

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

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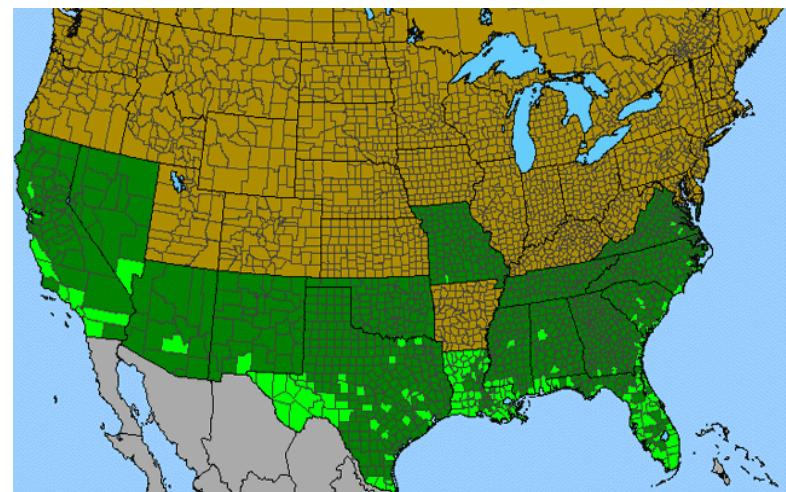
**TURFGRASS BREEDING
& GENETICS**





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





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 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 ($2n=2x=18$).

cv. 'Raleigh'

x

cv. 'Seville'

115 F₁ hybrids



Segregating for drought tolerance





Experimental design:

| Experiment | Greenhouse 2017 (GH17) | Greenhouse 2018 (GH18) | Sandhill field 2018 (F18) |
|------------|--|------------------------|--|
| Design | Three replicates at Randomized complete block design | | |
| Drought | Withhold water until 5% soil water content | | Annually recurring drought period in July and August |
| Traits | Leaf relative water content (RWC) | RWC | NDVI |
| | Green cover percent (GC) | GC | LF |
| | Leaf wilting (LW) | LW | |
| | Leaf firing (LF) | LF | |
| | Chlorophyll fluorescence (Fv/Fm) | Fv/Fm | |

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 platform:

- ❖ Genotyping-by-sequencing on Illumina HiSeq 2500

Linkage map:

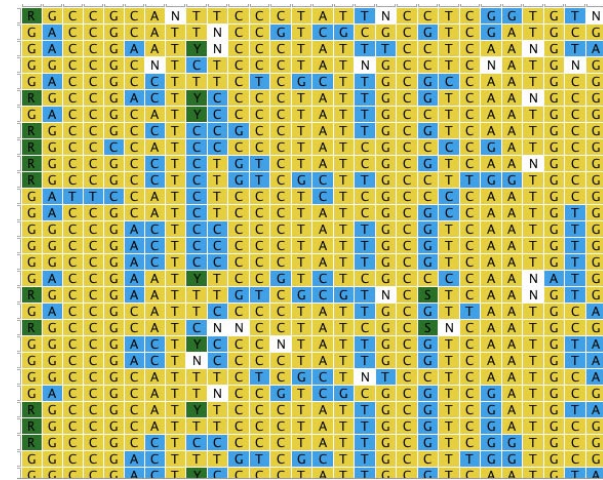
- ❖ Joinmap 4.0

QTL detection:

- ❖ MQM composite interval mapping on MapQTL 6.0

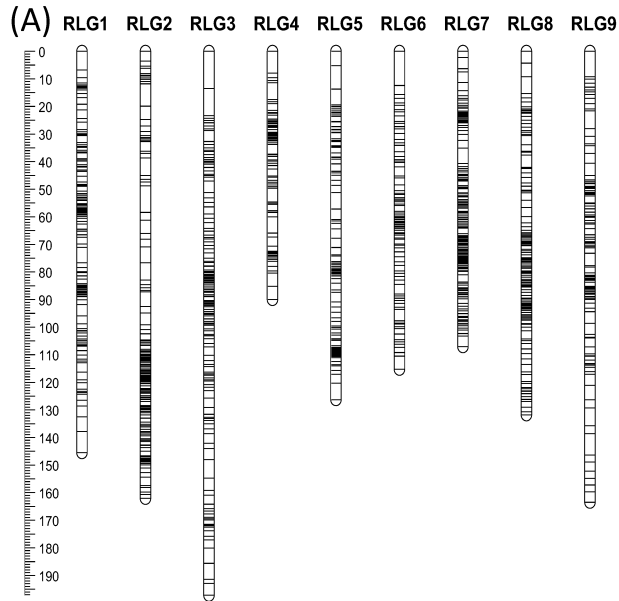
Statistics:

- ❖ SAS PROC MIX

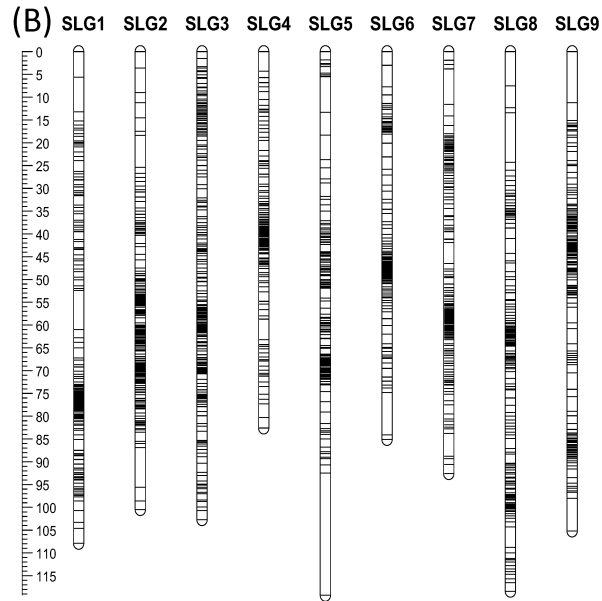




Raleigh map



Seville map

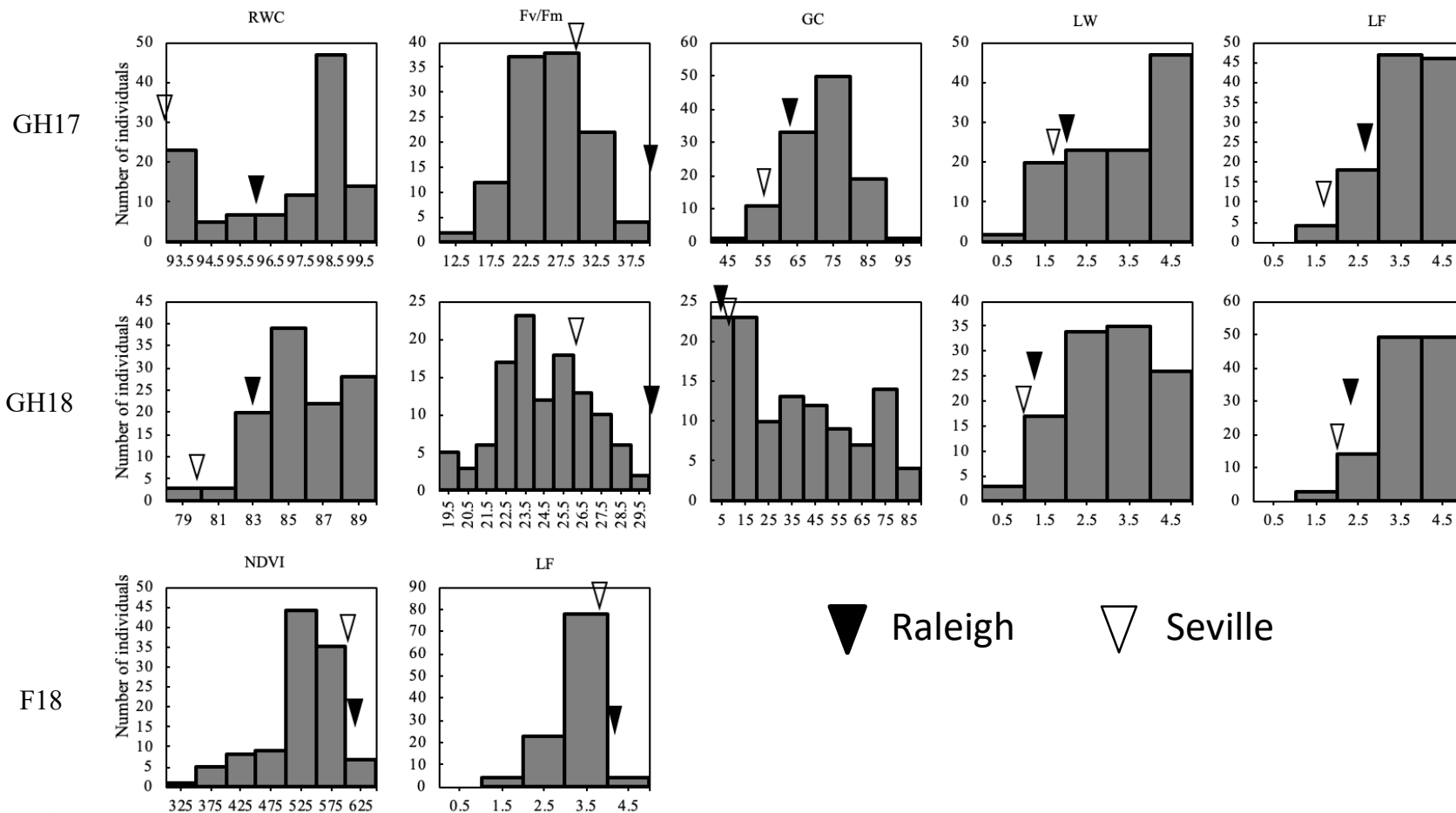


| Linkage group | Number of markers | |
|---------------|-------------------|---------------|
| | 'Raleigh' map | 'Seville' map |
| LG1 | 132 | 172 |
| LG2 | 148 | 189 |
| LG3 | 142 | 223 |
| LG4 | 78 | 150 |
| LG5 | 108 | 175 |
| LG6 | 93 | 128 |
| LG7 | 151 | 200 |
| LG8 | 129 | 164 |
| LG9 | 119 | 170 |
| Total | 1100 | 1571 |





❖ Distribution of each traits in *Raleigh* x *Seville* population





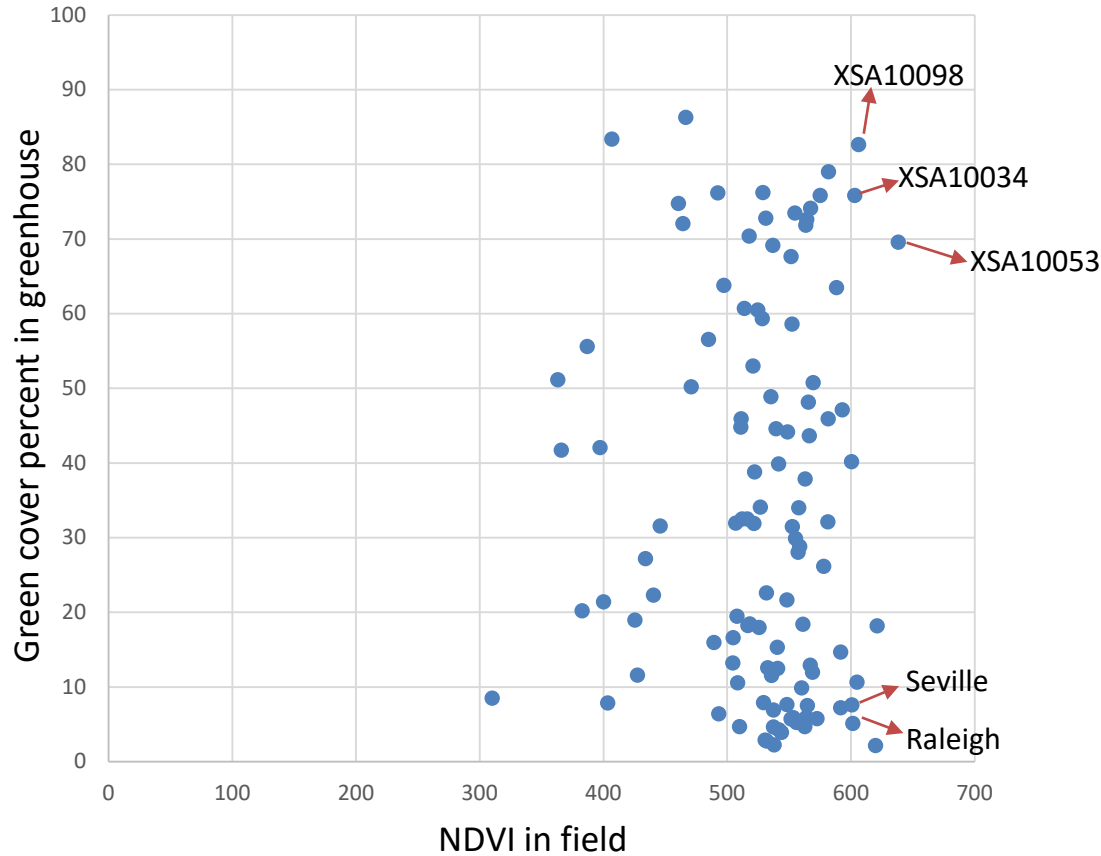
❖ Variance (genotype effect) of drought related traits in *Raleigh x Seville* population

| | GH17 | | | | | GH18 | | | | | F18 | |
|---------------------|-------|-------|-------|------|------|-------|-------|-------|------|------|--------|------|
| | Fv/Fm | GC | RWC | LW | LF | Fv/Fm | GC | RWC | LW | LF | NDVI | LF |
| Raleigh | 40.14 | 62.03 | 95.60 | 2.00 | 2.67 | 30.55 | 5.11 | 82.99 | 1.33 | 2.33 | 606.83 | 4.08 |
| Seville | 28.46 | 54.76 | 86.87 | 1.67 | 1.67 | 26.31 | 7.61 | 79.64 | 1.00 | 2.00 | 600.67 | 3.83 |
| Progeny Mean | 25.67 | 71.43 | 95.85 | 3.55 | 3.86 | 24.46 | 35.02 | 85.66 | 3.28 | 3.95 | 525.22 | 3.19 |
| Progeny Min | 12.33 | 46.10 | 77.72 | 1.00 | 1.33 | 19.00 | 2.15 | 79.01 | 1.00 | 1.67 | 310.00 | 1.25 |
| Progeny Max | 38.80 | 91.80 | 99.49 | 5.00 | 5.00 | 29.97 | 86.29 | 89.31 | 5.00 | 5.00 | 638.17 | 4.38 |
| Variance (genotype) | * | *** | ** | *** | *** | * | *** | ** | *** | *** | *** | *** |

*, **, *** Significance at p -value < 0.05, 0.01, 0.001, respectively.



Identification of top hybrids



QTL detection



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

| | | GC | LF | LW | NDVI | RWC | Fv/Fm | Total |
|-------------|-------|----|----|----|------|-----|-------|-----------|
| Raleigh map | R1 | 2 | | 5 | | | | 7 |
| | R2 | | 1 | 1 | | 1 | | 3 |
| | R3 | 2 | 2 | | | 3 | | 7 |
| | R4 | 1 | 2 | | | | 2 | 5 |
| | R5 | 2 | | 1 | | | | 3 |
| | R6 | 1 | 4 | 2 | | 1 | | 8 |
| | R7 | 1 | 1 | | 4 | | | 6 |
| | R8 | 2 | 1 | | | 1 | | 4 |
| | R9 | 2 | | | | 1 | | 3 |
| Seville map | S1 | | 2 | | | | | 2 |
| | S2 | 4 | 3 | | 2 | | | 9 |
| | S3 | 1 | | 1 | | 1 | | 3 |
| | S4 | 1 | | | | | | 1 |
| | S5 | | 2 | 3 | | | | 5 |
| | S6 | | | | | | | |
| | S7 | | | | | | | |
| | S8 | 2 | | 1 | | | | 3 |
| | S9 | | | | 1 | | | 1 |
| | Total | 21 | 18 | 14 | 7 | 8 | 2 | 70 |

QTL detection



❖ Location

| | | | | | | | | |
|------|---------|----|-------|---------------|---------|------|-----|------|
| GH17 | LW-R1.2 | R1 | 11.85 | 11.45 - 12.56 | SNP5027 | 8.17 | 6.2 | 0.54 |
| GH18 | LW-R1.2 | R1 | 12.26 | 11.65 - 12.56 | SNP5027 | 5.57 | 6.0 | 0.59 |

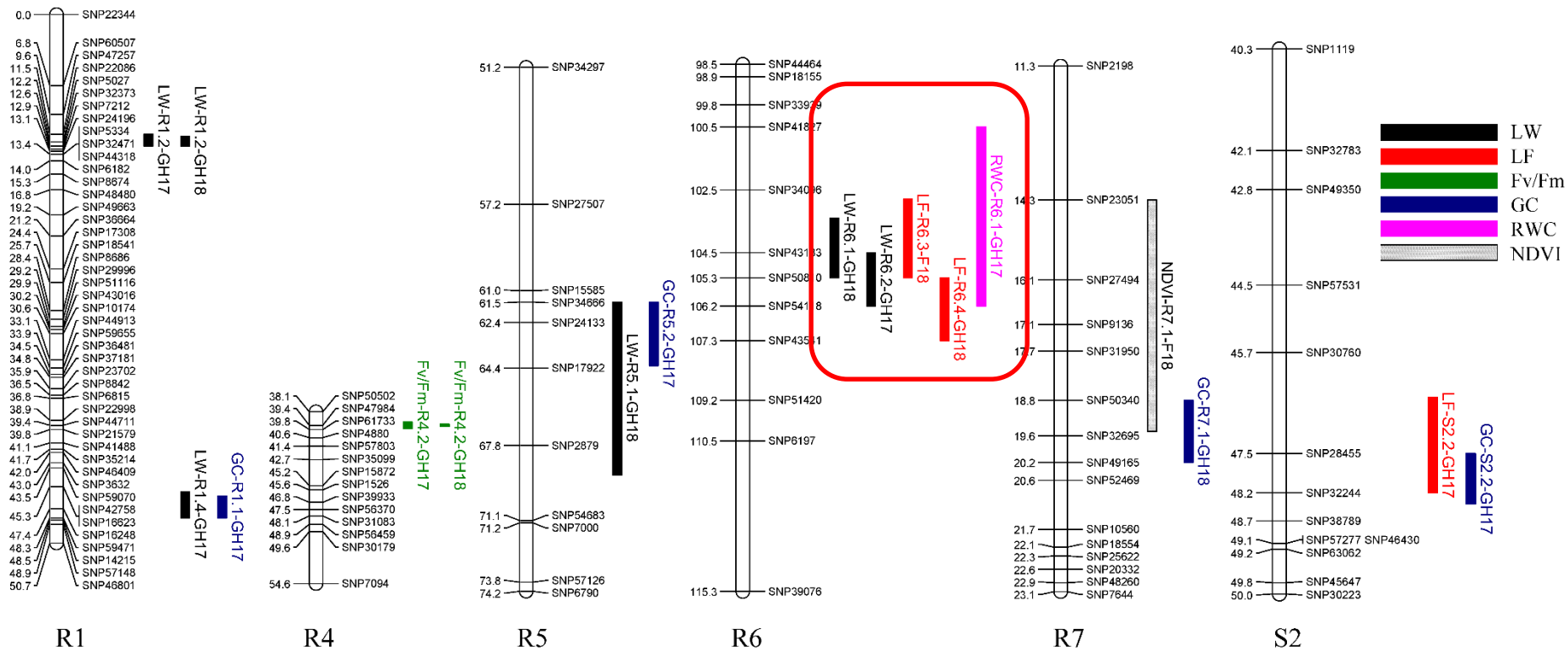
| | | | | | | | | |
|------|---------|----|--------|-----------------|----------|-------|------|-------|
| GH18 | LW-R6.1 | R6 | 104.50 | 103.38 - 105.30 | SNP43183 | 5.36 | 5.8 | -0.30 |
| GH17 | LW-R6.2 | R6 | 105.44 | 104.50 - 106.24 | SNP50810 | 14.52 | 12.9 | -0.53 |

| | | | | | | | | |
|------|------------|----|-------|---------------|----------|------|-----|------|
| GH17 | Fv/Fm-R4.2 | R4 | 39.39 | 39.09 - 39.69 | SNP47984 | 3.82 | 8.8 | 1.78 |
| GH18 | Fv/Fm-R4.2 | R4 | 39.39 | 39.29 - 39.45 | SNP47984 | 3.62 | 9.3 | 0.83 |

| | | | | | | | | | | | | | | | | | |
|------|---------|----|-------|---------------|----------|------|-----|-------|-----|-----------|----|-------|---------------|----------|-------|-----|---------|
| GH17 | GC-S3.1 | S3 | 33.00 | 32.69 - 33.10 | SNP13076 | 4.40 | 5.9 | -2.73 | F18 | NDVI-R7.2 | R7 | 76.29 | 76.28 - 76.39 | SNP45148 | 49.19 | 7.6 | -237.47 |
| | | | | | | | | | F18 | NDVI-R7.3 | R7 | 77.73 | 77.73 - 78.26 | SNP53130 | 47.80 | 7.1 | 233.17 |

| | | | | | | | | |
|------|---------|----|-------|---------------|----------|-------|------|-------|
| GH17 | LW-R1.4 | R1 | 47.58 | 45.80 - 48.28 | SNP16248 | 12.99 | 11.0 | -0.97 |
| GH17 | GC-R1.1 | R1 | 47.38 | 46.20 - 48.28 | SNP16248 | 4.67 | 6.3 | -2.89 |
| GH18 | LW-R5.1 | R5 | 67.84 | 61.45 - 69.14 | SNP2879 | 13.63 | 17.6 | 1.00 |
| GH17 | GC-R5.2 | R5 | 62.42 | 61.45 - 64.32 | SNP24133 | 5.00 | 6.8 | -2.50 |
| GH17 | GC-S2.2 | S2 | 48.20 | 47.48 - 48.40 | SNP32244 | 6.17 | 8.6 | 2.99 |
| GH17 | LF-S2.2 | S2 | 47.40 | 46.50 - 48.18 | SNP28455 | 19.55 | 20.4 | 0.64 |

Co-localization of QTL



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.



Acknowledgements

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NCSU TGB program members:

- ❖ Carolina Zuleta
- ❖ Esdras Carbajal
- ❖ Rocio van der laet
- ❖ Jessica Brown
- ❖ Sydney Graham

Field Trials:

- ❖ Sandhill Research Station

Funding support:



United States Department of Agriculture
National Institute of Food and Agriculture

