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) bermduagrass.*
3. *Transform and characterize bermdagrass 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 *Ophiosphaerella herpotricha* and *Ophioshpearella korrea.* 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 fluoro-genic 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 *0. 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 *0. 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 techinque.*
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.

Figure 7. Clones were planted on 3-foot centers.

Sixty clones that survived the mild winter of 1997-1998 in Missouri and exhibited good seed production were planted in turf plots in spring, 1998, for further evaluation. In addition, 56 clones that were brought from Rutgers University, in cooperation with both Drs. Funk and Meyer were planted in plots adjacent to the Missouri selections. The Rutgers material will be evaluated in both Missouri and New Jersey.

Several laboratory studies were conducted to characterize the germination process and evaluate factors affecting seed dormancy. The floret consists of a caryopsis that is covered by a lemma and palea that adhere tightly. The lemma is very thick, has thickened cell walls and a wax coating on the outer surface that may restrict water penetration. Naked caryopses (lemma and palea removed) germinated up to 80 percent at 22 °C, whereas intact florets germinate less than 10 percent. Florets that were cut transversely either at the base (below embryo) or at the tip (above embryo) germinated almost as well as naked caryopses.

These results suggested that water uptake is a dormancy factor. Water extracts of florets did not affect germination of naked caryopses, but inhibited germination of base and tip cut florets by 25 percent. The nature of the germination is not known. At 35 °C light and 20 °C dark, 90 percent of untreated florets germinated, even in the extract treatment. This indicates the need for high temperature in order for rapid germination to occur. Thirty-percent KOH scarification (15 min.) enhanced germination at 35/20 °C up to 98 percent. Seed treatments can overcome dormancy, which appears to be partially physical, and improve germintation. To understand the genetics of the process we developed 19 half-sib families. These will be evaluated for germination properties in the future.

On-Site Testing Putting Green Variety Trial

National Turfgrass Evaluation Program

Kevin Morris

Start Date: 1996
Number of Years: 5
Total Funding: $71,600

Objectives:

Provide bentgrass and bermudagrass putting green trials on golf course practice putting greens.

The Golf Course Superintendents Association of America (GCSAA), United States Golf Association (USGA) Green Section and National Turfgrass Evaluation Program (NTEP) jointly sponsored on-site trials for bentgrass and bermudagrass cultivars grown on USGA specification putting greens.

Location of Trial Sites. The sixteen sites, course superintendents, and research cooperators are named in Table 10. The bentgrass trial sites are established. The Snoqualmie Ridge Golf Course in Snoqualmie, Washington was the last to be selected and seeded. It is established and has received limited play. It should be in excellent shape for next season.

The bermudagrass sites are constructed and established. Progress on all trial sites is meeting expectations.

Site Visits. As of the date of this report, we have visited trials. All sites visited are doing well. Cooperation is outstanding and enthusiasm among researcher cooperators, host course superintendents, and golfers is extremely positive. Research cooperators will submit their data to NTEP for analysis later this winter. The NTEP will summarize the data and report the first year's results in spring, 1999. A meeting to discuss the on-site trials is scheduled for the GCSAA Conference and Show in Orlando. This meeting will serve as a "feedback mechanism", allowing comment and discussion from the researchers and host superintendents. We will use this information to make appropriate adjustments in data collection and analysis during the 1999-growing season.