NOVEMBER 1996
DEVELOPMENT OF IMPROVED TURFGRASS WITH HERBICIDE RESISTANCE AND ENHANCED DISEASE RESISTANCE THROUGH TRANSFORMATION

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 year we have accomplished several major goals.

As the first step in cultivar development of herbicide resistant creeping bentgrass, bialaphos-resistant progeny from crosses of original transformants and nontransformed plants have been planted in the field. These plants will be maintained as mowed spaced plants. Next spring and summer the Rutgers bentgrass breeder, Dr. William Meyer, can select those individuals with good overall turf qualities for incorporation into his breeding program. Progeny from crosses using new sources of nontransgenic plants are currently being screened for herbicide resistance and resistant plants will be placed in the field next spring.

We have made improvements in the efficiency of transformation by particle gun bombardment and have obtained plants transformed with the bacterio-opsin gene. In other species, this gene has conferred good broad spectrum disease resistance. Next spring these plants will be field tested to evaluate the effect of the transgene. We have experiments in progress on other disease resistance and abiotic stress tolerance genes.

Percentage of time devoted to the research program:

Faith Belanger	100%
Peter Day	10%

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. Here we report our progress over the past year towards the achievement of these goals.

I. Herbicide Resistance

A. Transfer of herbicide resistant plants to the Rutgers breeding program

During the past year we have begun transferring our bialaphos-resistant plants to Dr. William Meyer for incorporation into his bentgrass breeding program. Although AgrEvo has not yet made a decision regarding release of bialaphos-resistant creeping bentgrass, we consider it worthwhile to invest some effort towards cultivar development.

Over 250 progeny from the 1995 crosses are now in the field at Rutgers Horticulture Farm II (Fig. 1). These plants will be maintained as mowed spaced plants to allow evaluation under normal maintenance conditions. The planting was done, and maintenance of the plot will be carried out by Mr. Bill Dickson in consultation with Dr. Bill Meyer. Next spring and summer Dr. Meyer can select those progeny which have superior characteristics for use in crosses aimed at production of a commercially useful cultivar.

This past spring we carried out additional crosses of bialaphos-resistant plants with nontransgenic plants in the containment greenhouse. Viable seed were obtained from both the bagged crosses and from open pollination. We are beginning to screen seedlings from the crosses for herbicide resistance. Those progeny which are herbicide resistant will be planted in the field next spring. Since new nontransgenic germplasm was used in the 1996 crosses, these progeny will be a source of additional diversity for the breeding program.

B. Field test of fungicidal properties of Finale^{TM}

Uchimiya et al. (1993) reported that bialaphos could be used as a fungicide to prevent *Rhizoctonia solani* infection of transgenic bialaphos-resistant rice. In 1995 we established turf plots of three bialaphos-resistant creeping bentgrass clones in order to test whether use of the herbicide Finale^{TM} would also prevent fungal disease development. If a single chemical could be used for both weed control and fungal disease control, the commercial value of the transgenic plants should be increased.

Dr. Bruce Clarke designed an experimental protocol which would reflect the normal frequency of herbicide treatment used by a golfcourse superintendent. The plots were divided into four treatments which are described in the plot plan in Table I.

Ratings early in the season were suggestive disease suppression in the sprayed sectors. Because of the severe weed problem in the unsprayed sectors, it became impossible to do any comparisons of disease symptoms between the sprayed and unsprayed areas. However, by the end of the summer the plots which received the three herbicide sprays were clearly denser and in better condition than the others (Fig. 2). This may be due simply to the weed control provided by the herbicide. The Southshore and Cobra transgenic clones are weaker than the Emerald clone and were more susceptible to weed encroachment. We plan to repeat this field test next summer.

II. Disease Resistance and Abiotic Stress Tolerance

A. Improvement in transformation efficiency

Significant improvements have been made in four steps of the transformation protocol: 1) preparation of tissue for bombardment, 2) bombardment, 3) selection, and 4) regeneration. We expect these improvements to result in a major increase in transformation efficiency. Our most recent experiments incorporating all four improvements are not yet at the regeneration step. We are expecting improved efficiency in obtaining transformants from these experiments.

B. Bacterio-opsin transformants

In an experiment incorporating three of the four improvements discussed above, we have obtained transformed plants which carry the *bar* gene for bialaphos resistance and the bacterio-opsin gene. Fig. 3 is a photo of the PCR analysis confirming the transformation of four individuals. Bacterio-opsin is a proton pump protein from the bacterium *Halobacterium halobium*. Dr. Eric Lam of the AgBiotech Center discovered that tobacco and *Arabidopsis* transformed with the bacterio-opsin gene exhibit broad spectrum resistance to fungal and bacterial pathogens (Mittler et al., 1995). Rutgers has applied for patent protection on the use of bacterio-opsin for disease resistance.

C. Other genes of interest

We have constructed expression vectors and have experiments in progress for other potential disease resistance genes: 1) pokeweed antiviral protein (Hur et al., 1995), 2) PR5K receptor protein kinase (Wang et al., 1996), and 3) glucose oxidase (Wu et al., 1995). All three of these genes have shown good disease resistance against fungal pathogens in other species. Rutgers will have the rights to the first two.

We are constructing new expression vectors for mannitol 1-phosphate dehydrogenase (Tarczynski et al., 1993) and myo-inositol O-methyl transferase (Vernon et al., 1993), potential abiotic stress tolerance genes.

D. Use of elite genotypes for callus initiation

We have been using callus derived from germinating seeds for transformations. Because of the heterogeneity of bentgrass cultivars, this means we do not know the genotype of resulting transformed plants. Based on the suggestion of Dr. Kenna and on the report by Yamamoto and Engelke (1996), we have started callus initiation from stolon nodes of superior plants. Dr. Richard Hurley of Lofts Seeds, Inc., has given us eight bentgrass plants which he has identified in the field as having overall superior characteristics. Callus has begun forming from these plants. When we have enough callus we will

begin using this as our tissue source for transformation. As Dr. Bill Meyer identifies superior plants from his breeding program, we will use them as additional sources for callus tissue.

We expect this approach to be a big advantage in eventual cultivar development. Although the potential for somaclonal variation will exist, the variation due to mixed genotypes in a cultivar will be eliminated. In general, the genotype of transformed plants will be of higher overall quality and fewer cycles of breeding should be required to produce a commercially useful cultivar. This approach will also facilitate rigorous evaluation of the effect of the transgene since genotypically identical nontransformed plants will be available for comparison.

III. Summary

During the past year we have made significant progress towards the development of improved creeping bentgrass cultivars. We have obtained transformed plants carrying the bacterio-opsin gene. During the coming fall and winter we expect to obtain plants transformed with other disease resistance and abiotic stress tolerance genes. In the spring of 1997, transformants will be put in the field to evaluate their performance. We expect by next fall to have some preliminary analysis of the effectiveness of the genes described above.

IV. References

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Figure 1. Bialaphos resistant progeny from the 1995 crosses.
A. Photo taken shortly after planting. The field was over-seeded with fine fescue to facilitate mowing.
B. Photo taken following the first mowing.

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TABLE I

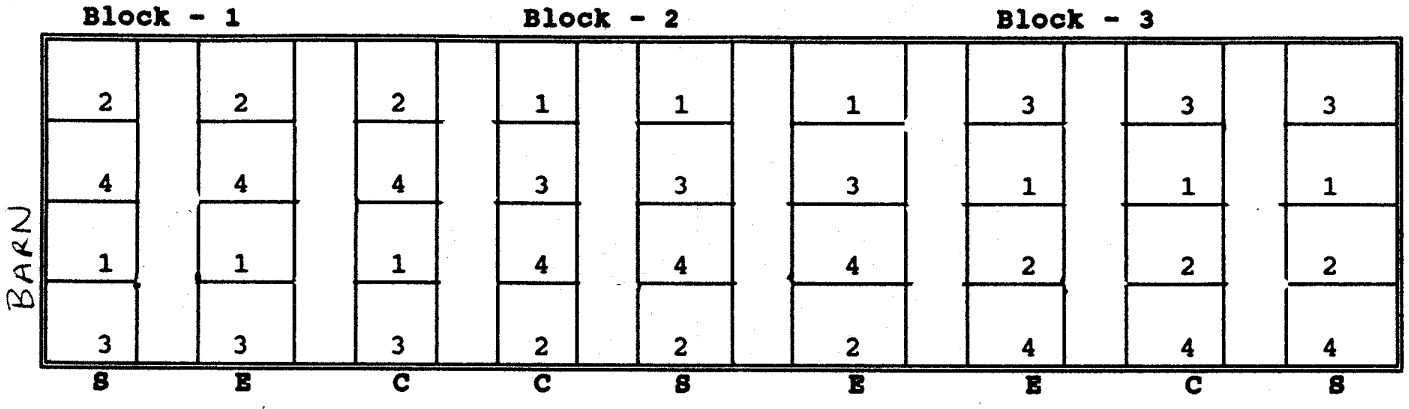
Bialaphos Resistant Bentgrass : Disease Field Study - 1996.

B. B. Clarke, F. Belanger and B. Dickson
Hort. Farm - II, North Brunswick, New Jersey

I. **Treatments :**

1. Bialaphos @ 1 X label rate once in late May
2. Bialaphos @ 1 X label rate in late May then repeat July 15 and August 30, 1996.
3. Unsprayed / Hand Weeded Check
4. Unsprayed / Nonweeded Check

II.



II. Bentgrass Variety Key :
S= Southshore
E= Emerald
C= Cobra