Validation of Quantitative Trait Loci Associated with Freeze Tolerance in St. Augustinegrass

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Background

- St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze) has good shade tolerance, aggressive growth habit, and grows in a wide range of soil pH. However, a general lack of freeze tolerance has prevented more widespread use of this warm-season grass.
- The industry standard for cold tolerance 'Raleigh' was released in the 1980s.
- The first complete linkage map of St. Augustinegrass was developed using a mapping population of 'Raleigh' x 'Seville'[1]. QTL on linkage groups 1, 3, 6 and 9 were identified for turf quality and freeze related traits[1].
- Yu et al. (2018) developed a high density linkage map with 2871 SNPs[2].

Figure 1 (right): 'Raleigh' x Seville' map Linkage Group 9[1] and genomic regions associated with turf quality and freeze tolerance.
Figure 2 (above): St. Augustinegrass plots in Raleigh, NC 2012
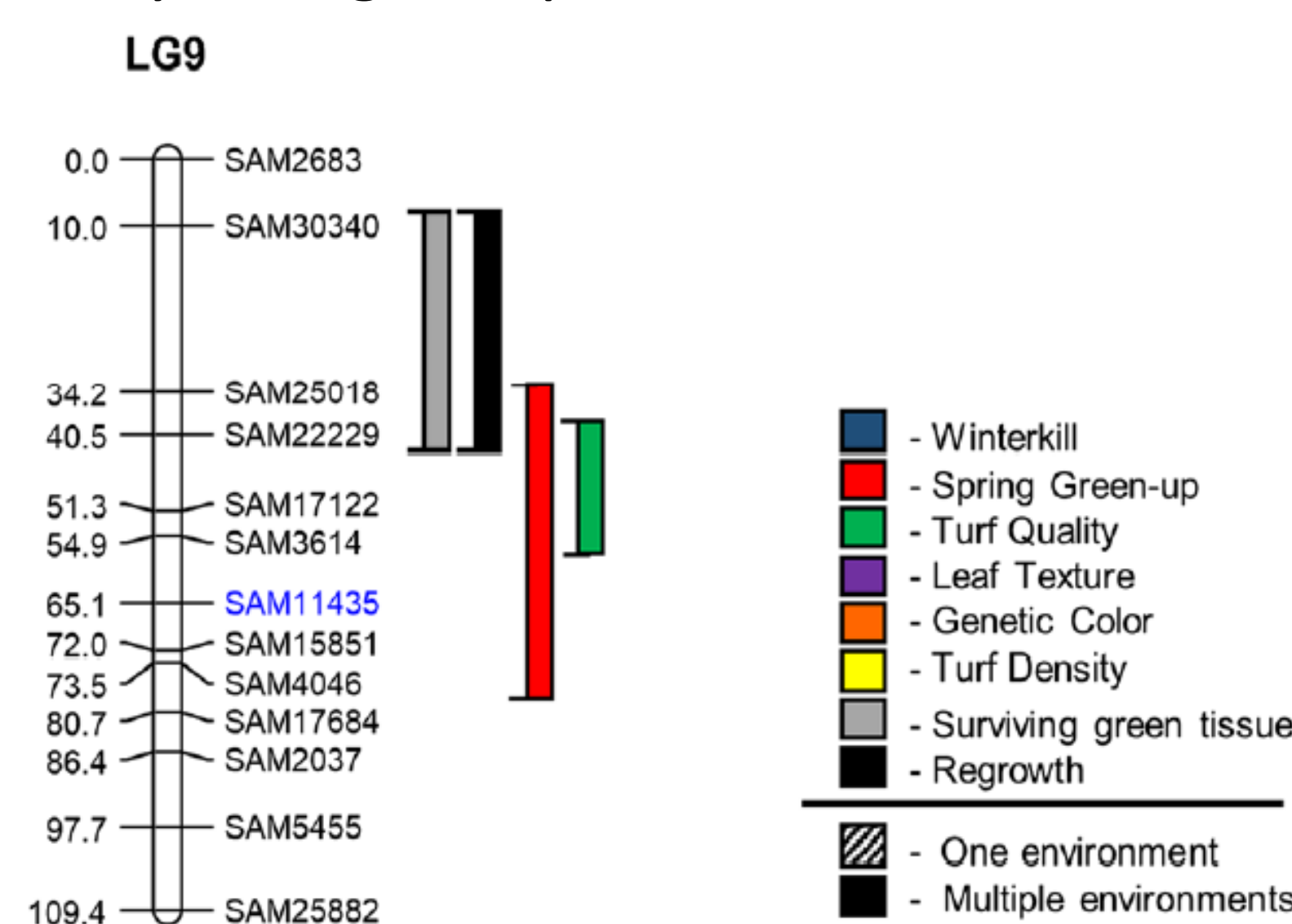
Objectives:

1. Use the high density SNP map to identify finer QTL associated with freeze tolerance in 'Raleigh' x 'Seville'
2. Develop a linkage map and identify freeze tolerance QTL for 'Raleigh' x 'Raleigh'
3. Compare QTL to validate candidate regions for marker assisted selection

Materials and Methods

Mapping Population:

- F_1 population (115 individuals) of 'Raleigh' x 'Raleigh' ('RxR') genotyped using 120 SSR markers.

Phenotyping:

- For lab freeze evaluations:
  - Acclimation treatments were no cold acclimation (NCA) or cold acclimation (CA) for 1 week at 13°C followed by 1 week at 3°C.
  - Freezing treatments were at both -3°C and -4°C for 10 hours.
  - Visual ratings were taken on surviving green tissue (SGT) and regrowth (RG) at weeks 3 and 6 post freezing.
- For field evaluations:
  - 3'x3' plots were planted at the Lake Wheeler Turf Field Lab (Raleigh, NC).
 - Spring green up (SGU) and winterkill (WK) were scored on a 1-9 scale in the spring of 2013-2015.

Linkage Mapping of RxR Population:

- Linkage mapping was completed in R Studio using the r/Onemap package[2].
- Markers were sorted into linkage groups (LG) using a default ~4.5 LOD score.
- 10 LGs were generated, one small LG (2 markers) was dropped. The order of markers within LGs was determined using the Kosambi and ripple functions.

QTL Identification:

- Both 'RxR' map and 'RxS' map from Yu et al. were used for QTL mapping[2]
- QTL mapping of least square means was done with MVQTLCIM[3].
- 1000 permutations were conducted to select genome wide logarithm of odds thresholds for significance (α = 0.05).

Results & Discussion
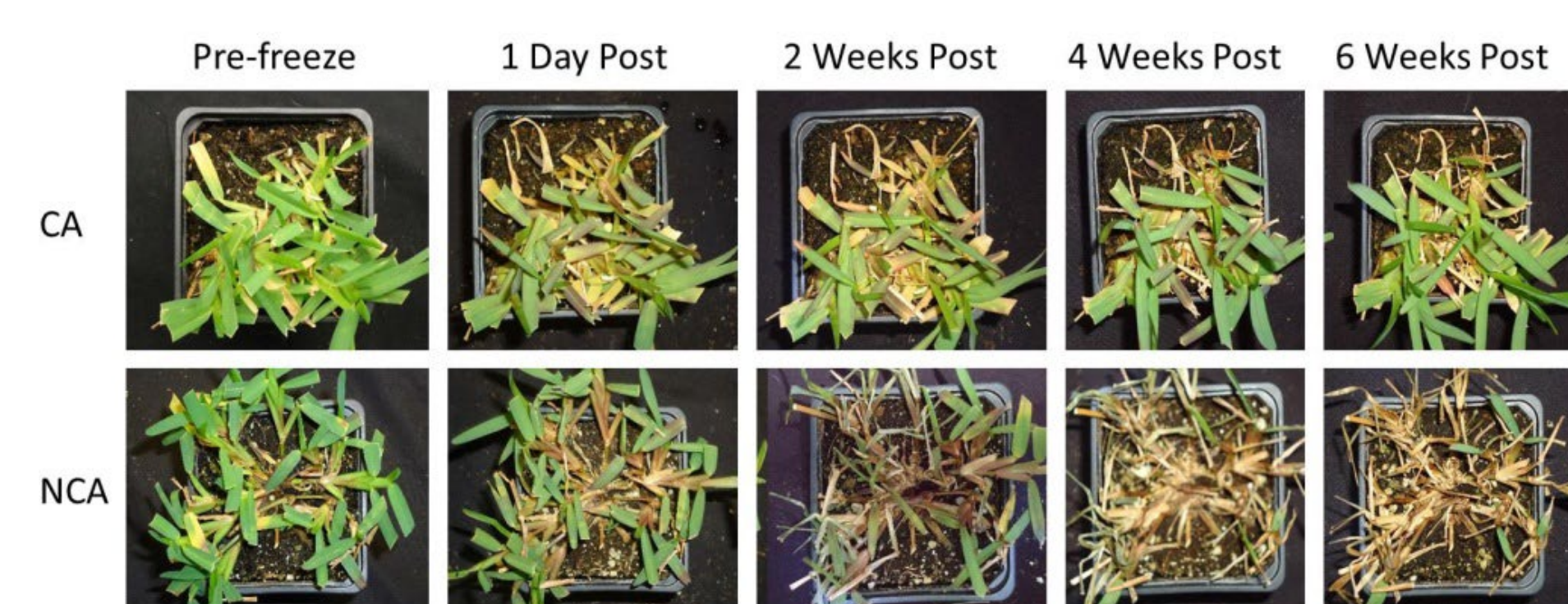
- Significant variation was observed among genotypes for freeze response both in the field and in freeze tests.
- 120 SSR markers were mapped into nine LGs.
- The RxR map spans 1650cM with an average distance between markers of about 14cM.
- 42 QTL identified in 'RxS' analysis and 4 QTL identified in 'RxR' analysis.

Significant QTL identified in 'RxR' population							
Trait	LG	Peak Position (cM)	Position Interval (cM)	LOD	LOD Threshold	Nearest Marker	Environment
SGT	3	52.341	52.341	17.661	17.633	*SSR3677	CA4
RG	9	108.76676	108.767-108.877	30.555	23.047	SSR17841	NCA3
RG	5	4.01	4.01	23.087	23.047	SSR1952	NCA3
RG	1	145.498	145.498	24.631	23.047	SSR15639	NCA3

Most significant QTL by LG identified in 'RxS' population							
Trait	LG	Peak Position (cM)	Position Interval (cM)	LOD	LOD Threshold	Nearest Marker	Environment
WK	1	48.306	40.529-52.366	7.755	3.830	SNP30999	Across
RG	2	82.673	77.135-87.919	9.426	3.530	SNP12185	Across
SGT	3	102.613	97.928-107.176	5.964	3.715	SNP9396	Across
WK	4	80.838	79.344-80.838	8.535	3.830	SNP18259	Across
WK	5	70.846	70.846-71.084	8.600	3.879	SNP54683	Across
WK	6	53.792	53.401-58.619	7.403	3.830	SNP8446	Across
WK	7	88.24	87.541-88.24	4.838	3.879	SNP7974	Across
RG	8	70.514	68.9-75.22	4.683	3.494	SNP18492	Across
SGU	8	0.01	0.01	4.253	3.762	SNP37566	Across
WK	8	0.01	0.01	4.112	3.879	SNP37566	Across
WK	9	39.975	39.975-40.63	6.100	3.879	SNP55431	Across
SGU	9	40.63	39.975-40.63	4.733	3.762	SNP55431	Across

Preliminary Conclusions

- There are many small effect QTL for freeze related traits and different freeze tolerance evaluations identified different QTL.
- QTL for WK and SGU are co-localized on LG's 8 and 9.
- QTL on LGs 5 and 9 have been implicated through three different analyses and make good potential targets for marker assisted selection.
- QTL analysis of independent environments for the 'RxS' population is underway and may identify QTL that are confounded across environments.
- Ongoing work:
  - Reference genome for 'Raleigh'
  - Transcriptomic analysis with RNA-Seq will help confirm which regions of the genome play an integral role in freeze tolerance and acclimation.

References

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3. Margarido, G. R. A., Souza, A. P. and Garcia, A. A. F. (2007) OneMap: software for genetic mapping in outcrossing species. Hereditas 144: 78-79.
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