GENETIC ENGINEERING: AN ULTIMATE SOLUTION TO THE TURFGRASS PROBLEMS

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Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) is a desirable species for use on golf courses throughout most of the United States due to its tolerance of low mowing heights, superior density, and exceptional turfgrass quality. Whether or not it is grown within or beyond its zone of adaptation, creeping bentgrass is limited by environmental stresses associated with drought and temperature extremes, and by biological organisms such as pathogenic diseases, insects and weeds.

The most promising approach to combating the stresses associated with creeping bentgrass and other turfgrasses is through the development of transgenic plants. These plants are created by the introduction of genes (fundamental units of heredity) into existing deoxyribonucleic acid (DNA), the primary carrier of genetic information. Thus, it would be advantageous to insert genes into creeping bentgrass that express greater resistance to these environmental and biological stresses.

Multi-Gene Transformation Studies

Initially, Dr. Sticklen's laboratory developed a genetic engineering system for creeping bentgrass using a marker (*gus*) blue gene to determine successful gene incorporation (Zhong et al., 1991; 1993). Then, her team successfully incorporated a gene for resistance to glufosinate (Finale™), a non-selective herbicide (Liu, 1996). Furthermore, Dr. Sticklen's team discovered that Finale™ has fungicidal, in addition to herbicidal, properties. As a result, her team has been able to simultaneously control weeds and diseases caused by the pathogenic fungi *Rhizoctonia solani* (brown patch) and *Sclerotinia homoeocarpa* (dollar spot) by spraying the herbicide on transgenic creeping bentgrass expressing this gene (Liu et al., 1998).

Dr. Sticklen's next challenge was to insert a chitinase gene cloned and characterized in her laboratory into creeping bentgrass. Chitinases are enzymes (proteins) that degrade chitin, a structural polysaccharide of fungal cell walls and insect exoskeletons. Since fungi cause the major pathogenic diseases of turfgrasses, expression of the chitinase gene in creeping bentgrass is expected to promote disease control via chitin degradation. Studies have shown that chitinase genes can make transgenic plants resistant to pathogenic fungi (Graham and Sticklen, 1994). The Sticklen laboratory team cloned and characterized a full-length chitinase gene which contains the necessary chitin-binding domain from a Dutch elm disease resistant American elm (*Ulmus americana*) (Hajela and Sticklen, 1993; Sticklen et al., 1993; Hajela et al., 1993). Then the team constructed a mini-gene containing this chitinase gene and successfully inserted this chitinase gene into creeping bentgrass (Chai, 1997). The collaboration was made with Dr. Vargas for laboratory and greenhouse levels inoculation studies of transgenic plants. These studies showed that two out of five independently transgenic turfgrass genetic lines were resistant to *R. solani* (Chai et al, 2000).

Dr. Sticklen's new lines of research include: (1) development of freeze tolerant transgenic turfgrass, and (2) discovery of functions of disease resistance genes via the Microarray techniques. To date several transgenic plants have been developed using three freeze tolerance genes. Work is in progress to test these plants for the functions of transgenes and for their freezing resistance.

Cross Breeding of Certain Lines of Transgenic Turfgrass

Michigan State University and Pure Seed Testing, Inc. entered into a license agreement whereby the Oregon research corporation conducted further testing of transgenic plants developed at Michigan State University. Pure Seed also cross bred our transgenic lines with their inbred lines and produced hybrid seeds. The Pure Seed Testing, Inc. also studied the distance needed to be kept between transgenic and the non-transformed turfgrass fields in order to avoid transfer of transgenic pollen grains to the surrounding fields.

Conclusions

At conclusion, we developed a very reliable system of genetic engineering for turfgrass, transferred multi-genes in plants, and tested transgenic plants for herbicide, disease and drought tolerance. Cross breeding was performed via cooperation with Pure Seed. Pure Seed confirmed the integration and expression of transgenes into the companies commercial lines.

Acknowledgement

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Generated Patent Applications

1. U.S. Patent in progress: MSU 4.1-153, Serial # 08/036,056, March 23, 1993. Method for isolating a grass plant with foreign DNA. On appeal.

2. U.S. Patent in progress: MSU 4.1-315, Serial # 60/015,485, April 15, 1996. Simultaneous control of weeds and turfgrass diseases with spray of Bialaphos on genetically engineered turfgrass. Provisional filed on April 15, 1997.

3. U.S. Patent in progress: MSU 4.1-395, ID 98-031, May 26, 1998. Disease resistant transgenic turfgrass containing a chitinase gene. Filed in 1998.

Generated Publications:

Chai, B. 1998. Transgenic creeping bentgrass expressing the elm chitinase and the mannitol-1-phosphate dehydrogenase drought tolerance genes. Ph.D. Dissertation. Michigan State University.

Chai, B., D. Green, R. Sabzikar, J. Vargas, and M.B. Sticklen. 2000. Brown patch resistance in transgenic creeping bentgrass with an elm chitinase gene. Phytopathology (submitted).

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