Genomic diversity in bermudagrass (*Cynodon* spp.)

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Introduction

- *Cynodon dactylon* var. *dactylon* (tribe Cynodonteae, subfamily Chloridoideae), a.k.a bermudagrass) is a warm-season perennial grass used as turfgrass, forage, and for soil stabilization.
- Limited knowledge of genetic diversity and genomic resources in bermudagrass (*Cynodon* spp.) is a hindrance for efficiently using the germplasm in the development of new bermudagrass cultivars.
- Previous genetic studies in bermudagrass have largely relied on single bi-parental or selfed populations and on small germplasm collections.
- To understand genetic diversity at genus level, a germplasm panel of 206 accessions was assembled for this study and genotyped.

Objective

- To assess genetic diversity, population structure, and relatedness among members of the bermudagrass germplasm panel.

Materials and Methods

- A diversity panel consisted of 193 common bermudagrass and 13 African bermudagrass accessions was assembled (Photo 1).
- DNA sequencing libraries were prepared following the genotyping-by-sequencing (GBS) protocol with *Ape*KI enzyme (Elshire et al., 2011) and sequenced with 101 bp single end reads on an Illumina Novaseq SP platform.
- Raw DNA sequencing data were processed with customized Perl scripts. SNPs were then called *de novo* using the UNEAK pipeline (Lu et al., 2013) of TASSEL 3 standalone.
- Population structure was assessed by principal component analysis (PCA) and by running ADMIXTURE software for K values from 1 to 10.
- Identity by state (IBS) matrix was calculated in TASSEL and a neighbor joining (NJ) tree was visualized in Interactive Tree Of Life (iTOL) v5.
- Genetic diversity parameters were calculated in TASSEL 5.0 and Fst statistic was calculated by using adegenet R package (Table 1).

Photo 1. A glimpse of diverse accessions in this germplasm panel which have been genotyped.

Results

Table 1. Diversity parameters for sub populations and germplasm panel.

Cluster	SegSites	Pi (π)	Theta (θ)	Tajima's D
Cluster 1	35343	0.21803	0.2114	0.11538
Cluster 2	25907	0.22819	0.22265	0.11522
Cluster 3	28240	0.17523	0.19011	-0.30685
Cluster 4	21782	0.18242	0.1872	-0.11822
Overall	37496	0.27197	0.1943	1.37043

Table 2. Pairwise Fst value among four subpopulations.

	Cluster 4	Cluster 1	Cluster 2
Cluster 1	0.3341		
Cluster 2	0.3437	0.2016	
Cluster 3	0.4553	0.3638	0.2336

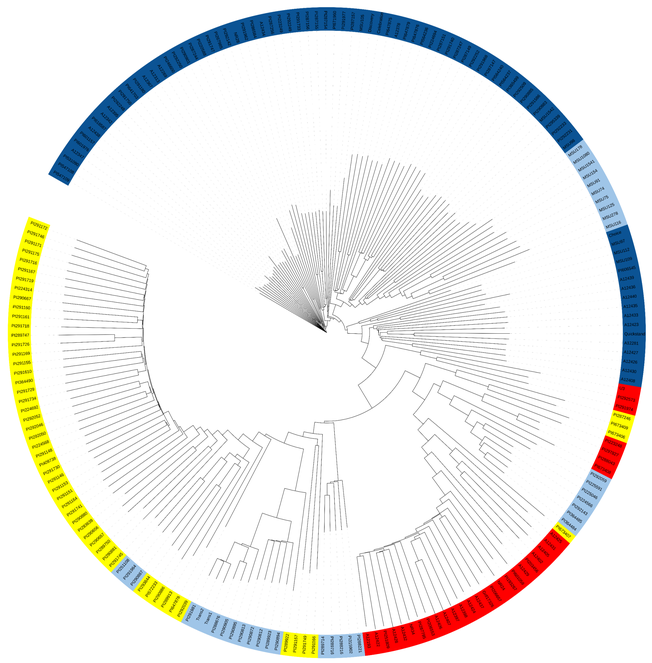

Fig. 3. Phylogenetic tree exhibiting relatedness and evolutionary relationship of accessions in the germplasm panel. These 206 *Cynodon* accessions are colored according to the four clusters which are obtained from ADMIXTURE results.

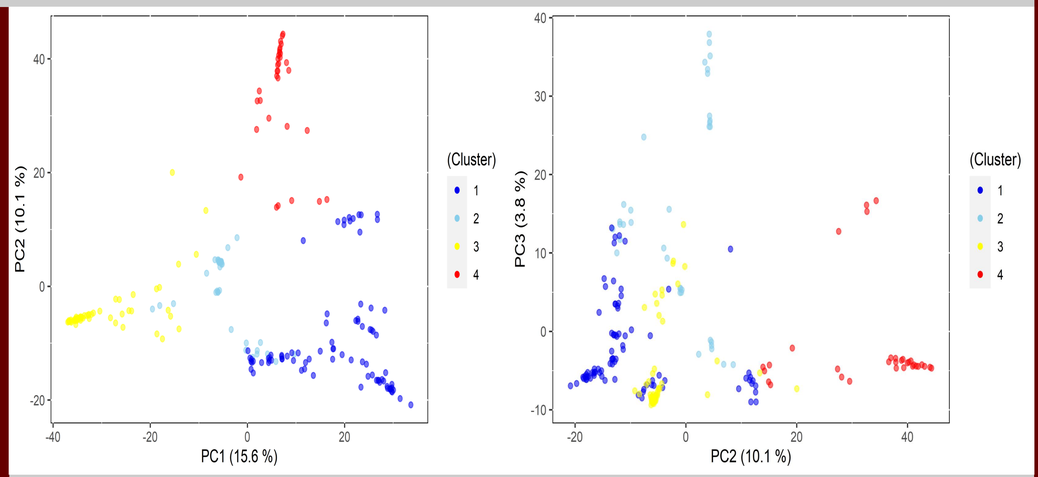

Fig. 1. Principal component analysis across the germplasm panel. Variance explained by each PC is in parentheses.

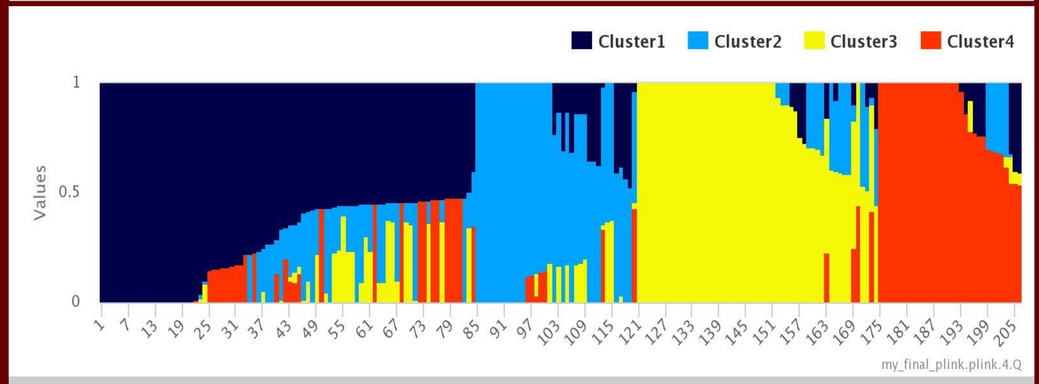

Fig. 2. Population structure of 206 accessions obtained from ADMIXTURE with K=4 revealed four subpopulations in the germplasm panel.

Results Summary

- A total of 600,380,494 reads were generated, with an average of 2.9 million reads per sample, and 537,127,057 reads were retained after trimming.
- A total of 37,496 raw SNPs were obtained with minor allele frequency of 0.05, minimum call rate of 0.5.
- Population structure analysis using ADMIXTURE revealed four subpopulations in this germplasm panel, and this was found to be consistent with principal component analysis (PCA) and phylogenetic analysis results (Figs. 2,3).
- Principal component (PC) analysis indicted that PC1, PC2, and PC3 explained 15.6%, 10.1%, and 3.8% of the genetic variation in the panel, respectively (Fig. 1).
- Overall composition and cause of four subpopulations:
  Cluster 1: Admixture of accessions from different continents
  Cluster 2: Majorly African bermudagrass (*C. transvaalensis*) accessions
  Cluster 3: *C. dactylon* accessions of African origin
  Cluster 4: Accessions from Oklahoma State University breeding program mainly
- Genetic diversity parameters including nucleotide diversity or average pairwise divergence (π), estimated mutation rate or expected nucleotide diversity (θ), Tajima's D statistic, and Fst statistic revealed substantial genetic variation in *Cynodon* genus (Tables 1, 2).

Future Studies

This diversity study will lay the foundation for association studies. Genotypic data generated from this study coupled with phenotypic data will be used to perform genome-wide association studies (GWAS) for important agronomic traits.

References

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Acknowledgements

This study is supported by USDA HATCH fund (MIS-213140) and Mississippi Department of Agriculture and Commerce (Award # G00006342). We also thank Geoffrey Lalk, Jason Ruffin, and Hannah Miller for maintaining greenhouse and field trials.