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.

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Summer bentgrass decline has been a major challenge for maintaining high quality bentgrass putting greens on many golf courses in warm climatic regions. The progress using either traditional breeding and genetic engineering to improve heat tolerance has been restricted by the lack of heat-tolerant germplasm or the genes that control heat tolerance. In recent years, breeders have become increasingly interested in exploiting genetic markers to use for marker assisted selection (MAS) as a new breeding methodology for genotypic selection. MAS has the potential to dramatically enhance the pace and efficiency of genetic improvement. MAS for stress tolerance, however, requires gene networks that are responding to stress or controlling stress tolerance.

We have recently discovered an *Agrostis* species, "thermal" *Agrostis scabra*, in the thermal sites at Yellowstone National Park, which demonstrates superior tolerance to high soil temperatures (45 - 50^{o} C at the surface 3-5 cm soil and 60-70^{o} C below 10 cm soil). The thermal *Agrostis* species is closely related to creeping bentgrass. *Agrostis scabra* plants were able to maintain much higher quality and physiological functions for extended periods of time compated to creeping bentgrass under high soil temperatures.

Using the differential display technique, we identified unique gene fragments or target DNA fragment (TDFs) that are present only in heat tolerant *Agrostis scabra* under heat stress (40^{o}C), but not present in control plants of the thermal species or control or heat-stressed 'Penncross' creeping bentgrass that is sensitive to heat stress. Therefore, these unique TDFs are considered heat tolerance genes.

Sequencing analysis showed that some TDFs are highly involved in stress signaling and regulation. One of the most interesting and important up-regulated TDFs is AsExp that encodes a gene controlling synthesis of expansin proteins in cell walls. Cells exposed to stresses develop rigid cell walls that restrict cell expansion and elongation. Expansin proteins act as loosening and extension agents to keep cell walls elastic and flexible. Wall stress relaxation reduces cell turgor and thereby creates the driving force for water uptake by growing cells.

To test the feasibility of using the unique TDFs in thermal *A. scabra* as heat-tolerance markers, we have started with AsExp TDF as a marker for selecting heat tolerant creeping bentgrass cultivars and another ecotype of thermal *A. scabra* (FTAS). The identified gene fragment AsExp was sequenced with the length of 646 bp. By comparing its translated protein sequence with the Genebank database (blastx), there is a region of 423-557 bp, which showed a high identity with the expansin gene in *Schedonorus pratensis.*

Based on the NTEP data from different locations, 11 creeping bentgrass cultivars that vary in summer performance were selected for the initial screening test. The ranking in summer rating of percent living ground cover for those cultivars (averaged over locations and years) were averaged as follows: 'Declaration' (96%) = 'Penn A-4' (96%) > 'Shark' (94%) > 'L-93' (93%) = 'Independence' (93%) > 'Putter' (92%, only one year data in one location available) > 'Kingpin' (85%) > 'Century' (70%) > 'Backpsin' (66%) > 'Bengal' (62%) > 'Pennlinks' (32%).

All creeping bentgrass cultivars and both ecotypes of thermal *A. scabra* were propagated into clonal materials and exposed to heat stress (40^{o}C) for 7 days in a growth chamber. Total cDNA was obtained and blotted on the nylon membrane. Hybridization was conducted by probing the up-regulated AsExp from thermal NTAS in the 11 creeping lines and in FTAS. The gene fragment of AsExp was found to be strongly up-regulated during heat stress in the thermal NTAS and FTAS.

The more tolerant cultivars 'Declaration' and 'Penn A-4' had strongest expression of the expansin gene, while those ranked lower in summer rating such as 'Putter', 'Kingpin', 'Century', 'Bengal', 'Backspin', and 'Pennlinks' had weak or no expression. 'Shark', 'L-93', and 'Independence' that are ranked intermediate for summer performance showed intermediate level of AsExp expression. The expansin gene (AsExp) from thermal *A. scabra* could be used as a molecular marker to select for heat-tolerant bentgrass plants.

We are developing the full-length AsExp and three other unique heat tolerance genes isolated from thermal *A. scabra*. We are constructing suppression subtraction hybridization (SSH) cDNA libraries from the heat-tolerant *A. scabra* and the heat-sensitive 'Penncross'. Multiple makers will be developed and a combination of markers will be used to screen for the best heat tolerance.

Summary Points

- Several unique heat tolerance genes were identified from thermal *A. scabra* adapted to geothermal sites in Yellowstone National Park using the differential display technique.
- The expression of a gene fragment encoding expansin proteins in cell walls, AsExp, was found to be highly correlated to heat tolerance in *A. scabra* and creeping bentgrass cultivars.
- The AsExp gene would be used as a molecular marker to select for heat-tolerant germplasm in bentgrass and other cool-season grass species.
- Suppression subtraction hybridization (SSH) cDNA libraries from the heat-tolerant *A. scabra* and the heat-sensitive 'Penncross' are being constructed to identify more heat tolerance genes or molecular markers from thermal *A. scabra*.