Physiological Characteristics and Molecular Basis of Heat Tolerance in Agrostis *species*

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Objectives:

1. To determine the physiological characteristics of thermal *Agrostis rossiae* and *A. scabra* exposed to high soil temperatures.
2. To compare the portfolios of heat-inducible proteins expressed in the thermal species, non-thermal species, and commercial cultivars, and advanced breeding lines of creeping bentgrass, and to identify potential candidates for proteins involved in the thermotolerant phenotype.
3. Identify and isolate heat-inducible genes responsible for heat tolerance in the thermal bentgrass species. The genes expressed to a greater extent in thermotolerant grass may be used for transformation of creeping bentgrass.

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

Our previous studies (addressing Objective 1) have shown that thermal *Agrostis scabra* collected from the thermal sites in Yellow Stone National Park was more tolerant to heat stress than *Agrostis stolonifera*. In 2005, we conducted a study to address Objective 2 and continued the work on identification of genes associated with heat tolerance.

Protein Degradation and Induction in Relation to Heat Tolerance

The induction and degradation of certain proteins may occur when plants are exposed to heat stress. However, changes in the amount and composition of soluble proteins in cool-season turfgrasses under increasing temperatures, especially in roots, have not been well understood. This study was performed to determine the critical temperature at which the changes of protein content and components start, and to compare protein profiles of both shoots and roots between two *Agrostis* species contrasting in heat tolerance.

Plants of thermal *Agrostis scabra* and two cultivars of *Agrostis stolonifera*, 'L-93' (moderately heat tolerant) and 'Penncross' (heat intolerant), were exposed to temperatures of 20, 30, 35, 40, and 45 °C in five growth chambers for 3, 7, and 14 days. The results from SDS-PAGE system showed that both roots and shoots of *A. scabra* had no obvious difference in their protein profiles under all temperatures at three days of stress, while the protein contents of both *A. stolonifera* cultivars significantly declined, and many proteins degraded at 45ºC in shoots and roots. Protein degradation occurred at 35ºC in 'Penncross' and at 40ºC in thermal *A. scabra*. Protein induction was observed at 40ºC in 'Penncross' and 35ºC in thermal *A. scabra*.

Our results suggest that 40°C was detrimental for both shoots and roots, causing protein degradation, particularly for heat-sensitive *A. stolonifera* plants. The thermal grass species had more thermal stable proteins and expressed new protein synthesis at a lower temperature. Both protein characteristics are important for plant tolerance to heat stress.

Rootzone temperatures in selection sites of thermotolerant Agrostis scabra *was at least 45ºC.*

Identification of Heat-inducible and Heat-tolerance Genes

This study was designed to compare the gene expression profiles between thermal *A. scabra* and *A. stolonifera* using differential display analysis (Delta Differential Display Kit). Total RNA was extracted from leaves exposed to a normal growth temperature (20ºC, control) or to 40ºC for a week (heat stress). With the transcript cDNA, 20 pairs of primers were tested.

Some genes were down-regulated and some were up-regulated in response to heat stress in both species. Sequence analysis of some genes fragments showed that the down-regulated genes are related to electron transport chain in photosystem II, but up-regulated genes are mainly signaling/regulatory components involved in the signaling pathway and target gene expression involved in the secondary metabolism.

One of the important genes that were up-regulated in thermal *A. scabra* encodes expansin proteins. Expansins are cell wall proteins controlling cell wall extensibility and cell enlargement. The up-regulation of expansins may contribute to heat tolerance of the thermal species.

Summary Points

- The study examined thermal *Agrostis scabra*, non-thermal *Agrostis scabra*, *A. rossiae*, and *Agrostis stolonifera* cv. L-93.
- Temperatures of 40°C or above were detrimental for both shoots and roots, causing protein degradation, particularly for heat-sensitive *A. stolonifera* plants.
- Thermal *A. scabra* plants had more thermal-stable proteins and expressed new protein synthesis at a lower temperature. Both protein characteristics are important for plant tolerance to heat stress.
- The up-regulation of a gene encoding expansins that control cell wall extensibility and cell enlargement was associated with heat tolerance of *Agrostis* species.