Development of Genetically Engineered Creeping Bentgrass Resistant to Fungal Diseases

Dr. Sam Ha

Virginia Polytechnic Institute and State University

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 over expression 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 using genetic engineering. The objectives are 1) to develop an efficient gene transfer system in creeping bentgrass and 2) to develop genetically engineered creeping bentgrass that is resistant to fungal diseases through over expression of chitinase genes.

An efficient gene delivery system for creeping bentgrass was developed during this research project. 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.

The research program successfully isolated three genomic clones of chitinase genes *(chi1, chi2, chi3)* from Kentucky bluegrass using adaptor-ligation polymerase chain reaction (PCR). The *chi1* and *chi2* genes encode full length chitinases of 340

and 320 amino acids, respectively. The *chi3* gene appears to encode a truncated chitinase (49 amino acids) due to the presence of a stop codon in the coding region.

Using reverse transcription and PCR, they found that both *chi1* and *chi2* were induced by ethylene, strongly indicating both genes were involved in plant defense responses.

Each of these two genes (*chi1* and *chi2*) were subcloned into the expression vector (plasmid). The plasmids containing the Kentucky bluegrass chitinase genes where then transferred into creeping bentgrass calli by particle bombardment. Transformed calli have been selected on the medium containing hygromycin. Transgenic plants are now being regenerated from these calli. Once transgenic plants are developed, those which exhibit a high level of chitinase expression will be screened and tested for resistance to fungal pathogens.