# *Hybrid Bermudagrass Improvement by Genetic Transformation*

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**Objectives:**

1. To develop and optimize tissue culture conditions in order to obtain embryogenic calli and to regenerate plantlets from bermudagrass.
2. To develop procedures to transform bermudagrass and to recover transgenic plants.
3. To obtain transgenic plants of bermudagrass expressing a potential nematode-resistance gene.

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

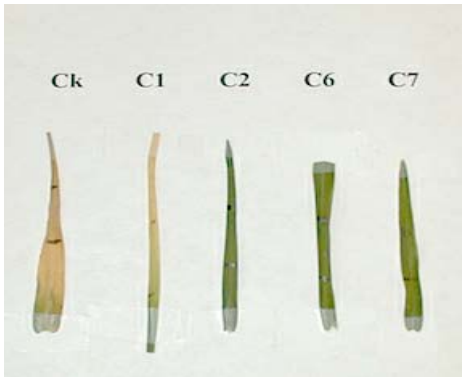
Bermudagrass is an important warm-season grass species for putting greens, tees, and fairways of golf courses in the southern United States. The ultimate goal of the research direction is to improve bermudagrass cultivars for the golf courses through biotechnology. The objectives of this project are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

Bermudagrass is a recalcitrant species in tissue culture, i.e., it is difficult to obtain callus regeneration at high frequency. In the past two years, by adjusting phytohormones in the culture medium, we have been able to develop highly regenerable callus lines from common bermudagrass variety J1224. By using these lines, we were able to transform common bermudagrass, for the first time, by both the biolistic method and *Agrobacterium*-mediated approach.

In the biolistic transformation, by using hygromycin B selection, four independent transgenic plants are confirmed by DNA hybridization and other analyses. By co-transformation with a vector which harbors both GUS reporter gene and a herbicide resistance gene, one of the transgenic plants showed GUS reporter gene activity and three are resistant to herbicide Finale. Multiple transgene copies in transgenic plants are observed.

*A bermudagrass callus showing origin of the yellowish, highly regenerable callus sector*

*Agrobacterium* transformation of bermudagrass was also performed using the highly regenerable callus lines. In addition to some transgenic calluses obtained from selection, two independently transformed plants are identified by DNA hybridization. By our estimate, one plant has one copy of transgene and the other plant has two. One of the plants showed GUS activity.




*Three of the four transgenic plants showing resistance to herbicide Finale. Ck: non-transgenic control.*

Transgenic plants sometimes showed off-type morphology. In two cases, wider and shorter leaves were observed. In another case, a plant showed a dwarf-like morphology.

Through a MTA (Material Transfer Agreement), we have obtained a nematode-resistance gene and have made the gene constructs for it to be expressed in bermudagrass. We have initiated experiments to transform bermudagrass with the gene construct using both biolistic and *Agrobacterium*-transformation approaches. Preliminary results indicated that ten hyg B-resistant calluses were obtained and one of them is regenerating. More experiments will be performed, and the transgenic plants will be subjected to sting nematodes to evaluate the resistance.

*A regenerated transgenic bermuadgrass plant obtained by* Agrobacterium *transformation*

## Summary Points

- Using highly regenerable callus lines developed in our lab, we have developed a protocol to obtain transgenic common bermudagrass plants by the biolistic (gene gun) method using hygromycin B selection. Four such plants are confirmed by DNA hybridization and other analysis. By co-transformation, one of them showed GUS reporter gene activity and three are resistant to the herbicide Finale. Multiple transgene copies in transgenic plants are observed.
- Using similar conditions, we also have been able to obtain two transgenic common bermudagrass plants by *Agrobacterium*-mediated transformation, which is confirmed by DNA hybridization.
- The nematode-resistance gene was obtained from a collaborator and gene constructs have been made. By using both biolistic and *Agrobacterium* approaches to deliver this gene construct, ten hyg B-resistant calluses were obtained. One of them is regenerating.