Functional Genomics of Stress Tolerance in Bermudagrass

Arron C. Guenzi
Oklahoma State University

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
Total Funding: $60,000

Over the past decade, major advances have been made in the molecular biology and genomics of host-microbe interactions with 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 finding of colinearity (synteny) among grass genomes, coupled with the release of the rice physical map and genomic sequence, now raises the possibility of rapid progress in the molecular analysis and manipulation of grass genomes. Unfortunately, many of the fundamental tools required for bermudagrass to benefit fully from genomics do not exist or are woefully incomplete.

Spring dead spot (SDS), caused by *Ophiosphaerella herpotricha*, is a serious disease of turf bermudagrass grown in the southern United States. The objective of our research is to utilize well-characterized genetic resources to dissect the molecular responses of bermudagrass to this soilborne fungal pathogen.

Bermudagrass cultivars have been extensively evaluated for SDS resistance. No immunity has been identified, however, genotypes have been identified with a wide range of phenotypic responses, from highly resistant to highly susceptible. These well-characterized genetic resources have been, and will continue to be, essential to dissect molecular interactions associated with this disease. Our long-range goals are to identify markers that can be utilized to select for resistance genes and to ultimately engineer increased levels of resistance not obtainable by genetic variation in this species.

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 fungal infection. To identify a maximal number of differentially expressed genes with limited financial resources, we constructed suppression subtraction hybridization (SSH) cDNA libraries from infected crown tissues from resistant (Yukon) and susceptible (Jackpot) cultivars.

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We have analyzed 834 clones from both forward and reverse normalized-subtraction libraries to insure that genes representing low-abundance transcripts were included. Sixty percent of the clones did not match current accessions in NCBI GenBank, which is nearly two-fold greater than the average number (30%) of new sequences usually discovered in SSH libraries.

Subsequent sequence analysis assembled these clones into 154 genes (contigs). Features belonging to the same contig were also treated as replications for expression analysis. Differential gene expression was evaluated during periods of fungal infection in the late fall and early spring.

During the fall and spring seasons, there were 80 and 66 genes, respectively, that displayed more than a two-fold differential expression between the two cultivars. These 107 responsive genes were grouped into six clusters according to their fall and spring expression profiles. The majority of differentially expressed genes had no homology to current accessions in NCBI GenBank.

These differentially expressed genes provide targets for future functional analyses to establish their role in disease development. However, this is extremely challenging for a species such as bermudagrass in which many of the tools for functional analyses are missing or in the early stages of development.

We have decided to use a genetic approach to validate our microarray results. Expression profiles for eighteen bermudagrass cultivars that were phenotyped for SDS resistance from 1997 to 2002 will be established during the next year. A biomedical research approach of using microarrays to establish gene expression patterns associated with disease development across a number of individual cultivars will hopefully allow us to validate which genes are associated with resistance or susceptibility.

Summary Points

- 834 gene expression sequence tags associated with bermudagrass responses to SDS were submitted to NCBI GenBank.
- 107 genes were identified by microarray analysis which have differential expression in response to either fall or spring fungal infection.
- The most interesting classes of genes differentially expressed between the resistant and susceptible cultivars were those involved in signaling pathways and the oxidative burst defense mechanism.