Development of Improved Turfgrass with Herbicide Resistance and Enhanced Disease Resistance through Transformation

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Goals:

- Establish a transformation system for creeping bentgrass.
- Improve the utility of creeping bentgrass by incorporating genes to confer herbicide resistance or enhanced resistance to fungal pathogens.

This program aims to establish a transformation system for creeping bentgrass (*Agrostis palustris*) which will enable us to improve the utility of this important recreational turfgrass by incorporating genes to confer herbicide resistance or enhanced resistance to fungal pathogens. While still providing high quality playing surfaces for golf, these improvements will help conserve natural resources by reducing chemical treatments against major fungal pests and remove weeds using available broad-spectrum and environmentally friendly herbicides.

Two milestones for successful turfgrass transformation are: 1) establishment of a plant regeneration system to produce regenerants at high efficiency, and 2) development of a high frequency transformation system to obtain large numbers of transgenic plants.

Using surface sterilized seed as explant material for callus initiation, we developed a turfgrass tissue culture and regeneration system. Embryogenic callus lines with high regeneration potential were established from eight commercially important creeping bentgrass varieties. Suspension cultures were initiated from embryogenic callus lines. Both embryogenic callus cultures and suspension cells were used as targets for stable transformation using a gene gun.

The *E. coli* ß-glucuronidase (GUS) gene was used as a scorable marker and the *bar* gene, which confers resistance to the herbicide bialaphos (*Basta*™), was used as a selectable marker. We optimized various parameters to improve transient expression of GUS in cultured bentgrass to high

levels, and we have developed a turfgrass transformation system with particle bombardment. Transformations were obtained from EMERALD, PUTTER, and SOUTHSHORE creeping bentgrass. Experiments incorporating several herbicide resistance genes in other cultivars are in various stages of completion.

Both plate and liquid selection were successful in obtaining herbicide resistant bentgrass. Transgenics have been confirmed by herbicide tests, polymerase chain reaction (PCR) assay and southern blot hybridization to show the presence of the transgenes.

We are also developing a protoplast transformation system. Regenerants were obtained from protoplasts through direct DNA uptake with polyethylene glycol (PEG) or electroporation. These plants will be tested for herbicide sensitivity by spraying in the greenhouse.