Functional Genomics of Stress Tolerance in Bermudagrass

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Objectives:

1. DNA microarray analysis of 18 cultivars in the 1997 National Turfgrass Evaluation Program (NTEP) with well-characterized 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 of DNA microarrays to identify genes associated with cold tolerance.

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Project Duration: three years
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Over the past decade, major advances have been made in the molecular biology and genomics of stress tolerance mechanisms in model plant systems. 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. 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.

We initiated experiments to create SSH libraries representing genes differentially expressed during cold acclimation for two bermudagrass cultivars, 'MSU' (A-12195) and 'Zebra'(Figure 1). 'MSU' is very cold hardy (T_{mid} = -10.5°C) which was originally collected on the campus of Michigan State University, East Lansing, Michigan, and 'Zebra' is very sensitive (T_{mid} > -6°C) and is grown as an annual in field plots at Stillwater, Oklahoma.

Phytomers from each cultivar were transplanted into forty 14-cm diameter pots in the greenhouse and maintained for eight weeks. Plants were transferred to a controlled environment chamber and grown at 28°C day/24°C night and 10/14 hours light/dark for six weeks. Thirty pots of each cultivar were transferred to a low temperature growth chamber for cold acclimation at 8°C day/2°C night and 10/14 hours light/dark. Fifteen pots for each cultivar were sampled at 2 and 28 days.

Figure 1. *To identify a maximal number of differentially expressed genes, researchers constructed suppression subtraction hybridization (SSH) cDNA libraries from the extremely cold tolerant cultivar 'MSU' and the extremely cold sensitive experimental line 'Zebra.'*

Crown tissues were harvested, immersed in RNAlater™ to inhibit ribonucleases, and stored at -80°C as small manageable packets for mRNA isolation. Crown tissues for fifteen pots of each cultivar, maintained at 28°C day/24°C night and 10/14 h light/dark, were harvested with the same procedure and serve as the source of mRNA from non-cold acclimated tissues for each cultivar.

Total RNA was isolated with the Fenozol™ reagent (phenol, detergent, and chaotrophic denaturants) and mRNAs were isolated with the mTRAP™ procedure. This yielded 1 to 5 µg of high quality mRNA, largely free of contaminating rRNAs and genomic DNA, per gram fresh weight of crown tissue. These mRNA preparations were utilized for cDNA library construction.

Two to five percent of plant genes are involved in stress defense mechanisms. We expected to find a large number of bermudagrass genes induced or repressed in response to cold acclimation. cDNA libraries generated by SSH are very rich sources of sequences which are either unique or share partial homology with known genes. We randomly selected 800 clones from each library (2 acclimation treatments x 2 cultivars x 2 types (forward or reverse) of libraries x 800 clones per library = 6,400 clones). Clones were evaluated by colony PCR to establish insert size and homogeneity.

Homogenous clones (~3869) with inserts greater than 200 base pairs were sequenced in Dr. Andrew Paterson's laboratory at the University of Georgia. Approximately 3,852 sequences were deposited in dbEST Genebank for public availability. Test hybridization for cold treated and control tissues for MSU were performed. Scanning of initial arrays reveal high quality hybridization and signals.

Summary Points

- Crown tissues from non- and cold-acclimated crown tissues of a tolerant ('MSU') and sensitive ('Zebra') seeded bermudagrass were harvested from growth chamber experiments. Sufficient quantifies of mRNA have been isolated from these tissues for cDNA library construction and gene expression profiling.
- Establishment of a cDNA libraries containing a combined total of 3,959 clones enriched for sequence tags of genes up- and down-regulated during cold acclimation in 'MSU' and 'Zebra'.
- A total of 3,869 cDNA clones were sequenced at the University of Georgia by Andrew Paterson's laboratory.
- Microarray hybridization procedures and techniques were optimized.
- Microarrays printed with 4,620 spots at the microarray facilities at Oklahoma State University.
- High quality test hybridizations were performed revealing excellent print patterns and strong signal intensities.