Development of Genomic SSR Markers in *Cynodon dactylon*

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ABSTRACT

Bermudagrass, *Cynodon dactylon* (L.) Pers., is economically important as a major turf and forage crop in the southern United States. The grass is highly cross-pollinated. Consequently, individuals are highly heterozygous. Microsatellite, or simple sequence repeats (SSR), DNA markers are co-dominant and therefore are useful in bermudagrass genetic and breeding research. However, genomic SSR markers are not currently available for bermudagrass research. The objectives of this study were to construct and sequence SSR-enriched genomic DNA libraries, to identify SSR containing sequences, to design SSR primer pairs, and to verify the designed SSR primer combinations. A genomic DNA sample was extracted from the bermudagrass genotype 'Zebra' using IBI Scientific Plant Genomic DNA Maxi Kit. Five libraries enriched in core SSR CA-, GA-, ATG-, AAC- and CAG-sequences were constructed using the pUC19 plasmid. The SSR-enriched DNA inserts are being sequenced at the Oklahoma State University Core Facility. The software program 'SSR Locator' was used to identify SSR sequences, and to design SSR primers, which will be used in the polymerase chain reaction (PCR) to amplify and characterize SSR alleles in a panel of bermudagrass genotypes. Initial sequence data for the –CA library (N=759) resulted in, 87% long reads (>600bp), 6% medium reads (300-600bp), and 7% showed short reads (<300bp). 'SSR locator' found 96% of the sequences submitted contained an SSR of which 57% were perfect and 43% were compound. Primers were designed for 82% of the sequences that contained an SSR. A total of 65%, of the primers designed, were non-redundant and were used to screen genomic DNA from two bermudagrass species (*Cynodon dactylon* 'Zebra' and *Cynodon transvaalensis* 'Uganda'). The –CA library screening of 'Zebra' and 'Uganda' (391 primer pairs) produced 84.4% and 80.6% reproducible and strong amplification products, 3.6% and 3.6% amplification products of the non-predicted size, and 12.0% and 15.9% resulted in no reactions, respectively. The information obtained by this project will aid in the creation of a molecular map for this species.

MATERIALS AND METHODS

Genomic DNA Extraction

- 1.5 G of Greenhouse grown 'Zebra' & 'Uganda' leaf tissue was ground in liquid nitrogen to extract genomic DNA.
- Genomic DNA extraction was performed using the CTAB method or IBI Maxi Prep Kit .

Libraries/SSR/ Primer Design

- Genetic Identification Services (California) created five enriched SSR libraries using the pUC19 plasmid (CA-, GA-, ATG-, AAC- and CAG).
- Using Blue/White screening the libraries were screened and the SSR Locator program was used to find SSRs from sequences constructed by the OSU core facility.

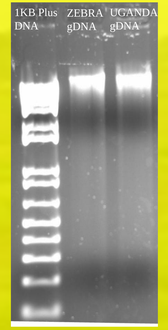
Analysis

- QV 20 score (AB Sequencer Software) was used to score the probability that the called base is correct in OSU core sequence data.
- Li-COR 4300 Gel Analysis System in conjunction with SAGA lite software was used to quantify SSR size and number.

Detailed Materials and Methods Available Upon Request

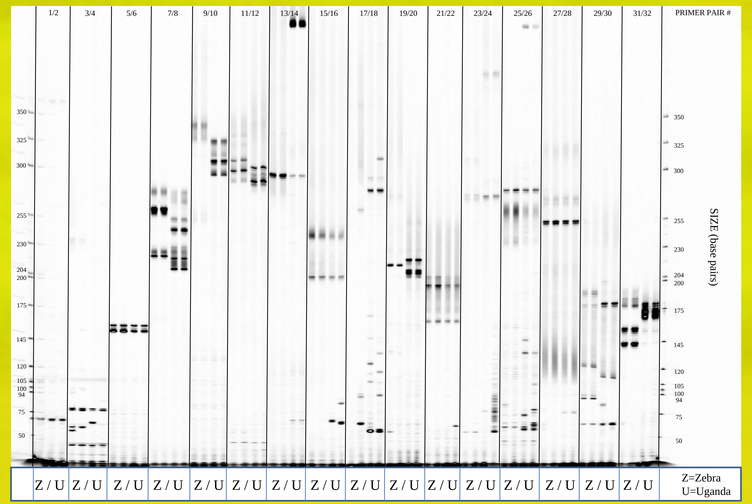
RESULTS

DNA



SSR

Total 96 well plates:	8
Total wells:	768
# of Controls:	4 (plus, 5 missed wells)
Total # of wells analyzed:	759
Total # good sequences:	759
Total # SSRs:	726
Total # primer pairs (PPs) designed:	602
Total # redundant PPs:	211
Total # non-redundant PPs:	391



CONCLUSIONS

Genomic DNA Extraction

- High Quality 260/280 1.8> using IBI Maxi Kit (for library construction) and/or CTAB method (for primer pair analysis).

Sequence Data Analysis

- Sequence reads were of high quality and reliable as indicated by a mean QV 20 score read of 906 bp

SSR Analysis

- 84.4% of SSRs were amplified strongly in *Cynodon dactylon* 'Zebra' while 80.6% were amplified strongly in *Cynodon transvaalensis* 'Uganda'.
- Data will aid in the construction of a genetic map and cultivar identification for *Cynodon dactylon.*

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