

# 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. 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.

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**Project Duration:** 3 years

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Heat stress is a major abiotic stress affecting creeping bentgrass growth, leading to quality decline during periods of prolonged heat in many turf areas. Improving heat tolerance in creeping bentgrass is critical for maintaining high quality turf during summer months. By identifying important genes controlling heat tolerance and developing markers to assist in selection, the severity of summer bentgrass decline could be significantly reduced.

Several potentially important genes associated with heat tolerance were identified using suppression subtraction hybridization method and proteomic profiling, including fructose 1,6-bisphosphate aldolase, inducible phenylalanine ammonia-lyase, protein disulfide isomerase, putative glutathione S-transferase, cysteine protease, HSP16 and expansin. Potential markers for these genes are being tested in a hybrid mapping population which consists of a creeping x colonial hybrid (TH15) crossed with another creeping line (9188).

Several methods of developing useful markers to detect polymorphisms are being employed. Using EST sequences in the NCBI database, allele-specific PCR-based markers have been tested for inducible glutathione S-transferase, protein disulfide isomerase, cysteine protease, HSP16, and expansin. This method has successfully uncovered polymorphisms in the cysteine protease gene that controls protein degradation. The mapping population is currently being scored to potentially add this gene to the linkage map. Unfortunately, this method did not work for the other genes, so other means are being explored.

Cleavage amplified polymor-

phism (CAP) markers have been tested on fructose 1,6-bisphosphate aldolase as well as HSP16. Previous experiments have shown that the HSP16 and expansin genes are important for heat tolerance. These genes from the parents of this mapping population are being sequenced to uncover useful polymorphisms for marker development.

Concurrent with marker development, heat stress trials on the mapping population are being carried out. Turf quality, normalized difference vegetative index (NDVI), chlorophyll fluorescence, chlorophyll content, and electrolyte leakage are being used to evaluate the heat tolerance of individuals in the population. Tissue samples are also being collected to confirm expression levels of key genes.

Initial heat stress results show significant variation for a number of physiological parameters. Turf quality and NDVI, which both indicate overall turf health, as well as electrolyte leakage, a measurement of membrane stability previously found to be correlated with heat tolerance, are among the preliminary results analyzed. These parameters demonstrate that there is phenotypic variation in heat tolerance within the population.

In the near future, potential marker development for several of the key genes will be finished, marker development for genes discovered to be of potential importance based on current proteomic research will begin. Markers will be



Plants exposed to 20 days of heat stress (38°C), showing genetic variations in heat tolerance.

screened and confirmed using the mapping population, and successful markers will be added to the linkage map expanding its coverage.

Confirmation of these genes' roles in heat tolerance will also help develop our understanding of heat stress on the molecular level. Eventually, these will result in quick and efficient methods to screen populations for heat tolerance and select tolerant individuals for cultivar development.

## Summary Points

- Several methods of detecting polymorphism have been tested.
- A potential marker for cysteine protease has been developed and is being scored to be added to the map.
- Parental DNA is being sequenced to uncover polymorphisms in two important heat tolerance genes, HSP16 and expansin.
- Heat stress trials are underway to confirm candidate genes importance and expand QTL maps
- Initial heat stress results show there are significant phenotypic variations within the population.