

1996 RUTGERS Turfgrass Proceedings



THE NEW JERSEY TURFGRASS ASSOCIATION

In Cooperation With

RUTGERS COOPERATIVE EXTENSION
NEW JERSEY AGRICULTURAL EXPERIMENT STATION
RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
NEW BRUNSWICK

Distributed in cooperation with U.S. Department of Agriculture in furtherance of the Acts of Congress of May 8 and June 30, 1914. Cooperative Extension work in agriculture, home economics, and 4-H. Zane R. Helsel, Director of Extension. Rutgers Cooperative Extension provides information and educational services to all people without regard to sex, race, color, national origin, disability or handicap, or age. Rutgers Cooperative Extension is an Equal Opportunity Employer.

1996 RUTGERS TURFGRASS PROCEEDINGS

of the

**New Jersey Turfgrass Expo
December 10-12, 1996
Taj Mahal Casino-Resort
Atlantic City, New Jersey**

The Rutgers Turfgrass Proceedings is published yearly by the Rutgers Center for Turfgrass Science, Rutgers Cooperative Extension, and the New Jersey Agricultural Experiment Station, Cook College, Rutgers University in cooperation with the New Jersey Turfgrass Association. The purpose of this document is to provide a forum for the dissemination of information and the exchange of ideas and knowledge. The proceedings provide turfgrass managers, research scientists, extension specialists, and industry personnel with opportunities to communicate with co-workers. It also allows these professionals to reach a more general audience, which includes the public. Articles appearing in these proceedings are divided into two sections.

The first section includes lecture notes of papers presented at the 1996 New Jersey Turfgrass Expo. Publication of the New Jersey Turfgrass Expo Notes provides a readily available source of information covering a wide range of topics. The Expo Notes include technical and popular presentations of importance to the turfgrass industry.

The second section represents performance of turfgrass cultivars and selections in New Jersey turf trials. The primary objective of these papers is to facilitate the timely dissemination of original turfgrass research for use by the turfgrass industry.

Special thanks are given to those who have submitted papers for this proceedings, to the New Jersey Turfgrass Association for financial assistance, and to those individuals who have provided support to the Rutgers Turf Research Program at Cook College - Rutgers, The State University of New Jersey.

Dr. Ann B. Gould, Editor
Dr. Bruce B. Clarke, Coordinator

TURFGRASS BIOTECHNOLOGY

Dr. Faith C. Belanger, Cynthia L. Laramore, and Dr. Peter R. Day¹

Plant transformation technology offers the opportunity to introduce genes from unrelated organisms into a plant species in a manner that would not be possible using traditional breeding methods. Genes from organisms as diverse as viruses, bacteria, fungi, and higher plants are currently being identified which confer beneficial new traits when introduced into particular plant species. Plant transformation is thus a useful complement to conventional breeding in the development of new cultivars with improved disease and stress tolerance qualities.

We are using plant transformation to augment breeding efforts in the development of improved cultivars of creeping bentgrass (*Agrostis palustris*). Creeping bentgrass is an outstanding cool season turfgrass whose fine leaves, prostrate habit, and tolerance to low mowing have made it the principal species used for putting greens and fairways on the golf courses of temperate climates around the world. The excellent playing surfaces desired by golf course managers, however, require intensive turfgrass cultural practices for their maintenance. The extreme density of putting green and fairway turf requires the extensive use of fungicides for disease control. Creeping bentgrass also requires extensive irrigation.

The focus of our research is to produce transgenic creeping bentgrass plants that exhibit improved disease resistance and stress tolerance characteristics. We are working with several genes that have shown good results in other plant species. The selected transgenic individuals will be incorporated into the ongoing Rutgers bentgrass breeding program for eventual production of new and improved commercial cultivars. The production of transgenic creeping bentgrass cultivars that require fewer fungicide treatments should help to reduce dependence on chemicals with adverse environmental impacts. Cultivars with greater tolerance to drought or which could tolerate irrigation with brackish water will conserve precious water supplies.

We consider it wise to work with several genes at the same time. Our transformation system is efficient, so we can readily obtain transgenic plants containing the genes of interest. As new beneficial genes are identified in other plant species and microbes, we plan to incorporate them into turfgrasses in our program. The genes we are currently working with are described below.

1. Bacterio-opsin

Bacterio-opsin is a proton pump protein from the bacterium *Halobacterium halobium*. Mittler et al. (1995) reported that expression of bacterio-opsin in tobacco protected the plants from viral and bacterial pathogens. Transgenic plants expressing bacterio-opsin were able to block the replication of tobacco mosaic virus and the growth of the bacterial pathogen *Pseudomonas syringae* pv. *tabaci* (Mittler et al., 1995). The details of the mechanism of action of bacterio-opsin in conferring disease resistance are under investigation in Dr. Eric Lam's laboratory, AgBiotech Center, Rutgers University. He and his colleagues have found that expression of bacterio-opsin activates the plant's natural

¹ Assistant Research Professor, Laboratory Researcher IV, and Director, respectively, Center for Agricultural Molecular Biology, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903.

defense mechanisms resulting in expression of disease-suppressive proteins (Mittler et al., 1995). Such expression may thus be the mechanism of pathogen resistance.

We now have transgenic bentgrass plants which carry the *bar* gene for bialaphos (a herbicide) resistance and the bacterio-opsin gene. Over 80 transformed plants were recently transferred to soil and placed in the greenhouse. We now are ready to begin greenhouse and field tests to evaluate creeping bentgrasses, transformed with the bacterio-opsin gene, for their ability to resist turfgrass diseases.

2. Pokeweed Antiviral Protein

The poke weed antiviral protein (PAP) is a ribosome- inactivating protein from the plant *Phytolacca americana*. PAP expression in transgenic tobacco confers broad spectrum resistance to several plant viruses (Lodge et al., 1993). Expression of PAP in tobacco also shows dramatic protection against *Rhizoctonia solani*, a fungal pathogen (Zoubenko and Tumer, in preparation). PAP is thus another good candidate gene for inducing broad spectrum pathogen resistance in turf. We now have bentgrass plants containing the PAP gene and are ready to begin greenhouse and field tests of pathogen resistance.

3. Glucose Oxidase

Glucose oxidase is an active oxygen species-generating enzyme from the fungus *Aspergillus niger* (Frederick et al., 1990). It utilizes glucose and oxygen to produce gluconic acid and hydrogen peroxide. Wu et al. (1995) found that expression of glucose oxidase in potato resulted in resistance to the bacterial pathogen *Erwinia carotovora* subsp. *carotovora* and the fungal pathogen *Phytophthora infestans* (late blight of potato). Glucose oxidase is thus another good candidate gene for inducing broad spectrum pathogen resistance in turf. We now have bentgrass plants containing the glucose oxidase gene and are ready to begin greenhouse and field tests of pathogen resistance.

4. Other Disease Resistance Genes

We are also working with two additional genes that have been discovered by other Rutgers' faculty members to confer dramatic pathogen resistance in other plant species. We now have bentgrass plants transformed with one of these genes. For the other gene, we expect to have transformed plants soon.

5. Mannitol-1-Phosphate Dehydrogenase

Recently, we have been working with the mannitol-1-phosphate dehydrogenase (an enzyme) gene from *Escherichia coli*. In *E. coli*, the enzyme functions in the degradation of mannitol-1-phosphate to form fructose-6-phosphate (Tarczynski et al., 1992). When expressed in tobacco, however, the enzyme forms mannitol-1-phosphate, resulting in the accumulation of the sugar alcohol mannitol (Tarczynski et al., 1992). Accumulation of mannitol has been associated with protection of plant cells from drought stress. Dr. Hans Bohnert and co-workers have found that transgenic tobacco plants expressing the bacterial mannitol-1-phosphate dehydrogenase gene were tolerant to high concentrations (250 mM) of salt (Tarczynski et al., 1993). We obtained a mannitol-1-phosphate dehydrogenase clone from Dr. Bohnert and are currently attempting to

transform creeping bentgrass. We anticipate no difficulty in obtaining transgenic plants expressing mannitol dehydrogenase.

In summary, we feel that the Rutgers turf program offers a unique combination of biotechnology and plant breeding for the improvement of creeping bentgrass and other important turfgrasses. We currently have many transformed plants containing candidate genes for disease resistance. Moreover, we have the expertise to readily obtain transformants for other genes of interest. We are ready to begin evaluating the effectiveness of several genes and incorporating transformed plants into a breeding program for cultivar improvement.

Literature cited

Frederick, K. R., Tung, J., Emerick, R. S., Masiarz, F. R., Chamberlain, S. H., Vasavada, A., Rosenberg, S., Chakraborty, S., Schoptr, L. M., and Massey, V. 1990. Glucose oxidase from *Aspergillus niger* cloning, gene sequence, secretion from *Saccharomyces cerevisiae* and kinetic analysis of a yeast-derived enzyme. *J. Biol. Chem.* 265:3793-3802.

Lodge, J. K., Kaniewski, W. K., and Tumer, N. E. 1993. Broad-spectrum virus resistance in transgenic plants expressing pokeweed antiviral protein. *Proc. Natl. Acad. Sci. USA* 90:7089-7093.

Mittler, R., Shulaev, V., and Lam, E. 1995. Coordinated activation of programmed cell death and defense mechanisms in transgenic tobacco plants expressing a bacterial proton pump. *Plant Cell* 7:29-42.

Tarczynski, M. C., Jensen, R. G., and Bohnert, H. J. 1992. Expression of a bacterial mtID gene in transgenic tobacco leads to production and accumulation of mannitol. *Proc. Natl. Acad. Sci. USA* 89:2600-2604.

Tarczynski, M. C., Jensen, R. G., and Bohnert, H. J. 1993. Stress protection of transgenic tobacco by production of the osmolyte mannitol. *Science* 259:508-510.

Wu, G., Shortt, B. J., Lawrence, E. B., Levine, E. B., Fitzsimmons, K. C., and Shah, D. M. 1995. Disease resistance conferred by expression of a gene encoding H₂O₂-generating glucose oxidase in transgenic potato plants. *Plant Cell* 7:1357-1368.