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Objectives:

- DNA microarray analysis of 17 cultivars in the 1997 National Turfgrass Evaluation Program (NTEP) with wellcharacterized resistance to spring dead spot (SDS) to identify and confirm gene expression patterns associated with resistance.
- 2. Establish expressed sequence tagged (EST) libraries representing genes associated with acclimation to cold in tolerant and sensitive bermudagrass cultivars.
- 3. Use DNA microarrays to identify genes associated with cold tolerance.

Start Date: 2003 Project Duration: three years Total Funding: \$60,000

Over the past decade, major advances have been made in the molecular biology and genomics of stress tolerance mechanisms in model plant systems. Until very recently, relatively little investment has been directed to the grasses, and investment in bermudagrass has lagged behind other grass species of economic importance. The long-range goal of this project is to establish collections of DNA sequences representing genes involved in bermudagrass response to cold and the fungal disease spring dead spot.

This research summary is for the

cold tolerance objective. To identify a maximal number of differentially expressed genes, we constructed suppression subtraction hybridization (SSH) cDNA libraries from the extremely cold tolerant cultivar 'MSU' and the extremely cold sensitive experimental line 'Zebra.'

'MSU' was collected on the campus of Michigan State University and 'Zebra' is an annual when grown in the transition zone represented by Stillwater, Oklahoma. 'MSU' and 'Zebra' represent the most tolerant and susceptible seeded bermudagrasses, respectively, when evaluated for cold tolerance in controlled laboratory experiments at Oklahoma State University. We expect to find a large number of bermudagrass genes up- or down-



Winterkill of bermudagrass is a serious problem for golf courses in the transition zone. Research at Oklahoma State University is investigating the molecular genetics of cold tolerance with the ultimate goal of producing hardier cultivars

regulated in response to cold acclimation and, more importantly, that the expression patterns of these genes will differ between bermudagrass cultivars with different levels of cold tolerance.

Plants were cold acclimated in growth chambers and crown tissues were sampled at 2 and 28 days from both acclimated and non-acclimated plants. DNA libraries enriched for expressed sequence tags (ESTs) representing up- or down-regulated genes during acclimation are currently being sequenced at the University of Georgia. Validation of the gene expression profiles will be accomplished using DNA microarrays during the final year of funding for this project.

Genes discovered in this study will have value in developing molecular markers to assist in breeding for cold tolerance and provide candidate genes for manipulation to improved cold tolerance in this turfgrass.

Summary Points

• Establishment of a cDNA library containing 926 clones enriched for sequence tags of genes up-regulated during cold acclimation in MSU

• Establishment of a cDNA library containing 1,058 clones enriched for sequence tags of genes down-regulated during cold acclimation in 'MSU'

• Establishment of a cDNA library containing 1,116 clones enriched for sequence tags of genes up-regulated during cold acclimation in 'Zebra'

• Establishment of a cDNA library containing 595 clones enriched for sequence tags of genes down-regulated during cold acclimation in 'Zebra'

• Validation of the gene expression profiles will be accomplished using DNA microarrays during the final year of funding for this project.