Project Title: Utilizing Molecular Technologies to Develop Zoysiagrass Cultivars with Improved Cold Tolerance
USGA ID#: 2015-03-518
Investigators: S.R. Milla-Lewis, Aaron Patton, and Brian Schwartz

Objectives: The overall objective of this project is to improve the efficiency of selecting for cold tolerance in zoysiagrass breeding by identifying genomic regions controlling this trait and associated molecular markers that can be used for selection. Phase I of the project focused on evaluation of a mapping population for i) field winter survival at Laurel Springs, NC, and West Lafayette, IN and ii) DNA markers useful for creating a map of the zoysiagrass genome. In Phase II, genotypic marker data was used with field data collected 2014-2016 to create a high-density DNA marker map and to identify genomic regions controlling cold tolerance in zoysiagrass and associated molecular markers.

Progress Update and Results: A mapping population of 175 individuals derived from the cross of cold-tolerant 'Meyer' and cold-susceptible 'Victoria' was developed. In June 2014, three replications of each single individual and nine controls including the two parents were planted in a randomized complete block design (RCBD) in 3 x 3 ft plots at the Upper Mountain Research Station in Laurel Springs, NC, and the William H. Daniel Turfgrass Research and Diagnostic Center in West Lafayette, IN. Additionally, the population was replanted at these two locations in June 2015 for secondary evaluations during the 2015-2016 winter season These four copies of the population and two years of evaluation created six unique environments for winter injury testing. Digital imaging was used to evaluate establishment and winter injury. Winter survival data was taken in spring 2015 and 2016 and significant variation in winter injury was observed within the population, including ten lines that performed as well as or better than Meyer across all environments. This variability in winter injury seen in the progenies was integral for the identification of markers associated with cold tolerance in zoysiagrass.

DNA marker data was collected on the population and used to generate a linkage map of the zoysiagrass genome. One-hundred-twelve simple sequence repeat (SSR) markers and 2,306 sequencing-derived single nucleotide polymorphism (SNP) markers were used to construct a high-density genetic map of the zoysiagrass genomes. The map covers 323 mega basepairs (Mbp) and 1973.1 centimorgans (cM) as well as all 20 chromosomes of the zoysiagrass allotetraploid genome. It is the first SNP-based map of the *Zoysia japonica* genome. This map was used in conjunction with winter injury data from six environments to identify genomic regions associated with winter injury. A total of ninety-three genomic regions associated with winter injury were identified across six environments and on all 20 chromosomes. Nine of these genomic regions had major effects and were observed in two or more environments. These genomic regions and markers linked with them could be valuable in implementing marker assisted selection for winter hardiness in a zoysiagrass breeding program.

Progress Update and Results:
- A mapping population of 175 individuals has been developed crossing cold-tolerant cultivar 'Meyer' and cold susceptible cultivar 'Victoria'.
- The mapping population was established in June 2014 in Laurel Springs, NC, and West Lafayette, IN in 3'x3' plots in three replications in randomized complete block design (RCBD). Additionally, the population was replanted at these locations in June 2015 for secondary evaluations during the 2015-2016 winter season.
- The mapping population was evaluated for winter injury and survival using digital image analysis in 2014-2016. Significant variation in winter injury was observed including ten lines that performed as well as or better than Meyer across all environments.
- A total of 2,418 DNA markers were used to construct a high-density genetic map of the zoysiagrass genome. The map all 20 chromosomes and is the first high density genetic map of the *Zoysia japonica* genome.
- Using the genetic map and field data, ninety-three genomic regions associated with winter injury were identified. Nine of these genomic regions had major effects and were observed in multiple environments. These genomic regions and markers linked with them will be valuable in implementing marker assisted selection for winter hardiness in a zoysiagrass breeding program.

	NC,A, 2015	NC, A, 2016	NC, B, 2016	IN, A, 2015	IN, A, 2016	IN, B, 2016	Average Overall
11-TZ-4720	✓	✓		✓	✓		✓
11-TZ-4738	✓	✓		✓	✓		✓
11-TZ-4755		✓	✓	✓	✓		✓
11-TZ-4778	✓	✓	✓	✓	✓		✓
11-TZ-4826	✓	✓		✓	✓		✓
11-TZ-4836	✓	✓	✓				✓
11-TZ-4851	✓	✓	✓	✓	✓		✓
11-TZ-4877	✓	✓	✓	✓	✓		✓
11-TZ-4890	✓	✓	✓	✓	✓		✓
11-TZ-4891	✓	✓	✓			✓	✓
MEYER	✓	✓		✓	✓		✓

Table 1: Zoysiagrass mapping population lines that suffered less than 25% winter injury in four or more environments. These ten lines performed as well as or better than Meyer in the winters of 2014-2015 and 2015-2016 at the Upper Mountains Research Station (Laurel Springs, NC) and the William H. Daniel Turfgrass Research and Diagnostic Center (West Lafayette, IN)
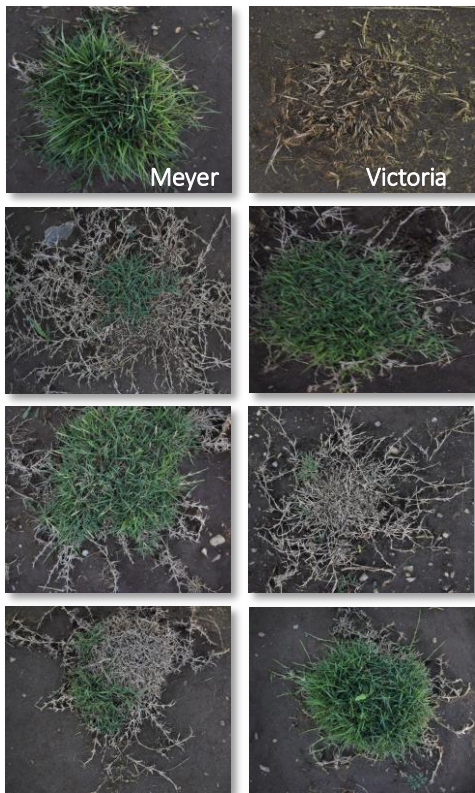

Figure 1: The mapping population and parents showed variation in winter injury levels in the winters of 2014-2015 and 2015-2016.
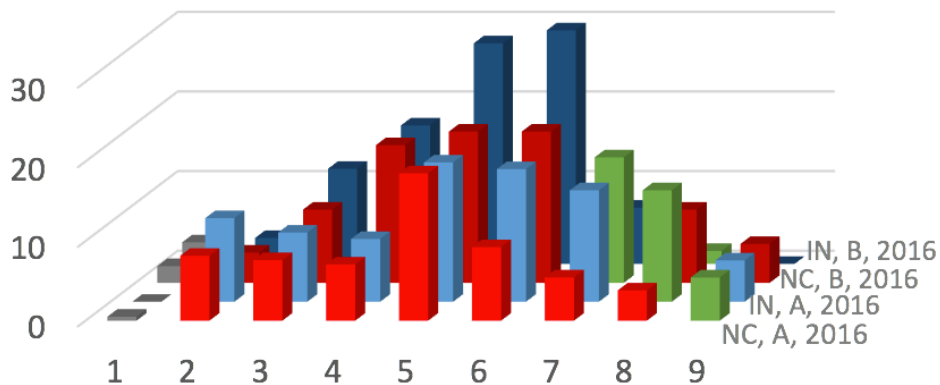
Figure 2: Winterkill distribution of lines for 175 progenies, Meyer, and Victoria for both copies of the population at Laurel Springs, NC and West Lafayette, IN in 2016. Winterkill is a measure of winter injury on a scale of 1 (completely dead) to 9 (no winterkill). Bars in grey and green indicate where Victoria and Meyer fell, respectively.
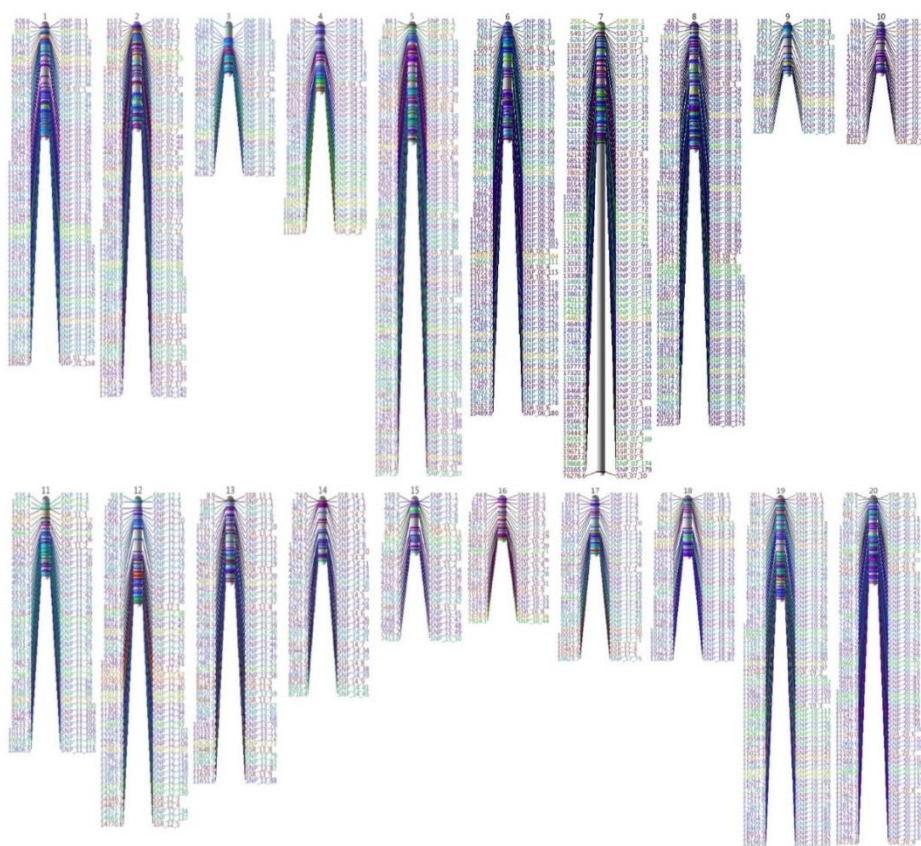

Figure 3: Genetic map of the zoysiagrass genome with 2,418 DNA markers. This high-density map was used to identify genomic regions controlling freeze tolerance.
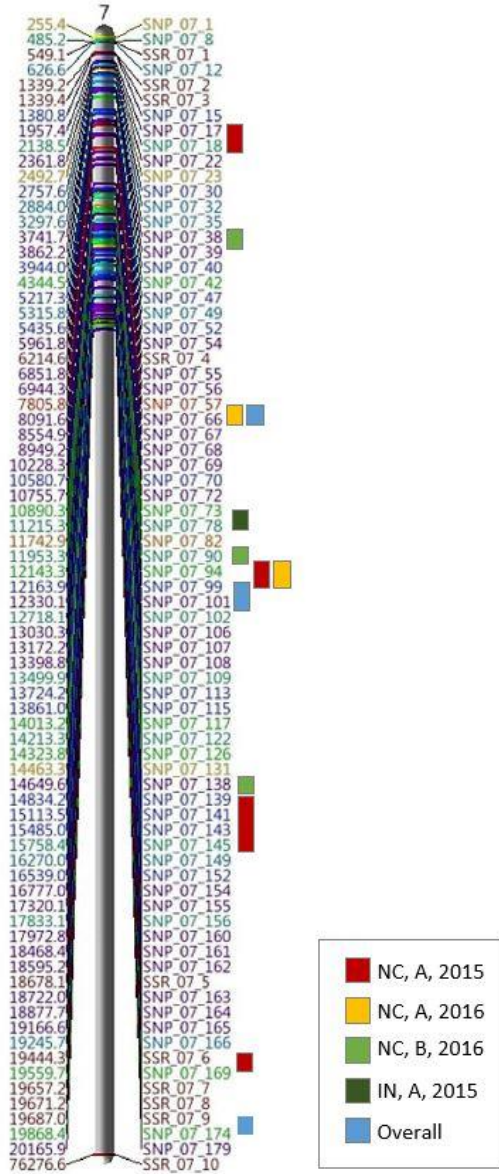
Figure 4: A marker map of chromosome 7 with genomic areas of interest that were identified in individual field environments and across all environments highlighted.