Highly specialized equipment is used to shoot foreign DNA into bentgrass plant cells.

Herbicide-Resistant Creeping Bentgrass

by LISA LEE, CHRISTINA HARTMAN, CYNTHIA LARAMORE, NILGUN TUMER, and PETER DAY
Center for Agricultural Molecular Biology, Cook College, Rutgers, The State University of New Jersey, New Brunswick, New Jersey

HERBICIDE-RESISTANT creeping bentgrass, a product of laboratory experimentation, may become a useful tool for golf course superintendents in their daily encounters with weed problems in the future.

Golf course managers use a variety of cultural practices in maintaining golf course turf, including the use of pesticides. The potential for negative effects from such materials on golf courses is a concern through exposure to golf course superintendents and their staffs, the general public, and the environment. Production of turfgrasses that can use safe herbicides and that require fewer fungicide treatments may help to reduce these potential problems. Biotechnology allows the insertion of foreign genes into turfgrass, a process that can lead to the production of new cultivars that require less use of herbicides and fungicides.

Some herbicides used on golf courses are not environmentally friendly chemicals. However, the application of herbicide products to many golf course areas is required for the maintenance of excellent playing surfaces. At Rutgers AgBiotech Center, we are developing creeping bentgrasses that are resistant to a safer herbicide, glufosinate. The source of resistance is a fungal gene for resistance, *bar,* that has been shown to be effective in the transformation of both narrow- and broad-leaved plants.

This group of herbicides, glufosinate and its tripeptide bialaphos, have trade names such as Final,™ Ignite,™ Basta,™ or Herbiace.™

They inhibit the enzyme glutamine synthetase, causing rapid accumulation of ammonia and cell death. The *bar* gene encodes an enzyme (PAT) that inactivates the active ingredient of the herbicide PPT; thus, transformed plants that carry the *bar* gene are resistant. We have used two methods to transform creeping bentgrass — biolistic bombardment and protoplast transformation. In this article, we describe how transgenic creeping bentgrass with herbicide resistance was obtained and what the implications of our work are to golf course superintendents.

Creeping Bentgrass Tissue Culture and Regeneration

To transform creeping bentgrass, we first had to develop a tissue culture regeneration system. About 6 to 8 weeks after surface sterilized seeds were placed on callus initiation medium, embryogenic callus cultures (cell masses with embryos), were selected from germinating seedlings. These were established from seedlings of seven creeping bentgrass cultivars: Cobra, Emerald, Pennlinks, Providence, Putter, Southshore, and SR1020. Depending on the cultivars, between 5% and 30% of seeds can produce embryogenic callus cultures. Upon transfer to regeneration medium (MS medium without hormone), around 200-400 plants can be obtained from each gram (fresh weight) of callus. The callus cultures were used to establish suspension cultures by placing approximately 1-2 grams of callus into liquid medium in 250 ml flasks in the dark on a rotary shaker (125 rpm). By subculturing to fresh medium twice a week, suspension cultures with small cell clusters were established for transformation. Both embryogenic callus and suspension cultures were used as target tissues in transformation.

Biolistic Transformation

Biolistic transformation was carried out using a Bio-Rad PDS-1000/He Biolistic Delivery System. This is a device that uses a pulse of helium at high pressure to accelerate very small (1-3 μ) metal particles coated with transforming DNA to hit target plant cells placed in their path. Some particles enter the cell nuclei and in a small proportion of these, some of the DNA carried on the particles also enters the nucleus and becomes integrated on a plant chromosome. Target tissues, either suspension cells or callus cultures, were placed on sterile filter disks in dishes containing medium prior to bombardment and kept in the dark. Foreign DNA was constructed in a plant expression vector as a plasmid which will amplify in *E. coli* cells cultured in a broth medium to produce enough DNA for transformation. In the biolistic experiments, purified DNA was mixed with gold particles.

Herbicide was added to the tissue culture medium to select transformed cells from bombarded materials. Selection commenced 3-4 days after bombardment and continued for 8 weeks. The bombarded tissues were then transferred to regeneration medium. Regenerants appeared within 2-8 weeks. Shoots were transferred to Phytatrays™ (a presterilized clear polystyrene sundaecup-like vessel for plant culture) with regeneration medium, and roots appeared within 2-4 weeks. Plants were transplanted to soil and were tested for herbicide resistance in the greenhouse. It takes about 6 months from tissue bombardment to obtain plants in soil.

Protoplast Transformation

Another method used to insert desirable genes into plant cells is a process called protoplast transformation. In this process, the walls of four-day-old suspension culture cells were removed by enzyme digestion to release protoplasts that are able to take up DNA. Protoplasts were transformed with foreign DNA by polyethylene glycol (PEG)

Table 1

List of Transgenic Creeping Bentgrass Lines Produced by Particle Bombardment

Cultivar	Particle Bombardment Number	2.0 mg/ml Herbiace	Tissue* Clone	Phenotype
Emerald	1 14-3	3	EB.5	erect
	2 14-4	1	EB.5	erect
	3 14-18	14	EB.5	erect
	4 16-27	—		
	5 19-15	5	EBmm	creeping**
Southshore	1 16-24	4	SSB.2	petite
	2 19-25	8	SSB.2	petite
	3 19-28	20	SSB.2	petite

*Each clone derived from one seedling

**Creeping phenotype is equivalent to w.t. of two cultivars

Herbicide resistance of transgenic plants and controls at an application rate of 2 mg/ml Herbiace. Plants were photographed two weeks after herbicide application. Resistant plants remained green, while others and controls were killed.

treatment. PEG is used to enhance the uptake of DNAs. We have developed a system for culturing the protoplasts back to cells and subsequently to embryogenic callus cultures which then form plants by regeneration.

Transformed protoplasts were treated with herbicide 16 days after protoplast isolation, and resistance colonies of cells were detected about 3-4 weeks after selection. Plants were regenerated after transfer of resistant colonies to regeneration medium and, once rooted, were transferred to soil. It takes about 5-6 months from protoplast isolation to the production of transgenic plants in soil.

Greenhouse Herbicide Tests

All regenerants were treated with Herbiace. Rates were determined by applying different concentrations of Herbiace to control plants by painting with a brush. Herbicide at 2 mg/ml was used, as this led to plant death in susceptible hosts in all cases. The herbicide was applied at 120 ml/flat (24 plants/flat).

Creeping bentgrass clones resistant to Herbiace were obtained from three cultivars: Emerald, Southshore, and Cobra (Figure 4). Table 1 lists the herbicide-resistant creeping bentgrass lines produced from the biolistic bombardment experiment. In five experiments, involving 12 independent bombardment events, some 900 plants were regenerated for testing. Of these, 55 plants survived. The transformation frequency for herbicide-resistant plants ranged from 0% to 13.7%. Thirty Cobra plants were obtained in 3 later bombardment experiments.

Cobra transgenic plants were also obtained from protoplast transformation. A total of 153 plants were regenerated from 2 resistant colonies obtained through protoplast transformation. All these plants survived the 2 mg/ml spray rate. More than 200 transgenic plants of Emerald, Southshore, and Cobra survived 2 mg/ml herbicide spray in greenhouse tests and are resistant to 5× the field rate.

Field Test

We conducted the first field test of herbicide-resistant creeping bentgrass in the USA in the summer of 1994 (Figure 5) at Rutgers' Research and Development Center in Bridgeton, NJ. A field test permit was obtained from USDA-APHIS. Transgenic plants from bombardment experiments of Emerald, Putter, and Southshore were tested for resistance to the herbicide Ignite at 1× (0.75 lb AI/A) and 3× (2.25 lb AI/A) the label rate (1.5-4 fluid ounces per gallon of water).

All plants that survived the 2 mg/ml greenhouse test were completely resistant to both 1× and 3× the field rate (Figure 6) in the field test. They remained green and unaffected like untreated plants in the control plot. No control plants (Emerald, Putter, and Southshore plants from seeds) survived. More than 30 (3 tissue clones) Emerald and Southshore creeping bentgrass lines are resistant to 3× the field rate.

The resistant transgenic plants will be vernalized in the field and will be moved to a containment greenhouse next spring for pollination and seed production to determine the inheritance of herbicide resistance. Suitable resistant clones will be used as parents in a traditional breeding program before a resistant cultivar is made available commercially.

Implications

Ignite-resistant creeping bentgrass will be most useful in new golf course construction and for keeping unwanted species out of golf greens and fairways. Ignite will control undesirable grasses such as *Poa annua* at a very low rate but will not affect the transgenic bentgrass. The availability of a safe and biodegradable herbicide, such as Ignite, to deal with weed problems will aid superintendents and their staffs.

Our success in obtaining herbicide-resistant creeping bentgrass through transformation also provides us with a selection tool for introducing other agronomically important genes into turfgrass. We have inserted genes (such as chitinases) for resistance to fungi and are analyzing their expression in bentgrass plants. Transgenic turfgrass with enhanced disease resistance will require less use of fungicides. We believe herbicide-resistant and disease-resistant turfgrass cultivars will be available to the golf course industry in the near future.

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