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Project Title: Utilizing Molecular Technologies to Develop Zoysiagrass Cultivars with Improved Cold Tolerance

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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.

Start Date: January 2015

Project Duration: 2 years

Total funding: \$20,000

Summary Text:

Zoysiagrass generally requires fewer maintenance inputs compared to many cool- and warm-season grasses. In comparison with other warm-season turfgrasses, Zoysia species have moderate drought and shade tolerance, and lower fertilizer demands. Expanded use of this turfgrass could have a significant impact in making golf courses more environmentally sustainable. One factor limiting widespread use of zoysiagrass; however, is a relative lack of cold tolerance, especially when compared to cool-season grasses. Limited progress has been made in the development of additionally cold tolerant zoysiagrass cultivars since Meyer was released in 1951. This lack of improvement through breeding is in part due to the complexity of cold hardiness traits. The identification of molecular markers that are associated with cold tolerance is needed to improve the efficiency of selection when breeding for this trait. For this purpose, 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 and plots were also rated for pre- (July) and post-freeze (April) turf quality. Significant variation for all four traits was observed within the population (Figure 1), including lines that performed as well as or better than Meyer across all environments (Figure 2).

DNA marker data was collected on the population and used to generate a linkage map of the zoysiagrass genome. A total of 104 simple sequence repeat (SSR) markers and 2,359 single nucleotide polymorphism (SNP) markers were used to construct a high-density genetic map of the zoysiagrass genomes. The map (Figure 3) covers 324 mega basepairs (Mbp) and 2520 centimorgans (cM) as well as all the 20 chromosomes for the zoysiagrass haploid genome. Phenotypic data on winter injury, establishment, and turf quality collected in North Carolina and Indiana in 2014-2016 were used in conjunction with this map to identify quantitative trait loci (QTL) associated with winter hardiness. Fifty-seven putative QTL for winter injury, establishment, pre-freeze turf quality, and post-freeze turf quality were identified within and across six environments. Twelve QTL for winter injury, pre-freeze turf quality, and post-freeze turf quality were identified in two or more environments. Additionally, seven regions of interest where QTL for three or more of these traits co-located were found on chromosomes 8, 11, and 13 (Figure 4). Analysis with NCBI basic local alignment search tool (BLAST) indicated that within these regions are contained proteins that have been previously reported to be related to abiotic stresses including heat, drought, salt,

and cold. These genomic regions and markers linked with them could be valuable in implementing marker assisted selection for winter hardiness in a zoysiagrass breeding program.

Summary Points:

- 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 establishment, and turf quality pre- and post-freezing in 2014-2016. Significant variation in all four traits was observed in the population, including ten lines that performed as well as or better than Meyer in terms of winter injury across all environments.
- A total of 2,463 DNA markers were used to construct a high-density genetic map of the zoysiagrass genome. The map covers all 20 chromosomes and is the first high density genetic map of the *Zoysia japonica* genome.
- Using the genetic map and field data, seven regions of interest where QTL for three or more of the traits evaluated co-located were found on chromosomes 8, 11, and 13. These genomic regions and markers linked with them will be valuable in implementing marker assisted selection for winter hardiness in a zoysiagrass breeding program.

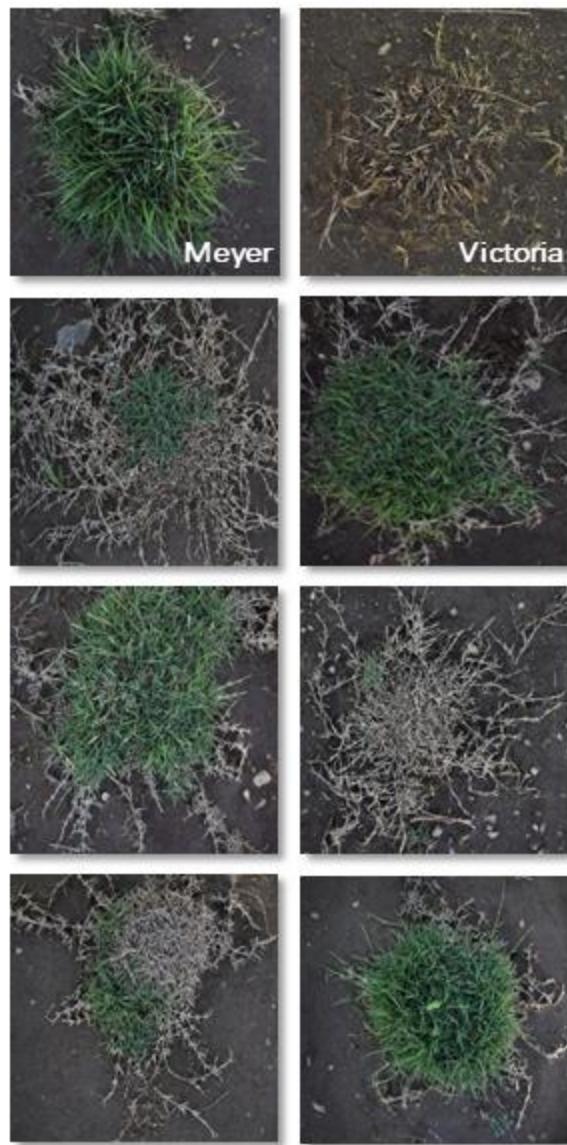


Figure 1: The mapping population and parents showed variation in winter injury levels in the winters of 2014-2015 and 2015-2016.

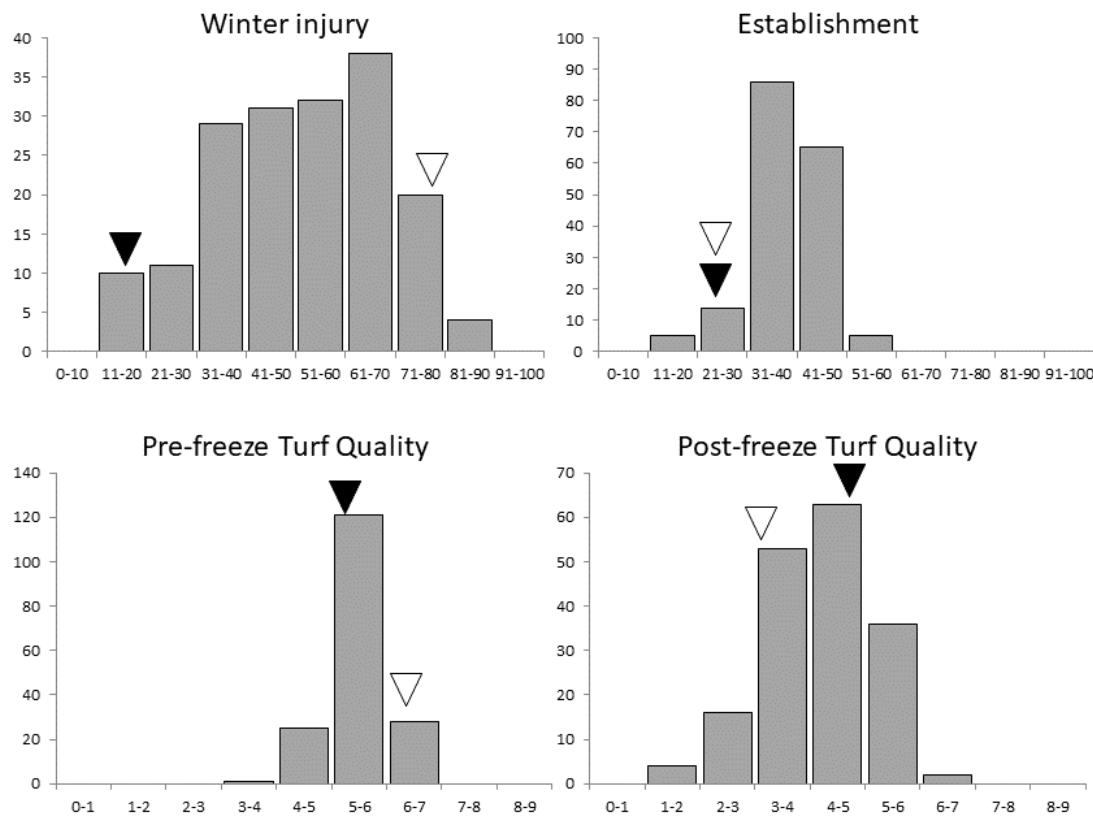


Figure 2: Distribution of winter injury, establishment, and pre- and post-freeze turf quality means for 175 evaluated at Laurel Springs, NC and West Lafayette, IN in 2016. The arrows indicate values of the two parents: 'Meyer' (black) and 'Victoria' (white).

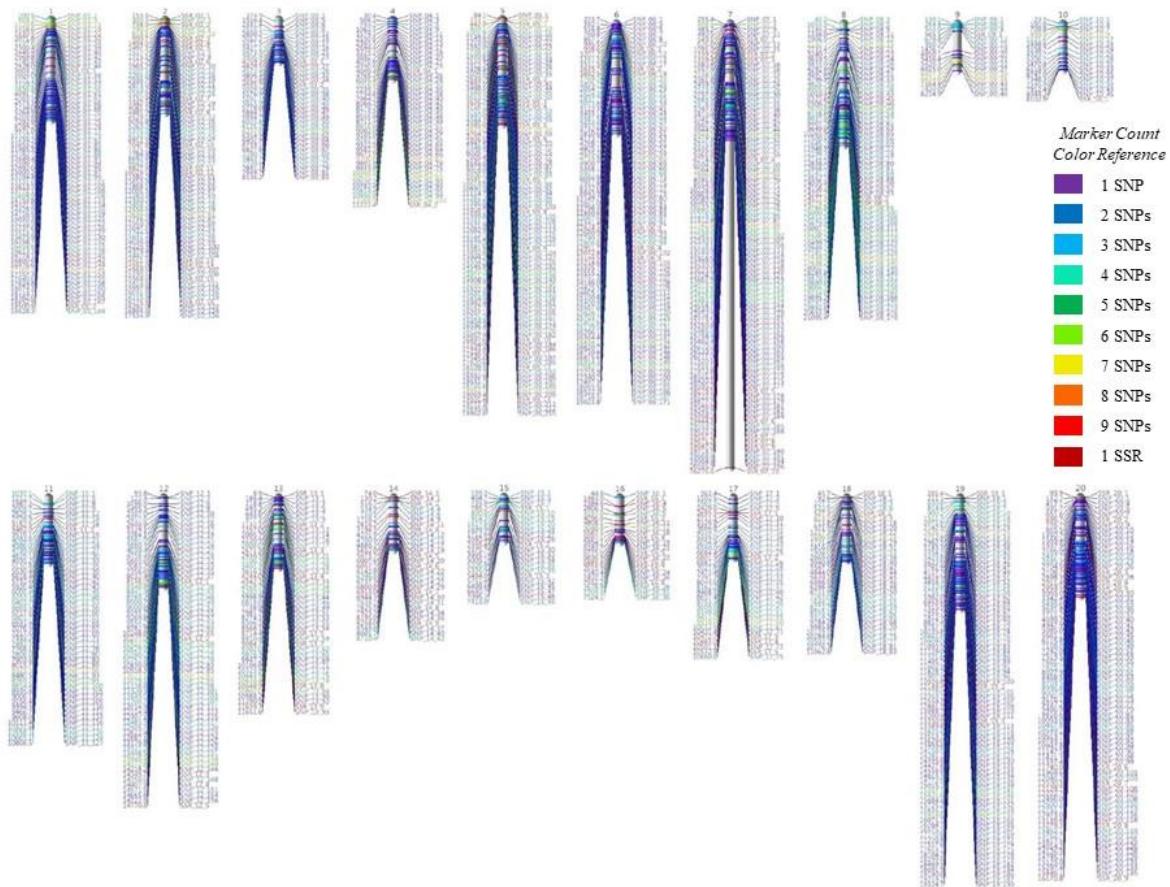


Figure 3: Genetic map of the zoysiagrass genome with 2,418 DNA markers. This high-density map was used to identify genomic regions controlling freeze tolerance.

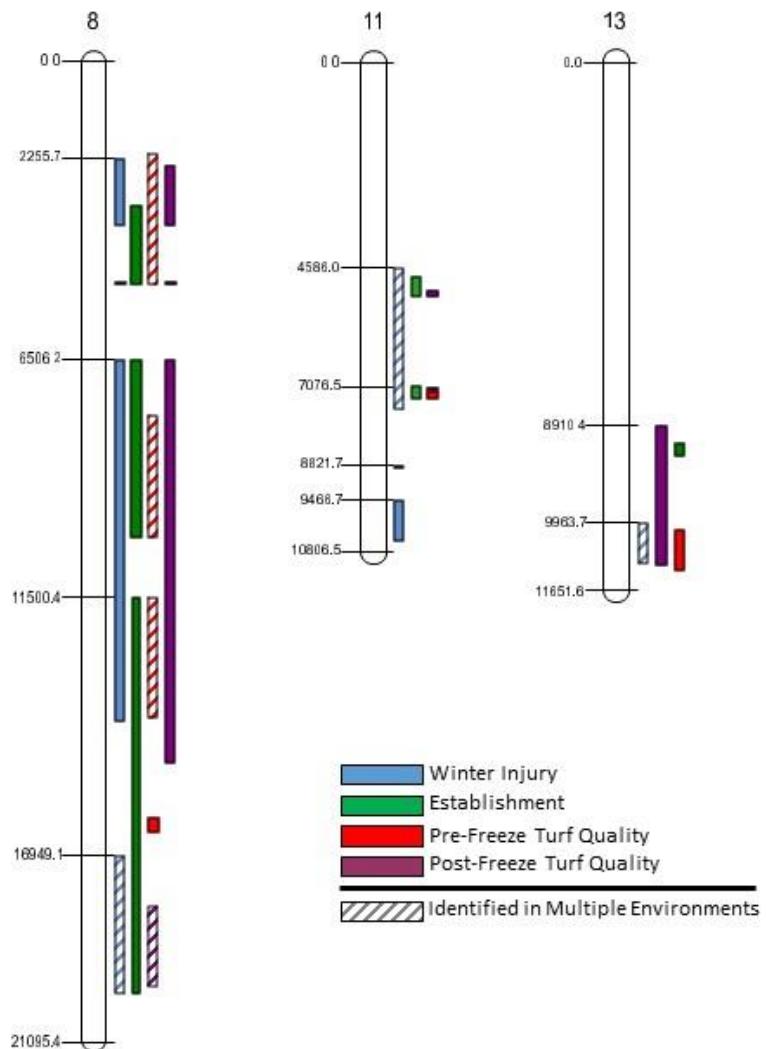


Figure 4: Chromosomal position of QTL associated with winter injury (blue), establishment (green), pre-freeze turf quality (red), and post-freeze turf quality (purple) across combined environments. Map positions for the closest markers are indicated on the left.