

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

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## Objectives:

1. *Develop PCR-based markers from heat-responsive genes.*
2. *Map heat responsive candidate genes on the present bentgrass genetic linkage maps.*
3. *To 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*

Drought and heat stress are primary abiotic stress causing the decline in growth of cool-season turfgrasses, including creeping bentgrass. Summer turf quality decline is a major concern in turfgrass management. Developing heat and drought tolerant turfgrass cultivars is indispensable for maintaining high quality turf with limited irrigation during the summer months. Our previous projects have identified and developed some molecular markers (both QTLs and gene-based markers) linked to drought and heat tolerance in bentgrass species. The proposed project will validate and characterize those markers for use in screening for newly-developed drought and heat tolerant lines of creeping bentgrass. The project will be carried out in field plots covered with rainout shelters in two different locations with different climatic conditions, Rutgers University in New Brunswick, NJ and University of Georgia (UGA) in Griffin, GA, to evaluate broad application and stability of molecular markers for drought and heat tolerance.

In the current project, we propose to 1) validate SSR markers linked to known heat/drought tolerance QTLs and gene-based markers that were developed in previous USGA-funded projects in a bentgrass breeding population with a wide range of variation in drought and heat tolerance in two different environments or locations; 2) determine the stability of known QTLs over a range of test cross parents and

**Figure 1. Field plots located at the University of Georgia to evaluate heat tolerance.**

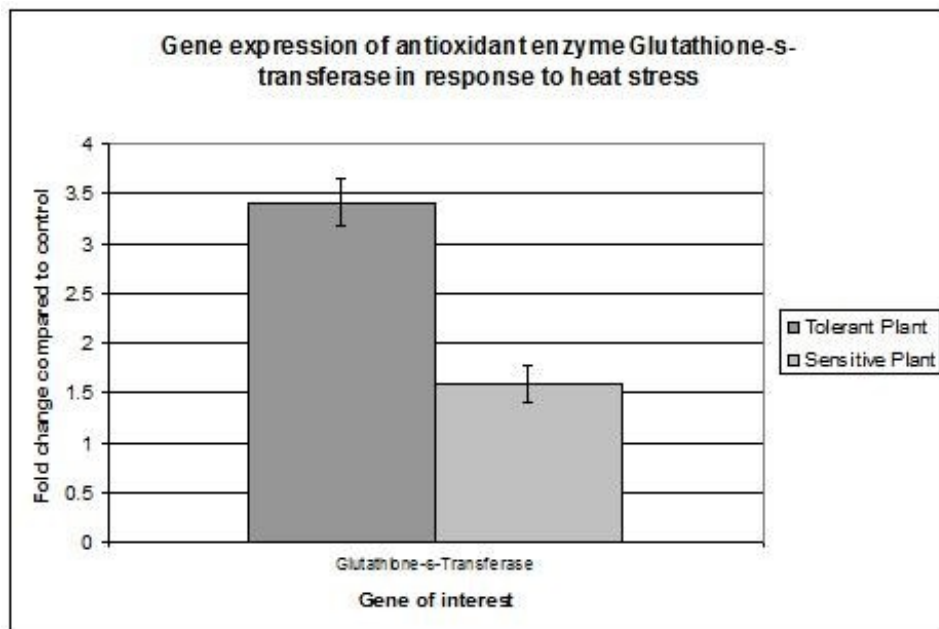


environments; 3) assess physiological traits (phenotypes) linked to these molecular markers in drought and heat tolerance; and 4) identify and characterize ideal phenotypes of newly developed drought and heat tolerant lines using verified markers to facilitate MAS in creeping bentgrass breeding programs. The overall goal of the current project is to enhance the evaluation, genetic characterization, and efficient use of recently developed new creeping bentgrass germplasm using available QTL and gene-based markers. Our ultimate goal is to provide useful markers to plant breeders to develop new cultivars of

creeping bentgrass that are superior to the "best" existing cultivars using marker-assisted selection.

Two populations of 143 individuals of creeping bentgrass from Rutgers breeding program and UGA breeding program were planted in field plots under rainout shelters in both universities. Plants were established in spring at UGA and in fall of 2013 at Rutgers. Data of summer performance of 143 bentgrass genotypes were collected at UGA, demonstrating that mean summer survival rate ranged from 2.6% to 100% and average survival across all genotypes was 57.3%. The top summer performing standard cultivars were 'Declaration' and 'Luminary' with 72.6% survival. Forty experimental bentgrass lines (27% of entries) performed better than the top performing standard cultivars. Four sethoxydim resistant lines (SR1A–SR4A) developed at UGA using tissue culture had extremely poor survival at only 2.6%.

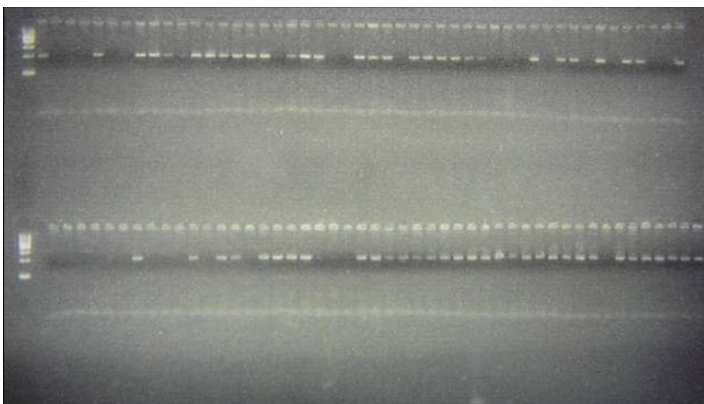
At current over 30 potential markers for candidate genes have been screened, of which 13 have successfully demonstrated useable polymorphism. In the creeping bentgrass mapping population the markers for 4 candidate genes have been screened for their addition to the linkage map. These genes include the important photosynthetic enzyme chlorophyll A/B binding protein, phenylalanine ammonia-lyase which is an important enzyme involved in phenylpropanoid metabolism which has been linked to abiotic stress tolerance, as well as several heat shock proteins, HSP-26, and HSP-70, which are protective chaperones induced by heat stress. In the creeping/colonial hybrid mapping population 8 candidate gene markers have been screened, which include catalase, cysteine



protease, expansin, glyceraldehyde-3-phosphate dehydrogenase, glutathione-s-transferase, heat shock proteins HSP-26, HSP-70 and HSP-101. Presently 6 of these 8 have successfully been added to the existing linkage map.

Additionally real-time PCR, also known as qPCR, has been performed to analyze the expression levels of candidate genes screened in the creeping/colonial hybrid population. By comparing a heat tolerant plant to a heat sensitive plant from this population under heat stress the importance of candidate genes in conferring tolerance can be confirmed. These results have confirmed anticipated results such as an increase in anti-oxidant enzyme expression in heat tolerant plants, or an increase in proteases responsible for protein degradation associated with heat induced senescence in heat sensitive plants.

### The photo provides evidence of marker polymorphism associated with heat tolerance.



### Summary Points

- Summer survival data in field plots confirmed genotypic variations in heat tolerance in two populations of creeping bentgrass.
- Thirteen SSR markers exhibited polymorphism in creeping bentgrass populations.
- Quantitative PCR analysis demonstrated that several candidate genes associated with polymorphic markers were linked to heat tolerance, which include genes underlying critical biological functions, such as antioxidant defense and heat shock protection, as well as photosynthesis.