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 (particle bombardment) method and to recover transgenic plants.
3. Obtain transgenic plants of hybrid bermudagrass that express nematode-reistance genes.

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

Hybrid bermudagrass is an important grass species for the greens, tees and fairways of golf couses in the southern United States. While the conventional breeding program has created the superior hybrid bermudagrass and will continue to contribute to the improvement of the species in the future, a biotechnolgoy approach will help the germplasm enhancement of the species as well as to directly contribute to the improvement of some important agronomic traits.

The successful applications of the biolistic method (particle bombardment) method and *Agrobacterium* infection in monocot transformation including transformation of creeping bentgrass, indicate that the time has finally come to improve turfgrass species including bermudagrass by genetic transformation.

Both methods require well developed tissue culture procedures as the basis of the technolgy. Despite the importance of the hybrid bermudagrass in golf courses, very little tissue culture work has been performed with the species.

The biolistic gun is used to shoot DNA-coated gold or platinum particles into embryogenic callus tissue.

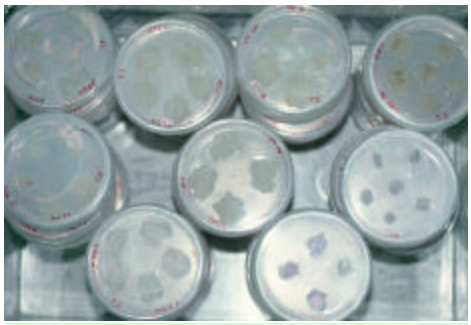
The ultimate goal of this research project is to improve turf-type bermudagrass cultivars for the golf courses through biotechnology. Specifically the project is intended to develop and optimize tissue culture conditions for hybrid bermudagrass, to develop genetic transormation procedures for the species, and to obtain nematode-resistant transgeic bermudagrass plants.

Overall, turf-type bermudagrass is recalcitrant for plant tissue culture. We mainly use the young inflorescences of hybrid cultivar 'Tifgreen' and a common bermudagrass cultivar 'Savannah' as tissue culture materials because their potential to regenerate.

So far, we have spent substantial efforts to improve the tissue culture responses of bermudagrass. We have showed previously that by supplementing 6-benzylaminopurine (BAP) and abscisic acid (ABA) in the callus induction medium we substantially improved somatic embryogenesis from bermudagrass callus.

Addition of gibberellic acid further improved the generation/regeneration of embryos. This year, we repeated the experiments and confirmed our observation. We also observed secondary somatic embryogenesis in bermudagrass culture which contributed to the regeneration. Moreover, we found a better combination of phytohormones for common bermudagrass tissue culture medium.

We have spent most of our efforts in the transformation experiments with emphasis on *Agrobacterium*-mediated transformation. A gene expression vector pRQ219 was constructed and eight strains were made. We found that some strains are more infectious in bermudagrass than others, and some bermudagrass cultivars are more susceptible than other cultivars. We also identified the optimal time to perform the infection.



The techniques to develop embryogenic bermudagrass callus will allow researchers at North Carolina State University to develop nematode resistant germplasm.

We performed 28 batches of agro-transformation experiments using young inflorescences and their derived callus. In addition, we established five suspension cell lines which have been used for transformation experiments. Using an intron-GUS reporter gene, we observed cell clusters showing GUS activity, indicating stable transformation of bermudagrass cells.

Summary Points

- It is difficult to derive embryogenic callus from bermudagrass.
- Supplementing BAP and ABA in the callus-induction media improved somatic embryogenesis.
- Giberellic acid further improved the generation/regeneration of embryos.
- Secondary somatic embryogenesis was observed.
- *Agrobacterium*-mediated transformation is being developed.
- The GUS-reporter gene is showing activity in five cell suspension lines.