Development and Application of Molecular Markers Linked to Heat Tolerance in Agrostis *Species*

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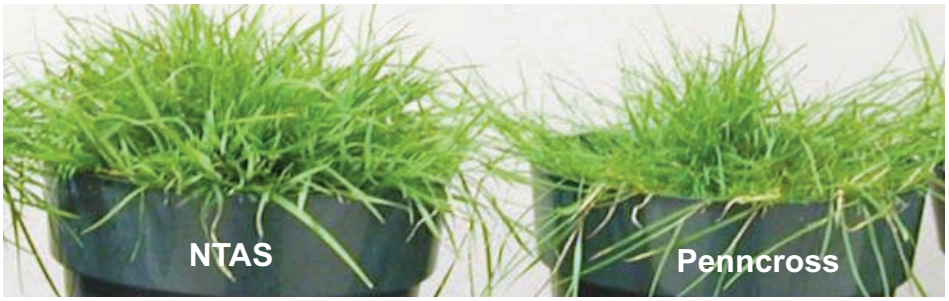
Objectives:

1. To identify molecular markers linked to heat tolerance for heat tolerance genes from a thermal *Agrostis* species.
2. To develop a marker-assisted selection system for improving heat tolerance in creeping bentgrass.

Start Date: 2003
Project Duration: three years
Total Funding: $90,000



Agrostis scabra *(NTAS) and creeping bentgrass (Cv. Penncross) exposed to 35^{O}C for 12 days in a growth chamber.*

Two independent studies, suppression subtractive hybridization (SSH) and proteomic profiling, were conducted to explore the molecular mechanisms of higher heat tolerance of *A. scabra* vs. creeping bentgrass at both RNA and protein levels. A total of 143 unique genes with higher expression level in heat-stressed thermal *A. scabra* were identified using differential display (DD-PCR) or suppression subtractive hybridization (SSH) approaches.

An ongoing study on proteomic profiling of heat-stressed vs. un-stressed *A. scabra* and creeping bentgrass has revealed 70 differentially expressed proteins. A large portion of identified genes have functions involved in protein and carbon metabolism, signaling / transcription, and stress defense. Therefore, these genes and their protein products are highly likely to be associated with the increased heat tolerance observed in thermal *A. scabra* compared to creeping bentgrass.

Several common genes with known functions in heat tolerance were identified from both gene expression and proteomic profiling, including those encoding for proteins involved in carbon metabolism (fructose 1,6-bisphosphate aldolase), stress and defense responses (phenylalanine ammonia-lyase, disulfide isomerase, and glutathione S-transferase). All of these genes code for products that are suggested to function in heat stress tolerance by either increasing glucose utilization or amplify signaling molecule biosynthesis. In addition, four heat-responsive genes encoding heat shock protein 70 (HSP70), heat shock protein 16 (HSP16), cysteine protease (AsCP1), and expansion (AsEXP1) were selected based on their heat-inducible patterns in both gene expression (northern blot) and protein (western blot) analyses. These genes may play the critical roles in the heat tolerance and could be used as potential candidate genes for marker development.

Eight heat-responsive genes were selected based on results from previous studies of differential gene expression analysis and proteomics. The apparent conservation of gene function across species provides a unique opportunity to translate discoveries in model species to creeping bentgrass. Availability of genome sequences from closely related species (rice, maize, wheat, and barley) in the public domain will dramatically shorten the effort to obtain the full length cDNA fragments of gene orthologs and increase the efficiency of creeping bentgrass marker development. Orthologous regions for the chosen candidate genes were identified from public databases.

SSR primer pairs were designed from the *A. scabra* EST sequences or the identified orthologous genes of related species. SSRs within each of the selected genes were found using the SSR identification tool Primer3 based on the *A. scabra* full-length cDNA. The same software was used to design primers amplifying fragments that contained the SSR region following main parameters and all other parameters in default settings.

Screening of two candidate gene primers (cysteine protease and expansin) indicated that one of the expansin primers showed polymorphisms. This primer pair amplifies a 166 bp, and a 188 bp fragment in 7418-3 and L93-10 genomes, respectively, which harbor the tetra-nucleotide repeat motif "AGCT".

Summary Points

- Suppression subtraction hybridization (SSH) cDNA libraries from the heat-tolerant *A. scabra* identified several important 'heat tolerance' genes or molecular markers from thermal *A. scabra*. Heat-responsive genes in thermal bentgrass were categorized into five functional groups: protein metabolism, signaling/transcription, carbon metabolism, stress defense, and other metabolism. Some genes have unknown functions. The largest group of heat-responsive genes is involved in stress/defense, followed by the group of genes related to protein metabolism.
- Proteomic profiling revealed that the up-regulation of sucrose synthase, glutathione S-transferase, superoxide dismutase, and heat shock protein Sti (stress inducible protein) may contribute to the superior root thermotolerance of *A. scabra.* Phospho-proteomic analysis indicated that two isoforms of fructose-biphosphate aldolase were highly phosphorylated under heat stress, and thermal *A. scabra* had greater phosphorylation than *A. stolonifera*, suggesting that the aldolase phosphorylation might be involved in root thermotolerance.
- Several PCR-based SSR markers from heat-responsive genes were developed, which could be used in marker-assisted selection of heat-tolerant bentgrass and other cool-season turfgrass species.