Confirmation and Utilization of Candidate Gene Markers for the Selection of Heat-Tolerant Bentgrass

Bingru Huang, Yan Zhang, Faith Belanger, and Stacy Bonos
Rutgers University

Scott Warnke
USDA-ARS

Objectives:

1. Develop PCR-based markers from heat-responsive genes.
2. Map heat-responsive candidate genes on the present bentgrass genetic linkage maps.
3. Test for co-localization of candidate genes with mapped heat-tolerance QTLs.
4. Confirm candidate gene markers for use in marker-assisted breeding of creeping bentgrass for improved heat tolerance.

Start Date: 2010
Project Duration: 3 years
Total Funding: $89,713

Summer bentgrass decline is a major issue affecting many turf areas during the warmer summer months. Insufficient heat tolerance in current bentgrass lines leads to major quality decline during periods of prolonged heat. By identifying important genes used for heat tolerance and then developing markers to assist in selection, the severity of summer bentgrass decline could be significantly reduced.

Several potentially important genes playing a role in heat tolerance have been identified using proteomic profiling and suppressive subtractive hybridization. Potential markers for these candidate genes are being tested in a hybrid mapping population which consist of a creeping x colonial hybrid (TH15) crossed with another creeping line (9188).

To date, markers have been tested for 18 candidate genes: catalase, chlorophyll a/b binding protein, cysteine protease, expansin, fructose 1,6-bisphosphate aldolase, glutathione S-transferase, glyceraldehyde 3-phosphate dehydrogenase, heat shock proteins (HSP 16, 26 70, 90, 90-1, 100, 101), phenylalanine ammonia-lyase, protein disulfide isomerase, Rubsico activase, and superoxide dismutase.

Several methods of developing useful markers to detect polymorphisms are being employed including using EST sequences in the NCBI database to create allele-specific PCR-based markers, as well as cleavage-amplified polymorphism (CAPs) markers which employ the use of restriction enzymes. Of the tested markers, eight have shown segregation in the mapping population they are being tested in, and of those eight, six have been added onto the existing linkage map which had previously been created using the mapping population. The six which have been mapped are catalase, cysteine protease, expansin, and heat shock proteins 26, 70, and 101 kDa.

Plants exposed to 20 days of heat stress (38º C), showing genetic variations in heat tolerance.

Phenotypic variations in heat tolerance in the creeping colonial hybrid x creeping population were tested through the evaluation of turf quality, green leaf biomass, chlorophyll fluorescence, chlorophyll content, and electrolyte leakage. These parameters revealed large range of variations for heat tolerance present within the population. For example, turf quality ranged from 1 to 7 under heat stress (38º C). This confirms the appropriateness of using this population, as well as gives valuable information about the population which can be used for later analyses, such as QTLs analyses.

We are continuing to search for genes of potential importance based on current proteomic and metabolic research and testing associated markers. A collection of multiple markers will be screened in the population and used to expand the existing linkage map. In addition, we will test for the expression level of genes linked to the markers using RT-PCR analysis. Confirmation of gene expression and marker association will enhance further understanding of heat-tolerance mechanisms and the efficiency of marker-assisted selection for heat tolerance.

Summary Points

- Several methods of detecting polymorphism have been utilized to develop markers for genes associated with heat tolerance, including using EST sequences in the NCBI database to create allele-specific PCR-based markers, as well as cleavage-amplified polymorphisms(CAPs) markers which employ the use of restriction enzymes.
- Potential markers for 18 candidate genes are being tested in a hybrid mapping population. Eight markers have shown segregation in the mapping population. Of those eight, six have been added onto the existing linkage map which had previously been created using the mapping population. The six markers are catalase, cysteine protease, expansin, and heat shock proteins 26, 70 and 101 kDa.
- Heat stress trials have confirmed phenotypic variation in heat tolerance in the mapping population and collected data can be used to expand QTLs.