Transformation of Bermudagrass for Improved Fungal Resistance

Oklahoma State University
Michael P. Anderson

Start Date: 1998  
Number of Years: 5  
Total Funding: $125,000

Objectives:

1. Isolate, identify, and characterize chitinases and glucanases and their genes that have high anti-SDS activity.
2. Develop an efficient protocol to transform (genetically engineer) bermudagrass.
3. Transform and characterize bermudagrass with the antifungal chitinase and/or glucanase genes directed against the spring dead spot casual organism.

A major disease commonly known as spring dead spot (SDS) causes significant economic damage to bermudagrass in the Southeastern United States. The causal agent for SDS throughout most of the United States is Ophiostoma herpotricha and Ophiostoma koreana. Both fungal species are very active in the fall and early spring when the temperatures are cool and moisture is plentiful. Infected areas appear as regular circular patches of dead and diseased turf that generally occurs in more mature stands of bermudagrass.

The long-term goal of this project is to increase resistance in bermudagrass turf varieties to SDS through gene transformation technology. This report describes the current progress and results for the development of the transformation system and the isolation and characterization of anti-fungal factors during 1998.

Bermudagrass Transformation. The use of high velocity micro-projectiles (biolistics) to deliver recombinant DNA into intact plant cells has been successfully utilized to transform many grass species, and is considered the method of choice for most grass species. The immature inflorescences of BRAZOS bermudagrass, a forage cultivar, were used to induce the formation of embryogenic callus tissue. BRAZOS was chosen for this experiment because it had previously demonstrated superior growth and plant regeneration potential in tissue culture. Tissue was transformed with a plasmid containing two chimeric genes of interests, the bar and uidA genes, under the control of ubiquitin promoters. The bar and uidA genes serve as a selectable marker and reporter gene, respectively. The GUS enzyme, coded for by uidA, can be assayed by accumulation of fluorogenic products by providing the enzyme substrate. PAT detoxifies bialaphos (the active ingredient in the herbicide Liberty) in the selective media; thereby allowing transgenic cells, and plants to continue to grow. Six hundred and seventy one putative transgenic plants have been recovered from this experiment. We are currently evaluating these putative transformants with PCR to determine if they contain the bar gene. PCR positive plants will be characterized by Southern analysis and enzyme assays for phosphinothricin acetyl transferase during 1999.

Anti-SDS Proteins. Living organisms produce many antimicrobial compounds to protect themselves from pathogens, or to give them a competitive advantage for nutrients. They range from the small molecular weight antibiotics and secondary metabolites to the larger macromolecular proteins and assorted polypeptides. Recently we discovered a bacterium that was strongly and persistently inhibitory towards O. herpotricha. The bacterium was identified to the genus taxonomic level with confidence by a GC-FAME and BIOLOG technology. This bacterium secreted many proteins into the extra-cellular matrix. Dialysis of the extra-cellular excretions suggested that the anti-fungal factor was a protein. Purification of the anti-fungal proteins on anion exchange, hydroxyapatite, and Mono Q chromatography resulted in the isolation of a 36 kD protein that is most likely expressed as multiple isoforms. Analysis of the purification results suggested that there are at least two distinct anti-fungal factors antagonistic against O. herpotricha secreted by the bacterium. Experiments are in preparation to identify, sequence, and characterize the 36 kD protein.

Selecting Seeded Zoysiagrass for Cold Hardiness

University of Missouri-Columbia  
Suleiman S. Bughrara

Start Date: 1998  
Number of Years: 5  
Total Funding: $91,535

Objectives:

1. Evaluate zoysiagrass germplasm for cold hardiness using the cold chamber technique.
2. Evaluate seed production of selected zoysiagrass strains under Missouri environmental conditions.
3. Our long-term objective will be to develop cultivars of zoysiagrass that can be seeded and that have desirable winter hardiness for the transition zone.

The zoysiagrass breeding program at the University of Missouri was initiated in Summer 1997 by planting over 500 clones in a spaced nursery at Bradford Farm, Columbia, Missouri. The main sources for these clones were the Georgia Plant Introduction Station, Bobbi Murray (Jack Murray’s widow) and some clones that collected from golf courses around Columbia, Missouri. Two genotypes with good turf and seed production characteristics are shown in Figure 7.