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Introduction

Zoysiagrass

(*Zoysia japonica* Steud. and *Zoysia matrella* (L.) Merr.)

Warm season, perennial turfgrass
Used in commercial and residential lawns



- ✓ Low maintenance
- ✓ Lower inputs
- ✓ Desirable dense texture
- ✓ Good drought & shade tolerance
- ✗ Poor cold tolerance
- limits use to South and transition zone



Meyer:

Industry standard for cold tolerance since its release in 1951
Excellent cold tolerance but poor establishment and coarse texture



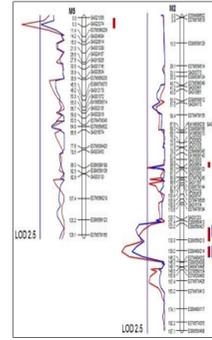
Molecular Markers

Linkage Mapping and QTL Analysis

Molecular markers are powerful tools for examining genetic variation and constructing linkage maps in plant populations

- **Simple sequence repeats (SSRs):** Efficient co-dominant PCR-based markers found throughout the genome
- **Single nucleotide polymorphisms (SNPs):** Abundant molecular markers helpful in creating high density maps
- **Genotyping by Sequencing (GBS):** Time efficient and cost effective method for rapid SNP discovery

Linkage maps can then be scanned for associations with particular phenotypes in order to identify genomic regions (QTL) controlling the trait of interest.



Marker Assisted Selection

Through MAS, the efficiency of selection can be improved by using DNA technology to determine the presence of desirable genes rather than waiting for gene expression. MAS can be used to transfer through crossing and selection specific genes into a desired genetic background.

Research Objectives

1. Phenotype a mapping population of Meyer x Victoria for winter survival
2. Genotype the population using SSR and SNP markers
3. Construct a linkage map which will be used to identify QTL controlling winter survival

Materials & Methods

Plant Materials



Meyer



Victoria

- ↑ cold tolerance
- Coarse leaf texture
- Slow establishment

- ↓ cold tolerance
- Finer leaf texture
- Faster establishment



175 progeny

- Pseudo-F₂ mapping population
- Planted in 3 replications in 3x3 ft plots in a randomized complete block design (RCBD) at 3 locations:



- William H. Daniel Turfgrass Research and Diagnostic Center, **West Lafayette, IN**
2014 High/Low: 92°F/-21°F
- Upper Mountain Research Station, **Laurel Springs, NC**
2014 High/Low: 82°F/-8°F
- University of Georgia Coastal Plain Experiment Station, **Tifton, GA**
2014 High/Low: 98°F/16°F¹

Phenotypic Evaluation

- **Winter injury, turf quality, percent coverage** were evaluated in 2014-2015 and will be evaluated in 2016-2017
- **Digital image analysis (FIJI)** using pictures taken of each plot periodically from March to October using a lightbox for uniformity of images²
- **Winter Injury** calculated using the formula: Winter Injury = $100 - [(Coverage\ after\ Winter\ Dormancy / Coverage\ before\ Winter\ Dormancy) \times 100]$ ³



Percent Coverage Evaluation using FIJI

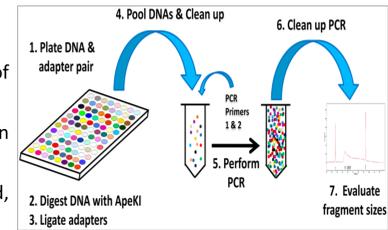
Genotypic Evaluation

- **SSRs**
 - DNA was extracted from 175 progeny and two parents using CTAB protocol⁴
 - The population was genotyped with approximately 125 SSR markers and evaluated on 12% polyacrylamide gel electrophoresis (PAGE) using LICOR sequencers.⁵

Genotypic Evaluation

- **SNPs: Genotyping by Sequencing (GBS)** for SNP discovery⁶

- Double digest with SphI and MluCI
- Multiplexed with 96 barcoded adapters
- Four Illumina indices allowed sequencing of 175 progeny + two parents simultaneously
- Sequence PCR amplified libraries on Illumina HiSeq 200SR platform
- Unique tags identified in Stacks, compiled, and used for mapping and SNP calling



Data Analysis

- **SAS:** to evaluate the variability of turf quality, percent cover, and winter survival within and between genotypes⁷
- **STACKS:** to call SNPs from 200M+ reads generated by GBS⁸
- **JoinMap:** to create a genetic linkage map with approximately 125 SSR markers, potentially over 10,000 SNPs, and the 175 genotypes of the mapping population⁹
- **MapQTL:** to identify QTL for cold tolerance based on genotypic and phenotypic data¹⁰

Results

Phenotypic Evaluation

The mapping population showed variation in color, texture, turf quality, aggressiveness, and winter injury in the winter of 2014.



- **Winter Survival & Winter Injury**

59% winter survival in Laurel Springs, NC
21% winter survival in West Lafayette, IN

Laurel Springs, NC		West Lafayette, IN	
11-TZ-4745	11-TZ-4815	11-TZ-4720	
11-TZ-4781	11-TZ-4779	11-TZ-4755	
Meyer	11-TZ-4757	11-TZ-4778	
11-TZ-4800	Zenith	11-TZ-4842	
11-TZ-4740	11-TZ-4768	11-TZ-4877	
11-TZ-4758	11-TZ-4840	Meyer	
11-TZ-4738	11-TZ-4724	Chinese common	
11-TZ-4720	11-TZ-4782		
11-TZ-4843	11-TZ-4890		
11-TZ-4726	11-TZ-4826		
11-TZ-4854	11-TZ-4819		
11-TZ-4794	11-TZ-4727		
11-TZ-4799	11-TZ-4884		
11-TZ-4877	11-TZ-4837		
11-TZ-4784	11-TZ-4753		
11-TZ-4851	11-TZ-4789		
11-TZ-4815	11-TZ-4836		

Tables 1 & 2: 36 lines suffered no winter injury in Laurel Springs, NC. Seven lines suffered no winter injury in West Lafayette, IN. Lines that suffered no injury at both locations are highlighted in red.

Winterkill 2014

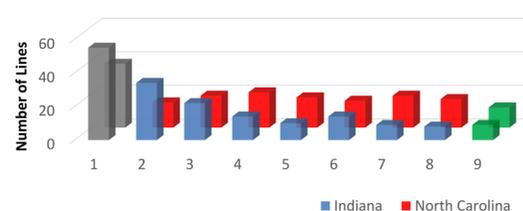


Figure 1: Distribution of winterkill for 175 progeny, Meyer, and Victoria at Laurel Springs, NC and West Lafayette, IN in 2014. Winterkill is a measure of winter injury on a scale of 1 (completely dead) to 9 (no winterkill). Bars in grey and green indicate where Victoria and Meyer, respectively, fell.

Winter Injury (%) 2014

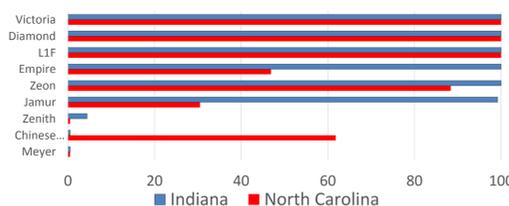


Figure 2: Winter injury of nine controls in Laurel Springs, NC and West Lafayette, IN in 2014. Winter injury is measured in percentages from 0-100%.

Genotypic Evaluation

- **SSRs**

- 125 primers screened for polymorphisms across the mapping population and will be mapped using JoinMap

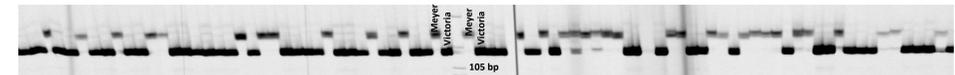


Figure 3: Amplified products generated from SSR b02d15 for selection of individuals from the Meyer x Victoria mapping population. This polyacrylamide gel is an example of screening the population for molecular markers.

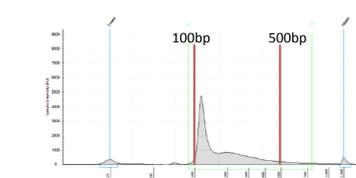


Figure 4: Bioanalyzer results for GBS library

- **GBS**

- Library was prepped and sent to the NCSU Genomic Sequencing Lab for sequencing
- Bioanalyzer analysis of library shows a concentration of size selected sequenceable fragments at 121bp.

Preliminary Conclusions :: Ongoing Work

- First year data showed wide range of trait expression within the population which is integral to the identification of quantitative trait loci (QTL), so the variability in winter survival seen in these progeny is promising for the identification of markers associated with cold tolerance in zoysiagrass.
- Winter injury, turf quality and percent cover data will be collected in 2015 and 2016
- Continued genotypic evaluation with SSRs using PAGE, and SNPs using GBS.
- The identification and mapping of SNP markers in addition to the SSRs will add depth to the linkage map.
- Increased coverage will improve our power to detect QTL for cold tolerance in zoysiagrass.