Hybrid Bermudagrass Improvement by Genetic Transformation

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Objectives:

1. Develop and optimize tissue culture conditions in order to obtain embryogenic callus and regeneration of hybrid bermudagrass plantlets.
2. Develop a procedure to transform the embryogenic callus by the biolistic method (particle bombardment) and to recover transgenic plants.
3. Obtain transgenic plants of hybrid bermudagrass that express nematode-resistance genes.

Start Date: 1998
Project Duration: 5 years
Total Funding: $125,000

Bermudagrass is an important warm-season grass species for putting greens, tees and fairways of golf courses in the southern United States. The ultimate goal of the research direction is to improve bermudagrass cultivars for golf courses through biotechnology. The objective of this project is to improve tissue culture response of bermudagrass, to develop transformation procedures for bermudagrass, and to introduce a nematode-resistance gene into bermudagrass.

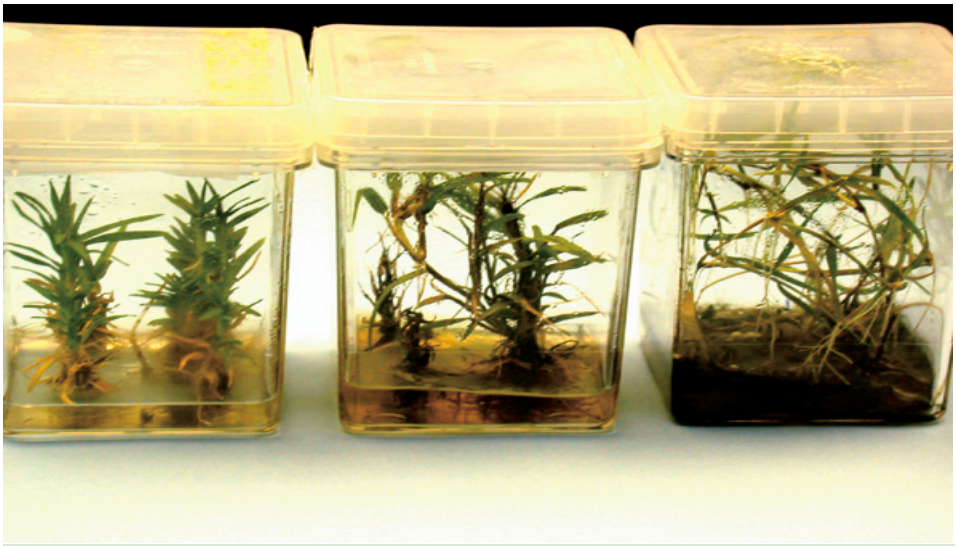
A successful tissue culture, especially the efficient regeneration of plants, is a prerequisite for genetic transformation of most plant species. Unfortunately, bermudagrass is a very recalcitrant species in tissue culture (i.e., it is difficult to obtain callus regeneration at high frequency). We have spent a great deal of efforts and have substantially improved callus regeneration of bermudagrass by adjusting the composition and levels of phytohormones in the culture medium.

Experiments completed in 2001-2002 yielded six highly regenerable callus lines from *Cynodon dactylon* cultivar J1224. The regeneration ability can last over more than a year. These callus lines provided ideal cell materials for subsequent transformation experiments. To longer preserve the callus lines, these lines were cryopreserved in liquid nitrogen. The cryopreserved callus lines were able to re-grow in culture medium. Some of them were still regenerable.

The biolistic method ("gene gun") was used to transform these highly regenerable callus lines. So far seven independent putative transgenic callus lines resistant to selection at 250 mg/L level of hygromycin B were obtained. Five lines regenerated into green plantlets, one formed albino plants and the other did not regenerate. The plantlets were resistant to 50 or 100 mg/L hyg B in rooting medium and some showed GUS activity. Variation in morphology of the putative transgenic plants was observed. Molecular analysis of the putative transgenic plants is underway.

Agrobacterium transformation of bermudagrass was also performed using the highly regenerable callus lines. Calli resistant to 5 to 7 mg/L bialaphos were obtained from 9 plates of a total of 150 inoculated plates. Calli from two such plates started to regenerate green plantlets or buds. Some others developed albino plantlets.

We are close to reaching a Material Transfer Agreement (MTA) with another university to obtain a nematode-resistance gene so we can introduce it into bermudagrass.



Three putative transgenic plants are growing in the rooting medium. Note the morphology variation among the plants.

Summary Points

- Bermudagrass is recalcitrant in callus regeneration in tissue culture, but we have made substantial improvements in the past four years.
- Six highly regenerable callus lines were developed using common bermudagrass (*Cynodon dactylon*) cultivar J1224. These lines were successfully cryopreserved.
- Using the highly regenerable callus lines and the biolistic method, seven independent putative transgenic callus lines resistant to hygromycin B (250 mg/L) were obtained, and five of them regenerated into green plantlets.
- Putative transgenic callus lines resistant to bialaphos (5 mg/L) were obtained after *Agrobacterium* transformation.
- We will introduce a nematode-resistance gene into bermudagrass after the MTA process is completed.