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

Bingru Huang
Rutgers University

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 (addressed 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 palustris*. All plants grown at 42 C had lower chlorophyll content than plants grown at 35^{o} and 20^{o} C. Differences in chlorophyll content between 35^{o} and 20^{o} C were not significant. Both ecotypes of thermal *A. scabra* had greater chlorophyll content than other *Agrostis* species at 35^{o} C. Canopy temperature for all species at 35^{o} and 42^{o} C increased over time as much as 6^{o} and 8^{o} C, respectively.

Thermal *Agrostis scabra* ecotypes had lower canopy temperatures than other *Agrostis* species after 25 days of 42 C. Photosynthesis rate, transpiration rate, and stomatal conductance decreased for all species and ecotypes over the course of the study. This decrease was most pronounced in a non-thermal *A. scabra* ecotype. This ecotype had the highest photosynthesis rate at 20^{o} C.

Thermal vents in Yellowstone National Park were used as the selection sites for high-temperature tolerant Agrostis scabra.

Differences between species were most pronounced early in the treatment period at 35^{o} C. One ecotype of thermal *A. scabra* had the greatest conductance and transpiration rate early in the study at 35^{o} C. The non-thermal *A. scabra* ecotype had the greatest photosynthesis rate over the first 25 days, but decreased rapidly.

Total root number for all species decreased over time at 35^{o} and 42^{o} C. Both non-thermal and thermal ecotypes of *A. scabra* had greater root number than other *Agrostis* species. Root distribution in thermal *A. scabra* ecotypes changed during the treatment period at 35^{o} C. New roots were produced 5 cm below the crowns, while roots at 15 and 30 cm below crowns decreased dramatically.

In 2004, we conducted a study to address Objective 3. This study was designed to compare their gene expression profiles between these two species using differential display analysis. The study examined thermal *Agrostis scabra* and *Agrostis palustris* cv. Penncross. Grasses were grown in sand in a growth chamber. Plants were watered daily and fertilized with full-strength Hoagland's solution once a week. Plants were exposed to 20^{o} C (control) or 40^{o} C(heat stress) for 10 days in a growth chamber. Leaves were sampled and frozen in liquid nitrogen immediately after cutting off from the plant, and stored for molecular analysis.

Total RNA was extracted from leaves exposed to a normal growth temperature (20^{o} C, control)) or to 40^{o} C for 10 days (heat stress). With the transcript cDNA, 20 pairwise combinations of P and T primers were tested. Eighty-six polymorphic fragments were identified and cloned individually. In *A. palustris*, 21 fragments were shown only in the control while 19 fragments were present only under heat stress. In *A. scabra*, 38 gene fragments were down regulated and 28 were up regulated under heat stress. In comparison of the two species, 17 down-regulated gene fragments were common, while 5 up-regulated genes fragments were shared in two species.

Rootzone temperatures in selection sites of thermotolerant Agrostis scabra *was at least 45^{o} C.*

Summary Points

- The study examined thermal *Agrostis scabra*, non-thermal *Agrostis scabra*, *A. rossiae*, and *Agrostis palustris* cv. L-93.
- Photosynthesis rate, transpiration rate, and stomatal conductance decreased for all species and ecotypes over the course of the study. This decrease was most pronounced in a non-thermal *A. scabra* ecotype.
- Total root number for all species decreased over time at 35 and 42 C. Both non-thermal and thermal ecotypes of *A. scabra* had greater root number than other *Agrostis* species. Root dry weight data showed the same trends as root number data.
- Among the 23 uncommon up-regulated genes, 20 fragments were only shown in heat-stressed *A. scabra*. Results suggests that some of these genes could be directly involved in heat tolerance since they are only found in heat-stressed thermal *A. scabra*.