Detection of Quantitative Trait Loci Associated with Drought Tolerance in St. Augustinegrass

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NC STATE
TURFGRASS BREEDING & GENETICS
St. Augustinegrass breeding

Properties:
- Warm-season grass
- Widely used as lawn grass in southern US
- Creeping growth habit via stolon
- Fast growing, establishes rapidly
- Relatively low input

Breeding goals:
- Aesthetic performance
- Drought tolerance
- Cold tolerance
- Resistance to chinch bug
- Resistance to grey leaf spot
Molecular breeding in turfgrass

Introduction

The areas of molecular breeding include:

- QTL mapping or gene discovery
- Marker assisted selection and genomic selection
- Genetic engineering and transformation

Application on turfgrass

- Conventional breeding methods still are the most applied and efficient methodologies
- Molecular breeding is becoming popular
- This is the time to equip turfgrass breeding programs with advanced genomic tools
Drought tolerance

Drought stress:
- One of the most important environmental factors limiting the growth of turfgrasses.
- The selection and development of species and cultivars of turfgrasses that resist drought stress and require minimal inputs of supplemental irrigation is a viable strategy to conserve water.

Drought tolerance evaluation:

Visual rating
- Turf quality
- Leaf wilting and firing
  - Morris and Shearman, 2006
  - Kim et al., 1988

Morphological traits
- Root depth
- Root dry weight
  - Huang et al., 1997
  - Su et al., 2008

Physiological response
- Chlorophyll content and fluorescence
- Relative water content (RWC)
  - Huang et al., 1997

Digital image analysis
- Green cover (%)
  - Steinke et al., 2010
Objectives

- Develop high density SNP-based genetic map for St. Augustinegrass
- Evaluate drought tolerance related traits in St. Augustinegrass
- Identify QTL controlling drought traits in St. Augustinegrass
Population was derived from cross between St. Augustinegrass cultivars ‘Raleigh’ and ‘Seville’, both on diploid level (2n=2x=18).

\[
\text{cv. ‘Raleigh’} \times \text{cv. ‘Seville’} \rightarrow 115 F_1 \text{ hybrids} \rightarrow \text{Segregating for drought tolerance}
\]
Drought tolerance evaluation

Experimental design:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Greenhouse 2017 (GH17)</th>
<th>Greenhouse 2018 (GH18)</th>
<th>Sandhill field 2018 (F18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Three replicates at Randomized complete block design</td>
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<td></td>
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<tr>
<td>Drought</td>
<td>Withhold water until 5% soil water content</td>
<td>Annually recurring drought period in July and August</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Traits</th>
<th>Greenhouse 2017 (GH17)</th>
<th>Greenhouse 2018 (GH18)</th>
<th>Sandhill field 2018 (F18)</th>
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</thead>
<tbody>
<tr>
<td>Leaf relative water content (RWC)</td>
<td>RWC</td>
<td>NDVI</td>
<td></td>
</tr>
<tr>
<td>Green cover percent (GC)</td>
<td>GC</td>
<td>LF</td>
<td></td>
</tr>
<tr>
<td>Leaf wilting (LW)</td>
<td>LW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf firing (LF)</td>
<td>LF</td>
<td></td>
<td></td>
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<tr>
<td>Chlorophyll fluorescence (Fv/Fm)</td>
<td>Fv/Fm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Traits measurement:

- RWC (%) = (Fresh weight – Dry weight)/(Turgid weight – Dry weight) *100.
- Chlorophyll fluorescence (Fv/Fm): MultiSpeQ v 1.0.
- Green cover (GC): Fiji image analysis.
- Leaf wilting (LW) and firing (LF): visual rating score 1-5.
- NDVI: TCM 500 Turf Color Meter.
Genotyping and QTL detection

Genotyping platform:
- Genotyping-by-sequencing on Illumina HiSeq 2500

Linkage map:
- Joinmap 4.0

QTL detection:
- MQM composite interval mapping on MapQTL 6.0

Statistics:
- SAS PROC MIX
Results

Genetic maps

Raleigh map

Seville map

Results

Distribution of traits

Distribution of each traits in Raleigh x Seville population

RWC

Fv/Fm

GC

LW

LF

RH17

Number of individuals

93.5 94.5 95.5 96.5 97.5 98.5 99.5

12.5 17.5 22.5 27.5 32.5 37.5

45 55 65 75 85 95

0.5 1.5 2.5 3.5 4.5

R18

GH18

Number of individuals

79 81 83 85 87 89

19.5 20.5 21.5 22.5 23.5 24.5 25.5 26.5 27.5 28.5 29.5

5 15 25 35 45 55 65 75 85

0.5 1.5 2.5 3.5 4.5

F18

Number of individuals

325 375 425 475 525 575 625

0.5 1.5 2.5 3.5 4.5

△ Raleigh  ▼ Seville
## Traits variance

- **Variance (genotype effect) of drought related traits in Raleigh x Seville population**

<table>
<thead>
<tr>
<th></th>
<th>GH17</th>
<th>GH18</th>
<th>F18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fv/Fm</td>
<td>GC</td>
<td>RWC</td>
</tr>
<tr>
<td>Raleigh</td>
<td>40.14</td>
<td>62.03</td>
<td>95.60</td>
</tr>
<tr>
<td>Seville</td>
<td>28.46</td>
<td>54.76</td>
<td>86.87</td>
</tr>
<tr>
<td>Progeny Mean</td>
<td>25.67</td>
<td>71.43</td>
<td>95.85</td>
</tr>
<tr>
<td>Progeny Min</td>
<td>12.33</td>
<td>46.10</td>
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<tr>
<td>Progeny Max</td>
<td>38.80</td>
<td>91.80</td>
<td>99.49</td>
</tr>
</tbody>
</table>

| Variance (genotype) | * | *** | ** | *** | *** | * | *** | ** | *** | *** | *** | *** |

* *, **, *** Significance at \( p-value < 0.05, 0.01, 0.001 \), respectively.
Identification of top hybrids

Results

Green cover percent in greenhouse vs. NDVI in field

- XSA10098
- XSA10034
- XSA10053
- Raleigh
- Seville
- Raleigh
- XSA10051
- Raleigh
- XSA10098

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Number of QTL distributed on each linkage group for each trait.

<table>
<thead>
<tr>
<th></th>
<th>GC</th>
<th>LF</th>
<th>LW</th>
<th>NDVI</th>
<th>RWC</th>
<th>Fv/Fm</th>
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<tr>
<td>Raleigh map</td>
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<tr>
<td>R1</td>
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<td>18</td>
<td>14</td>
<td>7</td>
<td>8</td>
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### Results

**Location of QTL associated with drought tolerance related traits in Raleigh x Seville population**

<p>| | | | | | | | | | | | | |</p>
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Co-localization of QTL

Results
Conclusions

Population containing 115 hybrids

- Develop high density genetic maps
- Evaluate drought traits in greenhouse and field
- Detect 70 drought associated QTL

1. Understand the molecular basis and genetic control of drought tolerance in St. Augustinegrass.
2. Improve drought tolerance breeding through marker assisted breeding.
Ongoing works

- Mining candidate genes and allelic variation with drought tolerance in putative QTL.

- Developing new mapping population using identified hybrids with different drought tolerance level to validate QTL.

- Conducting RNA-Seq analysis to illustrate gene expression profile under drought stress.
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- Rocio van der Laat
- Jessica Brown
- Sydney Graham

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