**Project Title:** Utilizing Molecular Technologies to Develop Zoysiagrass Cultivars with Improved Cold Tolerance
**USGA ID#:** 2015-03-518
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**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 (year 1) of the project focused on evaluation of a mapping population for field winter survival at Laurel Springs, NC, and West Lafayette, IN. The second objective of Phase I is to genotype the population with DNA markers in order to generate a map of the zoysiagrass genome.

**Progress Update and Results:** A mapping population of 175 individuals derived from the cross of cold-tolerant 'Meyer' and cold-susceptible 'Victoria' has been 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 planted at the University of Georgia Coastal Experiment Station in Tifton, GA for quality evaluations. Digital imaging was used to evaluate establishment and winter injury (Figure 1). The progenies exhibited great variability for color, texture, and aggressiveness (Figure 2). Winter injury and survival was analyzed for winter 2014-2015. Under the more severe winters of West Lafayette, IN, the population had a 21% survival rate (Figure 3). At Laurel Springs, NC, greater variation was observed in response to winter injury, with 59% survival. Thirty-three genotypes including Meyer and Zenith suffered no winter injury in Laurel Springs (Table 1) while seven including Meyer, Zenith, and Chinese Common suffered no winter injury in West Layafette. No significant winter injury was observed in Tifton, GA. A wide range of trait expression within a population is integral to the identification of DNA markers, so the variability in winter survival seen in these progenies is promising for the identification of markers associated with cold tolerance in zoysiagrass. Marker data is being collected on the population and used to generate a linkage map of the zoysiagrass genome. Two hundred simple sequence repeat (SSR) primers have been screened across the parents, Meyer and Victoria. Of those, 125 were found to be polymorphic and are being amplified across the progeny (Figure 4). Additionally, library construction for the genotype by sequencing (GBS) protocol has been initiated in order to generate single nucleotide polymorphism (SNP) markers to increase map density.

**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, West Lafayette, IN and Tifton, GA in 3'x3' plots in three replications in randomized complete block design (RCBD). Additionally, the population was replanted at these three 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 2015. Survival rate of the population was 21% at West Lafayette, IN and 59% at Laurel Springs, NC. A total of 33 lines suffered no winter injury in Laurel Springs, NC. Seven lines suffered no winter injury in West Lafayette, IN. Only three genotypes, including Meyer, had no winter injury at both locations.
- Out of 200 markers screened, 125 have been found to be polymorphic between the parents and are being amplified in the progeny in order to generate a linkage map.

**Table 1:** Zoysiagrass lines that suffered no winter injury during the winter of 2014-2015 at the Upper Mountain Research Station (Laurel Springs, NC) and the William H. Daniel Turfgrass Research and Diagnostic Center (West Lafayette, IN). Lines that suffered no injury at either location are highlighted in red.

| Laurel Springs, NC | | | | | West Lafayette, IN |
|---|---|---|---|---|---|
| 11-TZ-4720 | 11-TZ-4753 | 11-TZ-4784 | 11-TZ-4826 | 11-TZ-4884 | 11-TZ-4720 |
| 11-TZ-4724 | 11-TZ-4757 | 11-TZ-4789 | 11-TZ-4836 | 11-TZ-4877 | 11-TZ-4755 |
| 11-TZ-4726 | 11-TZ-4758 | 11-TZ-4794 | 11-TZ-4837 | 11-TZ-4890 | 11-TZ-4778 |
| 11-TZ-4727 | 11-TZ-4768 | 11-TZ-4799 | 11-TZ-4840 | Meyer | 11-TZ-4842 |
| 11-TZ-4738 | 11-TZ-4779 | 11-TZ-4800 | 11-TZ-4843 | Zenith | 11-TZ-4877 |
| 11-TZ-4740 | 11-TZ-4781 | 11-TZ-4815 | 11-TZ-4851 | | Meyer |
| 11-TZ-4745 | 11-TZ-4782 | 11-TZ-4819 | 11-TZ-4854 | | Chinese common |

**Figure 1:** Digital image analysis is used to estimate percent cover by quantifying green pixels.

**Figure 2:** The mapping population showed variation in color, texture, turf quality, aggressiveness, and winter injury in the winter of 2014-2015.

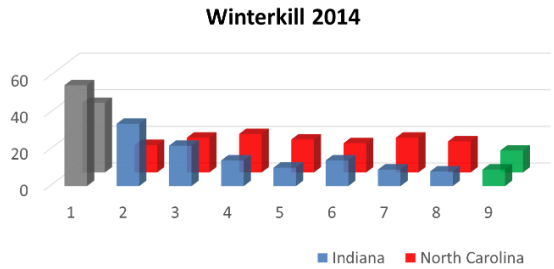


**Figure 3:** Distribution of winterkill for 175 progeny, Meyer, and Victoria at Laurel Springs, NC and West Lafayette, IN in 2014. Winterkill is a measure of winter injury on a scale of 1 (completely dead) to 9 (no winterkill). Bars in grey and green indicate where Victoria and Meyer, respectively, fell.

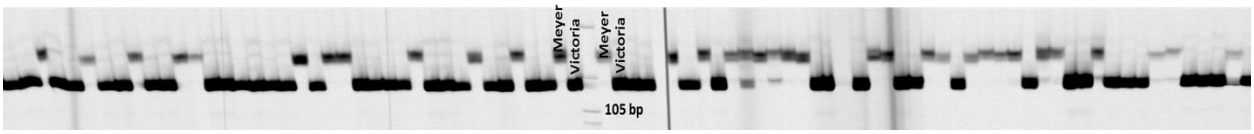


**Figure 4**: Polyacrylamide gel showing amplified products generated with SSR primer b02d15 across the Meyer x Victoria mapping population.