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

Investigators: Faith C. Belanger and Peter Day

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.

Through the Rutgers-Scotts-Monsanto partnership the development of a herbicide resistant commercial cultivar is actively underway.

The 2000 field test of creeping bentgrass transformants expressing potential disease resistance genes was successful. Some transgenic lines expressing the bacterio-opsin gene or the PR5K gene had delays of 2-6 weeks in development of dollar spot symptoms. These results are encouraging regarding the potential of biotechnology to contribute to development of improved cultivars of creeping bentgrass.

We have established a field test of transgenic plants expressing the barley HVA1 gene, a potential drought and salinity tolerance gene. The plants will be evaluated during the summer of 2001 for drought resistance.

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

Through the Rutgers/Scotts/Monsanto partnership development of a glyphosate resistant cultivar of creeping bentgrass is currently underway.

DISEASE RESISTANCE

We are currently working with four potential disease resistance genes developed by Rutgers faculty. Bacterio-opsin (BO) was developed by Eric Lam (Mittler et al., 1995). Pokeweed antiviral protein (PAP) and pokeweed antiviral protein II (PAPII) were developed by Nilgun Tumer (Hur et al., 1995; Wang et al 1998). PR5K was developed by Michael Lawton (Wang et al., 1996).

A field test of the transgenics was established in October 1999 using a new design developed by Dr. Bill Meyer. In this design a killed turf is used as the surface for tiller plots. Our plants were plugged into a dead turf surface which maintains a cover over the soil and a barrier for weeds. The test was established as a randomized complete block design with three replicates of each transgenic line and forty-two control plants. The plants established well and the field test this summer was successful. Below is a view of the field taken in August 2000.

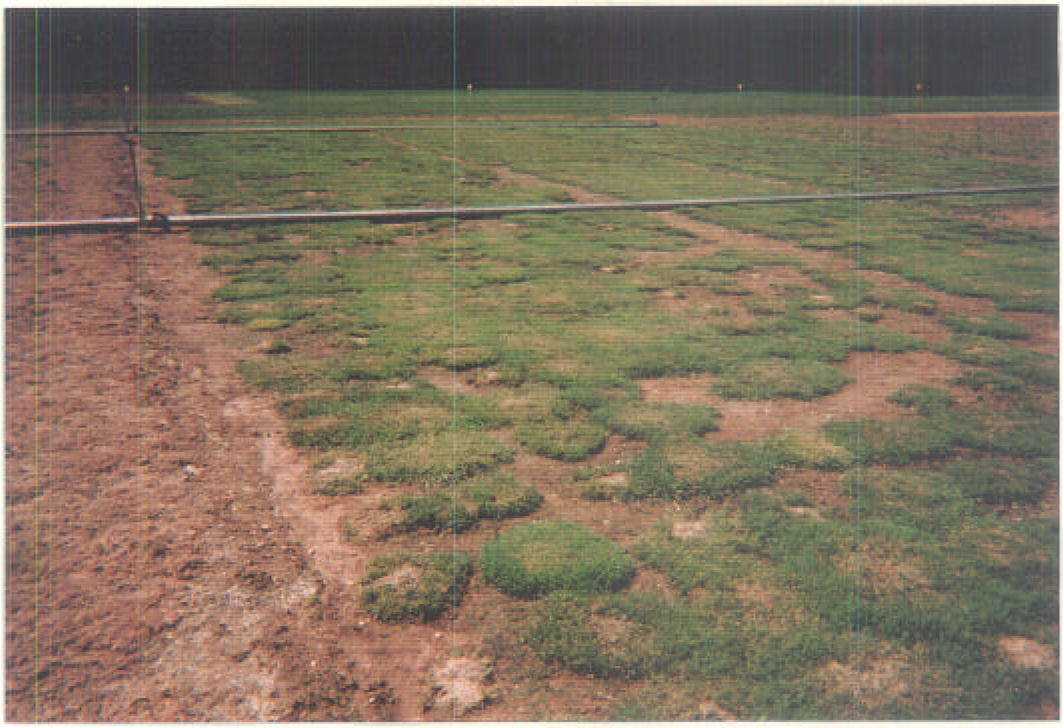


Figure 1. The 2000 disease resistance field test.

On June 26, the field was inoculated with the dollar spot fungus. Ratings for disease were started on July 11 and were repeated at almost weekly intervals through August 31. Some of the transgenic lines showed a delay of 2-6 weeks in disease symptom development. These results are encouraging regarding the potential of genetic engineering to improve disease resistance in creeping bentgrass.

Bacterio-opsin

Bacterio-opsin is a proton pump protein from the bacterium Halobacterium halobium. Expression of bacterio-opsin in tobacco and potato was reported to confer resistance to bacterial and fungal pathogens (Mittler et al., 1995; Abad et al., 1997).

Four of the transgenic creeping bentgrass lines showed a 4-6 week delay in dollar spot symptom development. Graphs of the ratings and standard deviations from July 11 to August 10 are shown in Fig. 2. A rating of 9 indicates no disease. After the August 10 rating, some of the transgenic replicates began to die. We don't know if this can be attributed to a direct effect of the bacterio-opsin expression. In tobacco, however, bacterio-opsin expression does cause lesion development due to the induced hypersensitive response (Mittler et al., 1995).

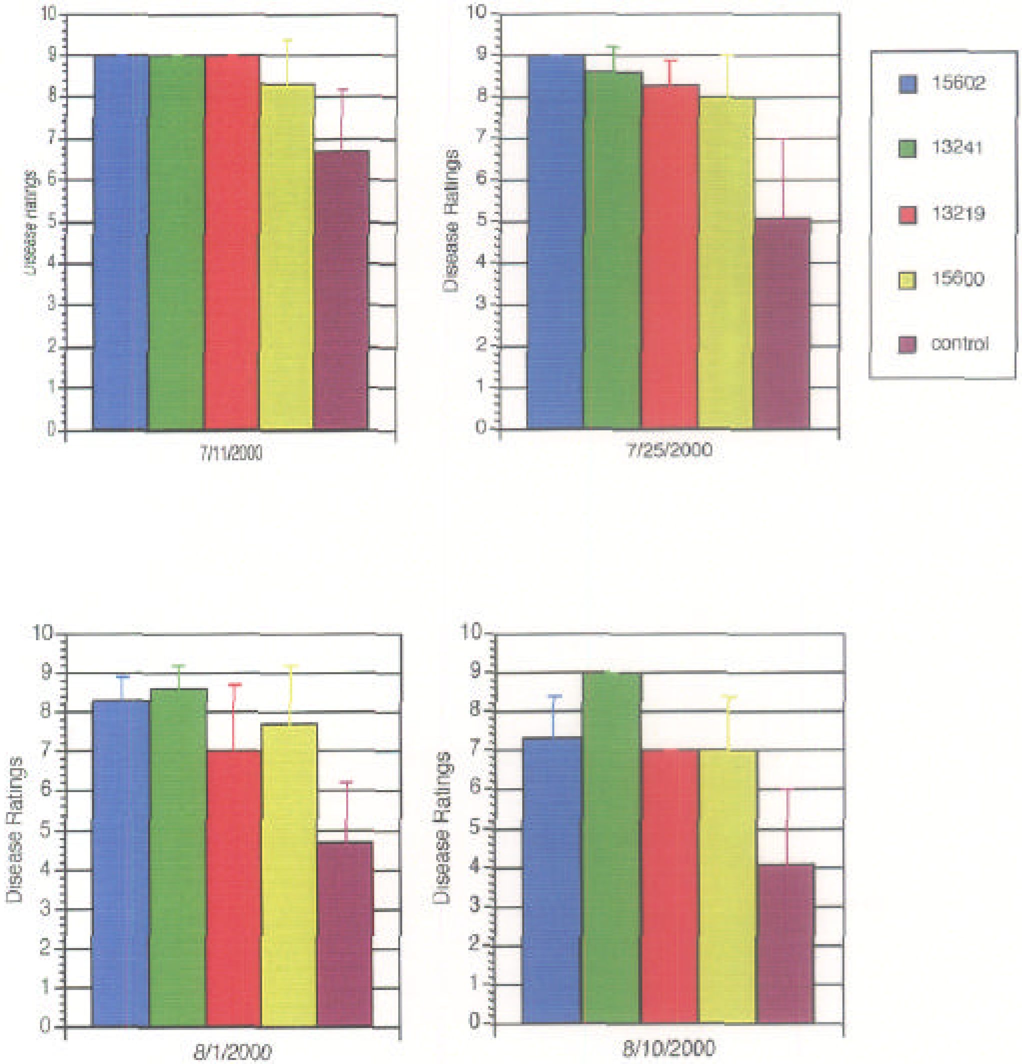


Figure 2. Disease ratings and standard deviations of BO transgenics and controls from July 11th to August 10th

PR5K

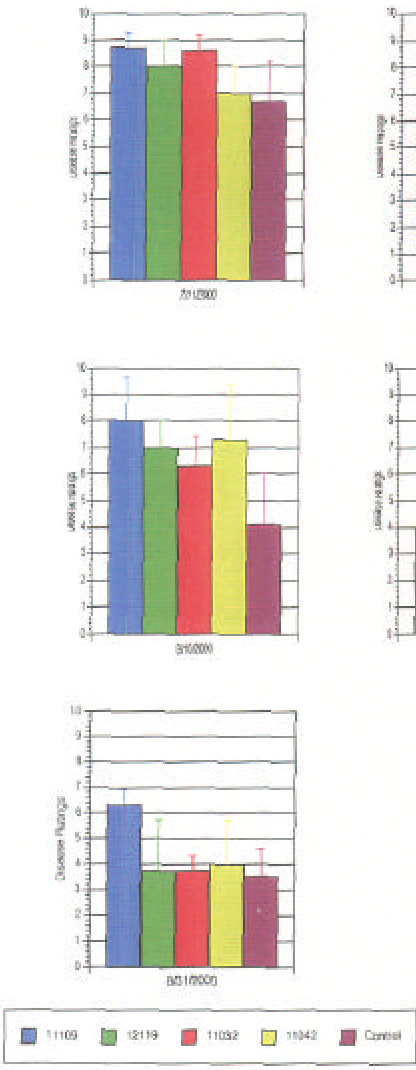
PR5K is a receptor protein kinase from *Arabidopsis thaliana*. It has an extracellular domain with similarity to the antimicrobial PR5 proteins and an intracellular kinase domain (Wang et al., 1996). Four of the transgenic creeping bentgrass lines showed a 5-6 week delay in disease symptom development. Graphs of the ratings at the different dates are shown in Fig. 3. After the Aug 10 rating, the lines began to exhibit disease symptoms similar to the controls. The PR5K transgenics did not die like many of the bacterio-opsin transgenics, but rather persisted throughout the season.

PAP and PAPII

The pokeweed antiviral protein (PAP) is a ribosome inactivating protein from the plant *Phytolacca americana*. Expression of the wild type form of PAP in transgenic tobacco was toxic to the plants (Lodge et al., 1993). A C-terminal deletion of the PAP coding sequence has reduced plant toxicity when expressed in transgenic plants, yet maintains the antiviral activity and also confers protection against the fungal pathogen *Rhizoctonia solani* (Zoubenko et al., 1997). Like PAP, PAPII is a ribosome inactivating protein from the pokeweed plant but its protein sequence is only 41% identical to that of PAP. The wild type form of PAPII has less toxicity when expressed in tobacco than does the wild type form of PAP and it also showed resistance to *Rhizoctonia solani* (Wang et al., 1998).

Our field test included transgenic creeping bentgrass plants expressing the C-terminal deletion form of PAP and the wild type form of PAPII. Some of the individual PAP and PAPII transgenic plants had high ratings. There were too many fatalities of the replicates, however, to obtain reliable data for those transgenic lines. The large number of fatalities may have been due to toxicity of the proteins. Our data does suggest, however, that a ribosome-inactivating protein may confer disease resistance in creeping bentgrass. We are therefore planning to test another PAP variant recently generated by Dr. Nilgun Tumer. This variant is a point mutation, which has lost all toxicity when expressed in tobacco yet, retains its antiviral activity. We would like to try this variant in creeping bentgrass since the other PAP forms with antiviral activity also had antifungal activity. As the first step in generating transgenic plants, we will soon begin construction of the monocot expression vector for the new PAP variant.

Figure 3. Disease Ratings And Standard Deviations Of PR5K Transgenics And Controls From July 11th To August 31st.



Delta-9-Desaturase

Delta-9-desaturase is a yeast gene being developed for disease resistance by Chee-Kok Chin at Rutgers. Expression of this gene in transgenic tomato has shown dramatic disease resistance (Wang et al., 1998). Expression of this gene results in increased levels of unsaturated fatty acids which either directly or indirectly enhance the disease resistance of the plants. We have produced transgenic plants containing the delta-9-desaturase gene which produce messenger RNA for the gene. From feeding studies conducted in Dr. Chin's lab, however, there was no production of the unsaturated fatty acids in the creeping bentgrass transgenics. One possible reason for this is the plants may not be producing adequate levels of the delta-9-desaturase protein.

We are investigating the possibility that the codon usage in the yeast gene is suboptimal for adequate protein production in a monocot. To do this Dr. Zhenfei Guo has constructed a synthetic gene which codes for an identical protein but utilizes the monocot-preferred codons in the DNA sequence. We now have small transgenic plants from bombardments with the delta-9-desaturase synthetic gene. We will soon begin analyzing the plants for alterations in the fatty acid levels.

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).

We have a number of creeping bentgrass transgenic lines expressing the barley HVA1 gene. In July 2000 a field test of these plants was established. The plants are now well established. The field will not be irrigated and will be evaluated for drought resistance during the summer of 2001. A view of the field test is shown below in Figure 4.

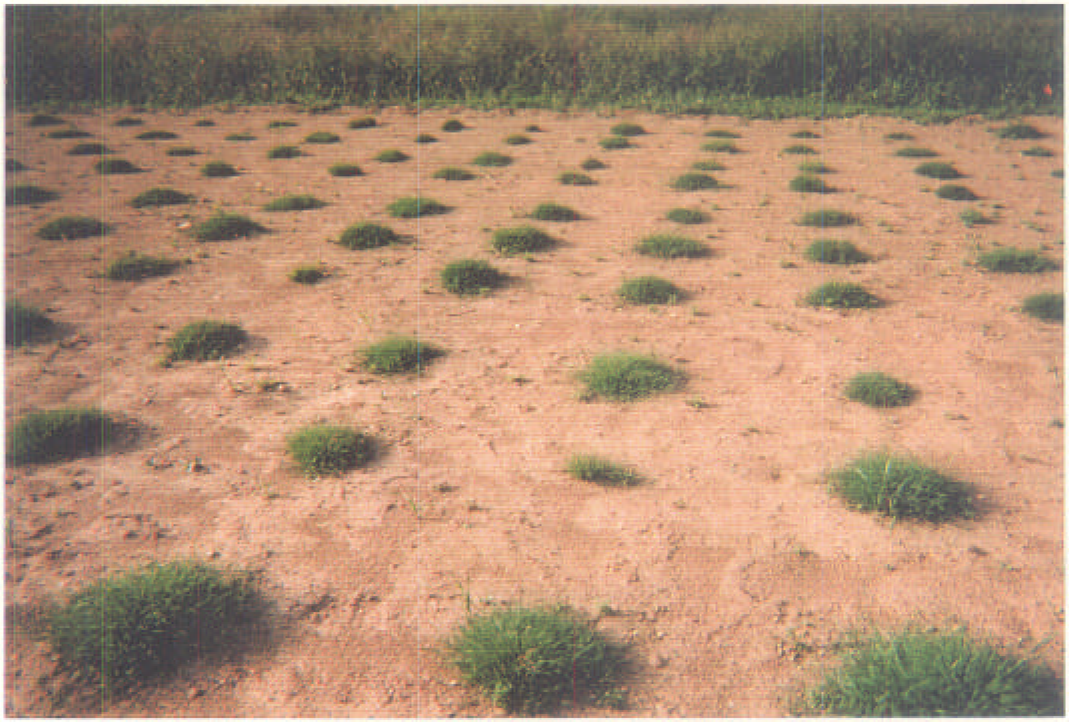


Figure 4. View of the field containing transgenic plants expressing the barley HVA1 gene.

SUMMARY

Our field test of creeping bentgrass transformants expressing some potential disease resistance genes was successful. The results of the field test indicate that genetic engineering can improve the disease resistance of creeping bentgrass. Some of the bacterio-opsin and PR5K transgenic lines had delays of 2-6 weeks is disease symptom development. The pokeweed antiviral proteins PAP (C-terminal deletion) and PAPII appeared to have some toxicity. The ratings on the surviving plants, however, are suggestive that ribosome-inactivating proteins may confer disease resistance. We will be generating additional transformants with a new variant of PAP which has no toxicity. We have also established a field test of the potential drought resistance gene HVA1 which will be evaluated during the upcoming summer.

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