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DEVELOPMENT OF IMPROVED BENTGRASS CULTIVARS WITH HERBICIDE RESISTANCE, ENHANCED DISEASE RESISTANCE AND ABIOTIC STRESS TOLERANCE THROUGH BIOTECHNOLOGY

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Executive Summary

The goals of this project are to produce improved creeping bentgrass cultivars through a combination of genetic engineering and breeding. Our aim is to provide golf course managers with more effective and selective weed control with herbicides by developing herbicide-resistant cultivars. We are also attempting to produce cultivars with improved disease resistance and abiotic stress tolerance which can be maintained in a more environmentally sound and cost-effective manner.

The effectiveness of genetically engineered herbicide resistance in creeping bentgrass has been demonstrated in multiple field tests. This trait is now ready to be incorporated into a commercial cultivar.

We currently have a total of fifty independent transgenic lines of creeping bentgrass expressing one of five potential disease resistance genes. We have established randomized replicated field trials of these plants which will be evaluated in the summer of 1999.

We also have transgenic plants from bombardments with HVA1, a potential drought and salinity tolerance gene. Plants found to be expressing HVA1 will be screened for effectiveness of the gene.

INTRODUCTION

The goals of this project are to produce improved creeping bentgrass cultivars through a combination of genetic engineering and breeding. Our aim is to provide golf course managers with more effective and selective weed control with herbicides by developing herbicide-resistant cultivars. We are also attempting to produce cultivars with improved disease resistance and abiotic stress tolerance which can be maintained in a more environmentally sound and cost-effective manner.

HERBICIDE RESISTANCE

Herbicide resistance is a trait which would be very useful in a commercial creeping bentgrass cultivar. Such a cultivar would give golf course superintendents a safe and effective means of controlling *Poa annua* on greens.

The effectiveness of genetically engineered herbicide resistance in creeping bentgrass has been demonstrated in multiple field tests. This trait is now ready to be incorporated into a commercial cultivar. Rutgers is close to an agreement with a patent holder on proceeding with this. We are planning to carry out crosses with Dr. Bill Meyer's best germplasm next spring.

DISEASE RESISTANCE GENES

The main characteristic we have been concentrating on during the past year is disease resistance. 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. 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 to confer striking fungal resistance when transformed into other plant species (Mittler et al., 1995; Hur et al., 1995, Wu et al., 1995). Bacterio-opsin (BO) and pokeweed antiviral protein (PAP) were developed by Eric Lam and Nilgun Tumer, respectively, other Rutgers faculty. Glucose oxidase (G O) was developed by scientists at Monsanto.

In the summer of 1997 a randomized, replicated field plot of the transgenics, along with nontransgenic controls, was established at Rutgers Horticulture Farm II. The preliminary ratings of disease obtained in the fall 1997 were encouraging. Disease in the field was from natural infection. At that time, a number of the transgenics had high ratings, compared to the nontransgenic controls.

In the spring of 1998, another randomized, replicated field test was established containing some of the same transgenic lines along with some new lines. The plot consisted of three replicates each of the following number of independent transgenic lines:

BO	3
PAP	6
GO	12
Gene A	8
Gene B	1

Genes A and B are not identified since if they are found to be promising, we may want to patent their use in transgenic plants.

Our intention was to inoculate the field with dollar spot fungus and rate the plants throughout the summer. The field was inoculated with the fungus in late June. Unfortunately, shortly after the inoculation, the field was accidentally sprayed with a toxic combination of herbicides. Many of the plants suffered severe injury from that spray. To minimize the risk that they would die from the combined effects of herbicide damage and disease, the field was then sprayed with a fungicide. In fact, some of the plants in the field never recovered from the effects of the herbicide spray and were lost. Since the plants were sprayed with a fungicide we were unable to obtain disease ratings during the peak disease season.

Later in the season the effects of the fungicide wore off and disease appeared in the field. The plot was rated four times between Sept. 22 and October 26, 1998, for the presence of dollar spot. The field was not reinoculated with the fungus so disease is from natural infection. The ratings were done by Stacy Bonos, a graduate student with Dr. Bill Meyer. A summary of those ratings is shown in Table 1.

This year the controls were not as severely diseased as last year and many of the transgenics have lower ratings than the controls. The stress effects of the toxic herbicide spray probably invalidate any comparisons. We do not feel we can draw any conclusions from the ratings obtained this year. Because of the problems encountered this summer we feel an additional year of evaluation should be carried out before coming to a decision on the usefulness of these genes. The field will be sprayed shortly with fungicide to allow the plants to recover. Next year we will proceed with the plan of inoculating and rating throughout the summer.

Since most of the plants in that field were injured from the herbicide spray, which may confound the evaluation, we decided to start a new field plot which contains the same transgenic lines with the addition of some new lines. This field was established September 21, 1998. Figure 1 is a photograph of the plot shortly after planting. This field is a randomized replicated plot consisting of the following independent transgenic lines:

BO	11
PAP	10
GO	9
Gene B	12

Both fields will be inoculated and evaluated in the summer of 1999. We expect that after the coming year we should be able to ascertain which, if any, of the transgenes are effective in conferring disease resistance.

STRESS TOLERANCE

In addition to herbicide resistance and disease resistance, we are also interested in approaches which may improve the abiotic stress tolerance of creeping bentgrass, in particular drought and salinity tolerance.

Dr. David Ho and colleagues have found that a barley protein, which they call HVA1, accumulates in developing barley seeds and in barley seedlings subjected to drought and salt stress (Hong et al., 1992). They found that when the HVA1 gene was expressed constitutively in transgenic rice, the plants were significantly protected from both drought and salt stress (Xu et al., 1996).

During the past year we constructed a monocot expression vector for HVA1 and carried out bombardments on bentgrass callus. We now have a number of transgenic plants from those bombardments. We are starting to analyze those plants for the presence of the HVA1 gene. Positive plants will be prepared for field testing next spring.

SUMMARY

During the past year we have focused on generating multiple independent transgenic lines of creeping bentgrass containing potential disease resistance genes. We have randomized replicated field plots established for evaluation of the transgenics. After the coming year's evaluations we should be able to decide if any of the transgenes are promising enough to warrant incorporation into the breeding program.

We are now focusing on generating new transgenic plants containing potential drought tolerance genes. We do have some plants in the greenhouse which will be prepared for field testing in the spring.

Figure 1. Field trial of creeping bentgrass transgenics containing potential disease resistance genes. This field was planted in September 1998 and will be evaluated for disease throughout the summer of 1999.

Table 1. Dollar spot resistance ratings of transgenic bentgrasses during the fall of 1998.

Gene	Clone	Rating*
Gene A	13217	8.4
Gene A	13285	8.3
L-93	Control	7.6
Gene A	13314	7.3
Gene A	13270	7.2
PAP	10907	7.1
Crenshaw	Control	7.0
Gene A	13316	6.8
Gene B	11032	6.5
GO	11615	6.4
Gene A	13262	6.4
GO	11725	6.2
Gene A	13267	6.2
BO	13243	6.0
PAP	10912	5.9
Gene A	13315	5.8
GO	11139	5.8
GO	10009	5.7
GO	10592	5.6
GO	9776	5.5
GO	12426	5.3
PAP	10831	5.3
GO	12401	5.1
GO	10099	4.8
GO	9963	4.8
GO	10710	4.7
GO	10649	4.3
PAP	10020	3.5
PAP	10017	2.7

* Ratings based on a 1-9 scale with 9 = least disease