Microsatellite Markers Development from EST Sequences of Bermudagrass (*Cynodon* spp.)

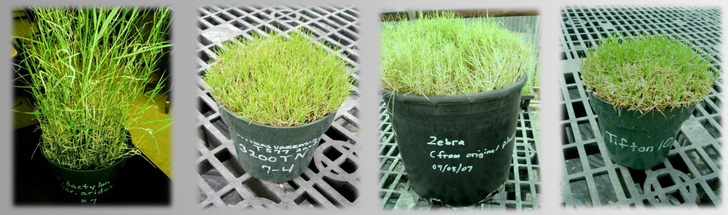
C.C. Tan, Y.Q. Wu, M.P. Anderson, T.D. Samuels, C.M. Taliaferro
Department of Plant and Soil Sciences
Oklahoma State University, Stillwater, OK 74078

Introduction

Bermudagrass is an important warm-season, perennial grass, widely used for turf and forage in the southern United States and other warmer temperate and tropical regions in the world. Microsatellites, or simple sequence repeat (SSR) markers, are highly useful in genetic studies and breeding, but are not currently available in bermudagrass. The objective of this study was to develop SSR markers from EST sequences of bermudagrass.

Materials and Methods

Plant materials



C. dactylon var. *aridus* (2n=2*x*=18) | *C. transvaalensis* 'T577' (2n=2*x*=18) | *C. dactylon* 'Zebra' (2n=4*x*=36) | *C. dactylon* cv. Tifton 10 (2n=6*x*=54)

Primer pairs design

A set of 20,237 bermudagrass EST sequences from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/) was used.

Using the program SSR Locator (da Maia et al. 2008): 1,303 SSRs were detected with a minimum of 5 for di-, tri-, tetra-, and penta-nucleotide motifs. Six hundred ninety two (692) unrepeated primer pairs were designed.

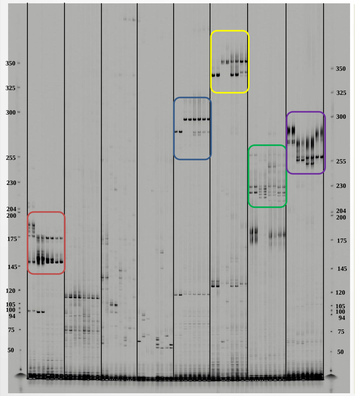
All primer pairs were custom synthesized by Integreated DNA Technologies (San Diego, CA, USA).

PCR amplification, gel electrophoresis and data analysis

Each genotype DNA with two replicates was amplified using each of the 692 primer pairs.

All PCR products were separated and visualized (Figure 1) using a Li-Cor 4300 DNA Analyzer.

Reproducible bands were scored based on target fragments, and band size was documented by Saga ^{GT} software version 3.3 (LI-COR Inc., Lincoln, NE) for determining the polymorphism of primer pairs.



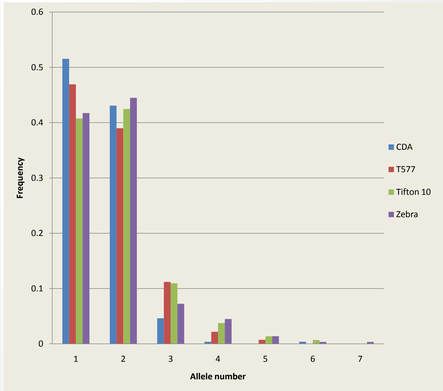


Figure 1 (left). A representative gel image showing polymorphic bands for selected primer pairs indicated by color boxes.

Figure 2 (right). Distribution of allele numbers of EST SSR markers for four bermudagrass genotypes (CDA is abbreviation for *C. dactylon* var. *aridus*).

Results

Out of 692 designed primer pairs, 303 (44%) were able to amplify target loci in at least one of the four bermudagrass genotypes. But the four genotypes had different percentages of primer pairs that produced clean repeatable bands (Table 1). In total, 237 primer pairs produced reproducible bands for all four genotypes.

Table 1.

Entries	Primer Pairs Analyzed	Primer Pairs Bands Present	% Bands Present
CDA	692	268	39
T577	692	283	41
Tifton 10	692	297	43
Zebra	692	295	43

Thirty-nine to forty-five (39-45%) percent of the effective EST SSR primer pairs produced double bands for all four bermudagrass genotypes, indicating the high polymorphism and significant value for SSR markers in application (Figure 2).

Reference

da Maia LC, Palmieri DA, de Souza VQ, Kopp MM, de Carvalho FI, Costa de Oliveira A (2008) SSR locator: tool for simple sequence repeat discovery integrated with primer design and PCR simulation. International Journal of Plant Genomics. DOI:10.1155/2008/412696

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