A Multigene-Transfer Strategy to Improve Disease and Environmental Stress Resistance in Creeping Bentgrass

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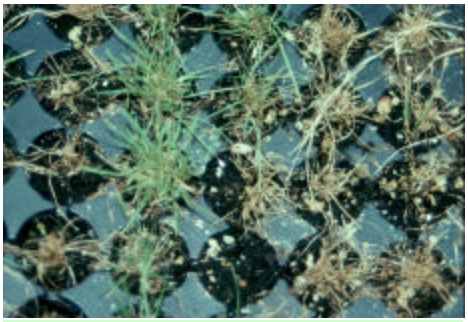
Objectives:

1. Enhance the expression (increase level of pest resistance) of the American elm chitinase gene in creeping bent grass.
2. Transfer two drought-resistance genes controlled by either a constitutive or an ABA-inducible promoter into creeping bentgrass.
3. Determine disease resistance of transgenic plants expressing different levels of the chitinase gene and transgenic plants containing single versus multiple-inserted genes grown under greenhouse and field conditions.
4. Determine environmental stress resistance of transgenic plants containing single versus multiple-inserted genes grown under greenhouse andfield conditions.
5. Evaluate transgenic creeping bentgrass clones for turf quality characteristics under field conditions.
6. Release transgenic creeping bentgrass germplasm with combined improvements in turf quality and pest and stress resistance to Pure Seed Testing, Inc., and/or other companies for use in their field testing and commercial breeding programs.

Start Date: 1998
Project Duration:3 years
Total Funding: $75,000

The most promising approach to combating the major biotic and abiotic stresses associated with creeping bentgrass and other turfgrasses is through the development of transgenic plants. These plants are created by the introduction of genes into existing DNA (deoxyribonucleic acid), the primary carrier of genetic information. Thus is would be advantageous to insert genes into creeping bentgrass that express greater resistance to stresses induced by extreme environmental conditions and pathogens.

Previously, Sticklen's research team developed creeping bentgrass clones that contain the glufosinate ammonia (Liberty or FinaleTM) resistant herbicide, a chitinae gene, a proteinase inhibitor gene, and a drought and salt tolerance mannitol dehydrogenase (mt1D) gene. Studies have shown that the chitinase genes can make transgenic plants resistant to patho genic fungi such as *Rhizoctonia solani.*



.At Michigan State University, scientists developed bialaphos resistant bentgrass clones.

This research confirmed that glufosinate ammonia has fungicidal as well as herbicidal properties. We have been able to simultaneously control weeds as well as turfgrass pathogens (mainly *Sclerotinia ulnocarpal* and *Rhizoctonia solani)* by spaying this herbicide on transgenic creeping bentgrass expressing the bar gene under greenhouse conditions.

Research by Dr. Vargas's laboratory demonstrated two out of 44 independently transformed lines of transgenic creeping bentgrasses transcribing the elm chitinase gene improved resistance of plants to *R. solani* under controlled environmental conditions.

Experiments performed by Dr. Baird's laboratory has shown no translation of transcribed Mt1D gene in certain lines of transgenic turfgrass. Of course, none of these transgenic lines tested for western blotting also showed accumulation of mannitol, as a sign for drought and/or salt tolerance. Not surprisingly, none of these transgenic lines showed any drought and/or salt tolerance either.

Since we made synthetic peptides and produced extra antibodies to the Mt1D protein, we plan to test the rest of Mt1D-transcribed transgenic lines for expression of Mt1D protein by western blotting. If positive, then once again we will perform

A bentgrass plant derived from Penncross contains the biolaphos resistance gene and a chitinase gene to help improve resistance to common turfgrass diseases.

mannitol test and will also test plants for drought and/or salt tolerance.

Should Michigan State University have a new turfgrass physiologist in place soon, this work will be performed with collaboration of the new physiologist.

Summary Points

- Developed herbicide (Finale) resistant strain of bentgrass.
- Confirmed fungicide efficacy of Finale used on herbicide resistant bentgrass.
- Two transgenic bentgrasses with the elm chitinase gene showed a 3 to 5 fold increase in tolerance to *Rhizoctonia solani.*
- None of the transgenic lines were resistant to dollar spot.
- The mannitol dehydrogenase gene (mtlD) did not increase drought resistance in transgenic bentgrass.
- Plasmid with Act1 promoter and intron developed.