	8	6
OP-F04	GGTGATCAGG	3	3
OP-F05	CCGAATTCCC	8	8
OP-F06	GGGAATTCGG	8	8
OP-F10	GGAAGCTTGG	8	7

Table 3. Simple matching coefficients of similarity determined from analysis using 172 RAPD loci.

Turfgrass	Tifdwarf	Tifway	Tifgreen	Paspalum	St. Augustin	Local bermud	Common bermud
Tifdwarf	1.00						
Tifway	0.78	1.00					
Tifgreen	0.75	0.92	1.00				
Paspalum	0.56	0.62	0.60	1.00			
St. Augustin	0.40	0.43	0.43	0.53	1.00		
Local B.	0.53	0.49	0.48	0.50	0.56	1.00	
Common B.	0.69	0.73	0.71	0.52	0.44	0.42	1.00

cultivars. This study illustrates that RAPD markers could be of high value for turfgrasses germplasm characterization and genetic maintenance. Other researchers have also come to the same conclusion^{1,10}. Moreover, The application of DNA tests to the turfgrass industry is a major challenge in an area of repeated vegetative propagation, triploidy and genetic instability⁴.

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