

Development of Genetically Engineered Creeping Bentgrass Resistant to Fungal Diseases

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Goals:

- Improve disease resistance of creeping bentgrass using a new genetic engineering approach.
- Introduce the chitinase gene into creeping bentgrass to develop varieties resistant to fungal diseases.

Chitinase is one of several anti-fungal proteins produced in plants upon fungal infection. This enzyme catalyzes the hydrolysis of chitin, a cell wall component of many fungal pathogens. It was shown that constitutive overexpression of the chitinase gene in transgenic tobacco plants resulted in enhanced resistance to fungal diseases.

This project is designed to improve disease resistance of creeping bentgrass via genetic engineering. The objectives are 1) to develop efficient gene transfer systems in creeping bentgrass and 2) to develop transgenic creeping bentgrass that is resistant to fungal diseases by overexpression of a chitinase gene.

For the second year of this project we focused our research efforts on developing a gene transfer system for creeping bentgrass and isolating a chitinase gene from Kentucky bluegrass.

We have developed an efficient gene transfer system for creeping bentgrass using particle bombardment. A hygromycin resistance gene was transferred into embryogenic creeping bentgrass cells by particle bombardment, and transformed cells were selected on the medium containing 150 or 200 mg/L of hygromycin. A total of 124 transformed calli were obtained from 27 bombarded plates, with an average of 4.6 hygromycin-resistant colonies per bombardment. Thirteen transgenic plants were regenerated from the resistant colonies. Southern blot analysis confirmed the integration of the transgene into the genome of the transgenic plants.

Using a PCR *in vitro* cloning method, we have isolated a chitinase gene from Kentucky bluegrass. We are currently determining the sequence of this gene.