

## **Towards Association Mapping of Drought Tolerance in Perennial Ryegrass**

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### **Objective:**

To determine relationship between drought tolerance traits and candidate genes of perennial ryegrass (*Lolium perenne* L.)

### **Rationale:**

Breeding a drought tolerant turf variety is one of the most important strategies to improve the water conservation of turfgrass. Grass improvement relies on genetic linkage mapping on the basis of segregating families. Conventional genetic linkage mapping for quantitative traits locus manipulation through marker-assisted selection have served plant geneticists and breeders. But one limitation of traditional linkage mapping is the low resolution. In recent years, association mapping has been developed as a novel and more powerful mapping technique. It uses a natural plant population to provide relationship between molecular markers and a phenotypic trait of interest and therefore serves as an alternative method for mapping quantitative traits locus.

Compared to linkage mapping in traditional bioparental populations, association mapping offers three main advantages: increased mapping resolution, reduced research time, and greater allele numbers. This complementary method results in a higher resolution than mapping with standard F2 populations and can evaluate a wide range of alleles rapidly. The genome-wide approach to association mapping requires an excessive number of molecular markers, but a candidate gene approach to association mapping could also be successful for identifying markers linked to the traits of interest.

Perennial ryegrass is a self-incompatible species that can potentially provide high-resolution association mapping. The use of natural populations may be particularly beneficial in perennial ryegrass since many potentially useful variations are still unexplored. Research on association mapping not only benefits grass breeders by providing molecular markers, but also improves our understanding of the genes underlying complex genetic traits.

### **Materials and methods:**

A set of natural populations of 192 perennial ryegrass accessions was obtained from the USDA National Plant Germplasm System at the Western Regional Plant Introduction Station in Pullman, WA. These accessions were chosen based on geographical location (from 43 countries) and included all core collections of perennial ryegrass. The accessions were planted in pots containing top soil with a pH of 6.9 in a greenhouse at Purdue University from March to April (Figure 1). Each pot contained the same soil volume and plant population and was initially watered to field capacity in order to achieve uniformity of experimental materials. Each accession had six replicates, and a total of 1200 pots were planted. A soluble fertilizer (N- P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, 24-8-16) (Scotts Inc., Marysville, OH) was used to provide 146 kg ha<sup>-1</sup> N.

The three replicates of grasses were transferred to the field at the W.H. Daniel Turfgrass Research and Diagnostic Center at Purdue University. Field plots were

established in the fall of 2007 (Figure 2). Phenotypic traits will be evaluated in the summer of 2008. Irrigation will be shut down from June to August 2008 field for evaluation of drought tolerance



**Figure 1.** Perennial ryegrass accessions



**Figure 2.** Field study for phenotypic traits

Genotyping 192 natural populations of perennial ryegrass with 105 microsatellite (SSR) markers were conducted. The PCR products were analyzed in the USDA genotyping lab at Kansas State University using ABI 3730 DNA Sequencer (Applied Biosystem, Foster City, CA). Population structure is being estimated using software package structure 2.1.

### Results to date:

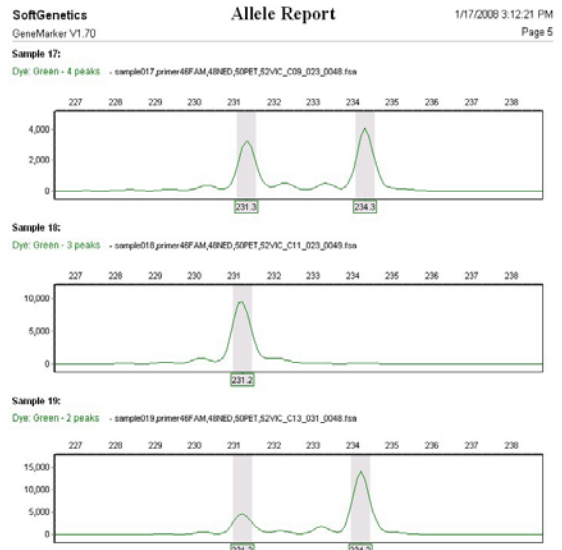
The initial screening drought tolerance in greenhouse showed that approximately 10 % and 12 % of these accessions exhibited good and poor drought tolerance, respectively (Figure 3). Genetic diversity are being analyzed (Figure 4) and population structure is being estimated.

### Acknowledgements

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**Figure 3.** Initial screening drought tolerance



**Figure 4.** Example of alleles for the specific SSR marker among accessions