Quantitative Trait Loci (QTL) Analysis of Freezing Tolerance in Zoysia Grass

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Introduction

Zoysia Grass (Zosia japonica Steud. and Zosia 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 us to South and transition zones

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 (SSR): 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

Phenotypic Evaluation

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

Genotypic Evaluation

- SNPs: Genotyping by Sequencing (GBS) for SNP discovery
  - Double digest with Spil and MluI
  - Multiplexed with 96 barcoded adapters
  - Four Illumina indices allowed sequencing of 175 progeny + two parents simultaneously
  - Sequence PCR amplified libraries on Illumina HiSeq 2000R 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 genotypes
- STACKS: to call SNPs from 200M+ reads generated by GBS
- JoinMap: to create a genetic linkage map with approximately 125 SSR markers, potentially over 10,000 SNPs, and the 175 genotypes of the mapping population
- MapQTL to identify QTL for cold tolerance based on genotypic and phenotypic data

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

Winterkill 2014

![Winterkill 2014 Graph](image)

Genotypic Evaluation

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

GBS

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

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 zoysia grass.
- 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 zoysia grass.

References:


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