DECEMBER 1997 FINAL REPORT

DEVELOPMENT OF IMPROVED TURFGRASS WITH HERBICIDE RESISTANCE AND ENHANCED DISEASE RESISTANCE THROUGH TRANSFORMATION

Principal Investigators: Peter Day and Faith Belanger

Executive Summary

This project seeks to improve creeping bentgrass through transformation to provide golf course managers with more effective and selective weed control with herbicides and more environmentally sound and cost-effective control of plant diseases with reduced use of fungicides. During the past two years we have made considerable progress towards accomplishment of these goals.

Our work with the herbicide resistant plants has progressed to the stage of incorporation into the breeding program. Herbicide-resistant progeny plants produced from crosses carried out in 1995 and 1996 are currently in the field for evaluation. In the spring of 1997, six of the herbicide resistant progeny were selected for crosses with breeding material from Dr. Bill Meyer.

We have also produced a number of independent transgenic lines of creeping bentgrass containing potential disease resistance genes. In the summer of 1997, some of these lines were put into a randomized replicated field test for evaluation. The plot includes six independent transgenic lines each containing the pokeweed antiviral protein and bacterio-opsin, five independent lines of glucose oxidase, and 22 nontransgenic controls. Disease in the plot was from natural infection. Our preliminary results from this test are very encouraging regarding efficacy of the genes. Overall, the transgenics are exhibiting considerably less disease incidence than the controls.

INTRODUCTION

This project was supported by a USGA grant from February 1996 to January 1998. The ultimate goals of the project are to produce improved turfgrass cultivars through a combination of plant transformation and breeding. The specific characteristics we are currently concentrating on are herbicide resistance, disease resistance, and abiotic stress tolerance. During the past two years we have made considerable progress towards the achievement of these goals, which we summarize here.

HERBICIDE RESISTANT CREEPING BENTGRASS

The Rutgers turfgrass biotechnology program previously developed transgenic creeping bentgrass lines carrying the *bar* gene which confers resistance to the environmentally safe herbicide bialaphos (Hartman et al., 1994; Lee et al., 1996). Bialaphos, or the related molecule glufosinate, are the active ingredients of the commercial herbicides Herbiace™, Ignite™, Finale™, and Basta™. Herbicide resistant creeping bentgrass clones were successfully field tested in 1994 and 1995 at Rutgers' Development and Research Center at Bridgeton, NJ. The bialaphos resistance gene is clearly very effective in creeping bentgrass. We are now focusing on incorporating this gene into a commercially useful cultivar. An herbicide resistant cultivar would give golf course superintendents a safe and effective means of controlling *Poa annua* on greens.

In 1995 and 1996 crosses between the original transgenics and nontransgenic germplasm were carried out. Progeny from the crosses were screened for herbicide resistance and planted at Hort Farm II. We currently have 598 herbicide resistant progeny in the field for evaluation of overall turf quality. In the spring of 1997 Dr. Bill Meyer selected six of the progeny plants for crossing with plants in his breeding program. Seed from these crosses was harvested in August, 1997.

Herbicide resistance has thus progressed from generation and testing of transgenic plants to incorporation into the breeding program. We expect development of a useful cultivar to require 4-5 cycles of breeding.

DISEASE RESISTANCE GENES

Creeping bentgrass is one of the most disease susceptible grasses maintained for turf purposes (Vargas, 1994). Currently, maintenance of bentgrass requires extensive use of fungicides. Golf courses throughout the country are under pressure to reduce their inputs of fungicides. The production of transgenic creeping bentgrass cultivars with enhanced disease resistance should help in reducing dependence on chemicals with potentially adverse environmental impacts.

We are working with three potential disease resistance genes which have been shown by other Rutgers faculty (bacterio-opsin, Eric Lam; pokeweed antiviral protein, Nilgun Tumer; and delta-9 desaturase, Chee-Kok Chin) to confer striking fungal resistance when transformed into other plant species (Mittler et al., 1995; Hur et al., 1995). The patents on the use of these genes will be owned by Rutgers. The use of the delta-9 desaturase gene has been licensed exclusively to Mycogen. We are also working with glucose oxidase, another potential disease resistance gene (Wu et al., 1995). During the past two years we have produced multiple independent transgenic lines of creeping bentgrass for each of these genes. We have confirmed by reverse transcriptase-PCR that the plants are producing messenger RNA for the new genes. In the case of pokeweed antiviral protein, we have confirmed production of the protein by immunoblot analysis.

In July 1997 a randomized, replicated field plot of the transgenics, along with nontransgenic controls was established at Rutgers' Hort Farm II. The plants are being maintained as mowed spaced plants, in a backgrouond of fine fescue, so they can be evaluated under normal use conditions. The plot includes six independent transgenic lines each of PAP and bacterio-opsin, five independent lines of glucose oxidase, and 22 nontransgenic controls, each started from a single seed. Our delta-9 desaturase plants were not ready for field testing this summer, but can be tested next summer.

Our preliminary evaluations from this test are encouraging. The plot has been rated three times for presence of dollar spot. The ratings were done by Stacy Bonos, a graduate student with Bill Meyer. Disease in the plot is from natural infection. Table 1 presents a summary of the ratings. The ratings are the averages of three individuals for each transgenic line.

The control value is the average of the 22 individual nontransgenic plants. Overall, the transgenics are exhibiting considerably less disease incidence than the controls. Representative plants from the test are shown in Fig. 1. After production of the transgenic bacterio-opsin plants, we discovered there is a point mutation in our construct so we cannot be sure these plants are producing the proper protein. We have new bombardments with a new construct in progress.

From our work so far, the transgenic approach to improving the disease resistance of creeping bentgrass looks promising.

DROUGHT AND SALINITY TOLERANCE GENES

The maintenance of creeping bentgrass typically requires extensive irrigation. Golf courses throughout the country, however, are under pressure to reduce the amount of water used for irrigation. The development of creeping bentgrass cultivars which require less irrigation, or which could tolerate irrigation with brackish water, would be of great benefit. The protection of plants from damage incurred by drought stress is being explored in many laboratories and some successful strategies have been reported. One strategy that has shown promising results is the engineering of transgenic plants to accumulate osmotically active metabolites (Bohnert and Jensen, 1990). Several genes have been identified which do confer drought or salt tolerance when transformed into other plant species. Since both salt and drought result in cellular osmotic stress, genes which confer salt tolerance are also candidates for conferring drought tolerance and *vice versa*. We have obtained a number of such genes from other researchers.

In collaboration with Dr. John Chen, we are working with a potential salt tolerance gene, betaine aldehyde dehydrogenase (BADH) from *Atriplex hortensis,* a salt-tolerant plant. The natural salt tolerance of *A. hortensis* is attributable to the accumulation of the protective osmolyte glycine betaine which is synthesized by betaine aldehyde dehydrogenase. The *A. hortensis* BADH gene has been cloned and transformed into rice (Guo et al., 1997). The resulting transgenic rice plants could survive the salt stress of 0.5% NaCl.

We have obtained the *A. hortensis* BADH gene, constructed a bentgrass expression vector, and performed bombardments. We now have three independent transgenic lines containing the BADH gene which are ready to be tested for drought and salinity tolerance.

SUMMARY

In summary, we have made considerable progress during the past two years of USGA support for this project. We now have herbicide resistant plants in the breeding program for cultivar development. We have produced a number of transgenic lines containing several potential disease resistance genes. Preliminary results from our field test are very encouraging regarding efficacy of the genes. Our work with potential abiotic stress genes is at an earlier stage than our work with the potential disease resistance genes. We currently have transgenic plants containing the salt tolerance gene betaine aldehyde dehydrogenase.

Table 1. Dollar spot ratings of transgenic creeping bentgrass clones in a field trial established at 1996 in North Brunswick, NJ.

Clonal line	Gene	Dollar Spot Oct 9 97	Dollar Spot Oct 14 97	Dollar Spot Oct 21 97	Dollar Spot Ave
		--------------------Rating (1-9 scale)†			------------------
11032	PR5K	9.0	9.0	9.0	9.0
10907	PAP	9.0	9.0	8.7	8.9
9963	GO	9.0	8.7	8.3	8.7
10083	BO	8.7	8.7	8.7	8.7
10831	PAP	9.0	8.7	8.0	8.6
9776	GO	8.7	8.3	8.7	8.6
10099	GO	9.0	8.0	7.3	8.1
10049	BO	8.7	7.3	7.7	7.9
10017	PAP	7.7	7.0	7.7	7.5
10020	PAP	8.7	7.0	6.7	7.4
10912	PAP	7.7	7.3	7.0	7.3
10009	GO	7.7	6.3	6.0	6.7
10329	BO	7.3	6.3	6.3	6.7
10144	BO	7.0	6.3	6.0	6.4
10710	GO	5.3	5.3	6.0	5.5
10066	BO	5.7	4.7	4.7	5.0
Control		4.7	4.0	4.0	4.2
$LSD_{0.01}$		2.9	3.6	3.8	3.3
CV, %		26	36	38	32

† Dollar spot disease ratings based on a 1-9 scale, (9 = No spots, very few leaf lesions, 1= large spots).

FIGURE 1. Representative plants from the field trial. The creeping bentgrass plants are circled in red. They are surrounded by a cover of fine fescue.

CONTROL PAP (10,907) GLUCOSE OXIDASE (9963)