Development of Transgenic Creeping Bentgrass Resistant to the Major Pathogenic Fungi

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Goals:

- Express our cloned chitinase gene in E. coli, and purify and collect chitinase protein.
- Identify the level of chitinase required to control three major turfgrass pathogens.
- Transform bentgrass with plasmids containing the chitinase gene.
- Evaluate the transgenic plants for resistance to major turfgrass pathogenic fungi.

A major problem associated with creeping bentgrass are pathogenic diseases. Most pathogens contain chitin in their cell walls, and therefore may be susceptible to the chitinase enzyme. The objective of this project was to develop fungal disease-resistant creeping bentgrass plants, initially by introducing a chitinase gene in this plant.

After the project began, we realized that one single gene may not necessarily work, and pathogens may develop resistance against a single gene product within a short period of time. Therefore, the effort has been supplemented with the following lines of research: 1) introduce a chitinase gene as proposed, 2) introduce a protease inhibitor gene in plants because these enzymes are essential for the survival of the pathogenic fungi, 3) introduce a drought resistance gene in plants to reduce the need for irrigation that would prevent growth and spread of disease, and 4) introduce a bialaphos resistance gene into creeping bentgrass that would simultaneously control weeds as well as certain pathogenic diseases.

During past year, we followed up on introducing a chitinase gene, a protease inhibitor gene, the bar (bialaphos resistance) gene, and a drought resistance (mannitol dehydrogenase) gene into creeping bentgrass.

As reported earlier, transgenic creeping bentgrass plants that express potato proteinase gene and the bialaphos-herbicide resistance were developed. These transgenic plants were sprayed with bialaphos, and their resistance to dollar spot, brown patch, and *Pythium* was evaluated. The results

confirmed that after we spray transgenic creeping bentgrass with bialaphos, we simultaneously control weeds, dollar spot and brown patch disease at the greenhouse level. This experiment will be repeated at the field level by Dr. Vargas next summer.

The chitinase gene and a drought resistance gene also have been introduced into creeping bentgrass. The selectable marker for this experiment also has been the bialaphos resistance gene. All plants regenerated are bialaphos resistant. Since

the construct containing the drought resistance gene was linked to the bialaphos resistance gene, it is believed the transformed plants now contain the drought resistance gene. Hundreds of transgenic plants have been produced from the chitinase/drought resistance experiment. Work is in progress to confirm the stable integration and expression of the chitinase gene and the drought resistance gene in these plants.