Evaluation of Off-Type Grasses in Hybrid Bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) Putting Greens Using Genotyping-by-Sequencing

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Introduction

- Off-type grasses (OTG) have been reported and observed in 'Champion', 'TifEagle', and 'MiniVerde' ultradwarf hybrid bermudagrass (UDBG; *Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) putting greens (Fig.1; Fig.2).[1,9,10,13,18]
- OTGs have different morphology and performance compared to the surrounding, desirable turfgrass.[2,3,4,18]
- Researchers using various molecular markers have had limited success in genetically distinguishing among these UDBGs and OTGs.[2,3,4,5,6,8,10,11,12,14,18,19,20]
- Genotyping-by-sequencing (GBS) is a high-throughput, next generation sequencing method capable of generating large numbers of single nucleotide polymorphisms (SNPs) on high diversity species.[7,16,17,18]
- The GBS technique can provide information about the entire genome, which could aid in understanding the genetic variation among OTGs and UDBGs. [7,16,17,18]
- GBS does this by targeting important regions of the genome such as non-coding, regulatory regions of DNA that are inaccessible to other molecular marker methods. [7,16,17,18]
- A complete reference genome sequence has not been developed for bermudagrass, but GBS does not require one because a reference map is developed around restriction sites. [7,16,17,18]
- GBS has the potential to investigate the genetic variation among OTGs and UDBGs based on the robustness of the technique and successful use in other grasses. [7,16,17,18]

Objectives

1)To explore the genetic variation among OTGs sampled from UDBG putting greens using GBS

2)To explore the genetic variation among UDBGs using GBS

Materials and Methods

Plant Material

- Desirable and OTGs were selected from 15 golf course putting greens located in five states in 2013 and UDBGs and progenitor species (*C. dactylon* and *C.* transvaalensis) were obtained from the University of Georgia (Tifton, GA) (Table 1).
- Plant material was cultured from single stolon transplants until stolons reached a minimum of five nodes in a glasshouse at the University of Tennessee (Knoxville, TN).
- Ploidy of all samples was confirmed using flow cytometry[10,15] (Table 1).

Genotyping-by-Sequencing

- Genomic DNA was isolated from leaf tissue using the Qaigen DNeasy Mini Kit (Qiagen, Velencia, California, USA).
- The GBS protocol was optimized using the ApeKI restriction enzyme to maximize the number of sampled genomic loci.
- Individual libraries were barcoded, multiplexed, and sequenced in duplicate on the Illumina HiSeq at the Cornell University Institute of Biotechnology according to methods described by Elshire et al. (2011).[7]

Table 1. Plant material used in genotyping-by-sequencing. Selections included 47 desirable and off-type bermudagrasses sampled from golf course putting greens, five ultradwarf bermudagrass (*Cynodon dactylon* (L.) x *C. transvaalensis* Burtt-Davy) cultivars, and two progenitor species (*Cynodon dactylon* and *C. transvaalensis*).

ID	Loc.	Selection	Ploidy	ID	Loc.	Selection	Ploidy	ID	Loc.	Selection	Ploidy	ID	Loc.	Selection	Ploidy
S1	TN	Champion	2n=3x=27	S17	AL	TifEagle	2n=3x=27	S32	MS	Off-Type	2n=3x=27	TG1-6	GA	Tifgreen	2n=3x=27
S2	MS	Champion	2n=3x=27	S18	TN	TifEagle	2n=3x=27	S33	TN	Off-Type	2n=3x=27	TD1-6	GA	Tifdwarf	2n=3x=27
S3	TN	Champion	2n=3x=27	S19	FL	Off-Type	2n=3x=27	S34	MS	Off-Type	2n=3x=27	TE1-6	GA	TifEagle	2n=3x=27
S4	TN	Champion	2n=3x=27	S20	FL	Off-Type	2n=3x=27	S35	AR	Off-Type	2n=3x=27	MV1-6	GA	MiniVerde	2n=3x=27
S5	TN	Champion	2n=3x=27	S21	TN	Off-Type	2n=3x=27	S36	TN	Off-Type	2n=3x=27	CH1-6	GA	Champion	2n=3x=27
S6	TN	Champion	2n=3x=27	S22	MS	Off-Type	2n=3x=27	S37	TN	Off-Type	2n=3x=27	TW1-6	GA	Tifway	2n=3x=27
S7	TN	Champion	2n=3x=27	S23	TN	Off-Type	2n=3x=27	S38	TN	Off-Type	2n=3x=27	TA1-2	GA	T-89	2n=4x=36
S8	MS	Champion	2n=3x=27	S24	TN	Off-Type	2n=3x=27	S39	AL	Off-Type	2n=3x=27	TB1-2	GA	T-797	2n=4x=36
S9	TN	Champion	2n=3x=27	S25	TN	Off-Type	2n=3x=27	S40	AL	Off-Type	2n=3x=27	DA1-2	GA	T-574	2n=2x=18
S10	MS	Champion	2n=3x=27	S26	TN	Off-Type	2n=3x=27	S41	TN	Off-Type	2n=3x=27	DB1-2	GA	T-617	2n=2x=18
S11	AR	Champion	2n=3x=27	S27	TN	Off-Type	2n=3x=27	S42	TN	MiniVerde	2n=3x=27				
S12	TN	Champion	2n=3x=27	S27	TN	Off-Type	2n=3x=27	S43	FL	Off-Type	2n=3x=27				
S13	FL	MiniVerde	2n=3x=27	S28	TN	Off-Type	2n=3x=27	S44	MS	Off-Type	2n=3x=27				
S14	FL	MiniVerde	2n=3x=27	S29	TN	Off-Type	2n=3x=27	S45	MS	Off-Type	2n=3x=27				
S15	TN	MiniVerde	2n=3x=27	S30	TN	Off-Type	2n=3x=27	S46	TN	Off-Type	2n=3x=27				
S16	TN	MiniVerde	2n=3x=27	S31	MS	Off-Type	2n=3x=27	S47	AL	TifEagle	2n=3x=27				

Fig. 1. Off-type grasses (lighter in color) present in an ultradwarf bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) putting green. Photo courtesy of Mr. Rodney Lingle.

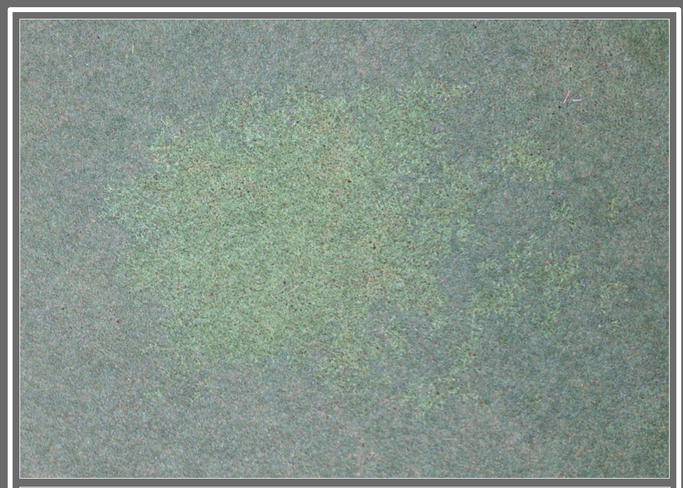

Fig. 2. Close-up of an off-type grass patch present in an ultradwarf bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) putting green.

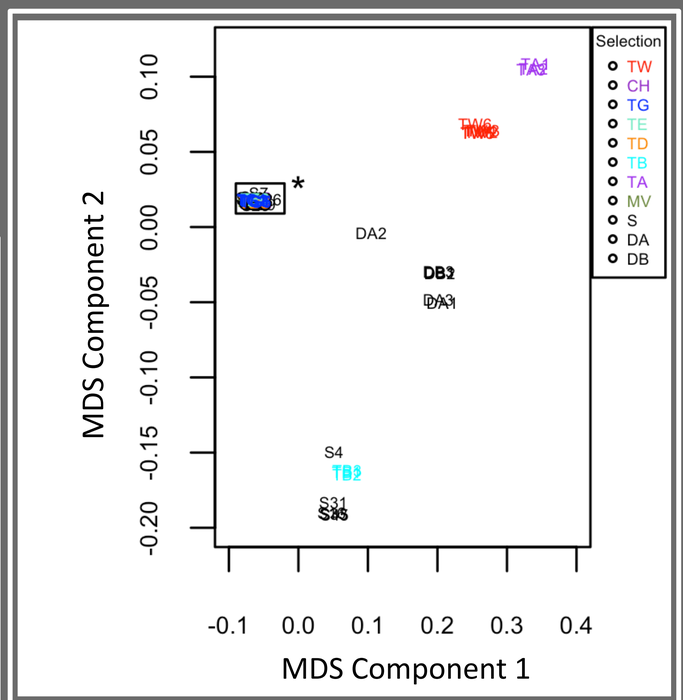

Fig. 3. Multidimensional scaling plot of single nucleotide polymorphisms from 47 desirable and off-type bermudagrasses sampled from golf course putting greens, five ultradwarf bermudagrass (*Cynodon dactylon* (L.) x *C. transvaalensis* Burtt-Davy) cultivars, and two progenitor species (*Cynodon dactylon* (L.) Pers. and *C. transvaalensis* Burtt-Davy).

Bioinformatics Analysis

- Selections S19, S28, S30, S32, and S44 were excluded from the bioinformatics analysis due to lack of read depth.
- Analysis was performed with the UNEAK pipeline from Cornell University designed for calling SNPs from GBS data on diploid organisms without a reference genome.
- Since UDBGs are triploid, SNPs were generated using Freebayes (Version 1.0.2-15; http://arxiv.org/abs/1207.3907), a multidimensional scaling (MDS) plot was calculated in Plink (Version 1.07; http://pngu.mgh.harvard.edu/purcell/plink), and then plotted in R (Version 3.1.1; http://www.r-project.org) to show the genetic variation among selections.
- Analysis of the combined datasets yielded 1,337,384 total SNPs for the triploid selections.
- Filtering SNPs using a minimum read depth of 40 yielded 128,476 potentially informative SNPs for triploid selections.

Results and Discussion

- The MDS plot (Fig. 3) revealed clear clustering of the progenitor species (DA1&2, DB1&2, TA1&2, and TB1&2) from UDBGs and the majority of OTGs; therefore, GBS is an effective method for differentiating diploid, triploid, and tetraploid bermudagrasses.
- The MDS plot (Fig. 3) also revealed clear clustering of 'Tifway' hybrid bermudagrass (TW 1-6) from UDBGs, OTGs, and progenitor species, so as a result, GBS is a technique that can differentiate between UDBG cultivars and other hybrid bermudagrasses.
- Of the 47 samples selected from golf course putting greens, only 5 (~11%) were genetically divergent from UDBGs; therefore, phenotypic variation among OTGs may be caused by differential gene expression.
- The 128,476 potentially informative SNPs may provide enough information about the genetic variation of UDBG cultivars to genetically distinguish them; however, more bioinformatics analysis is needed to ascertain which SNPs are potentially diagnostic.

Conclusions

- **The majority of OTGs sampled from ultradwarf putting greens are genetically similar to UDBGs.**
- **The 128,476 potentially informative SNPs for triploid selections may genetically distinguish among UDBGs.**

Future Research

- A complete reference genome sequence for bermudagrass would help determine if SNPs were potentially informative or diagnostic.
- RNA analysis on OTGs could determine if differential gene expression is causing the variation in phenotypes given their genetic similarity to UDBGs.

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