Hybrid Bermudagrass Improvement by Genetic Transformation

Rongda Qu
North Carolina State University

Objectives:

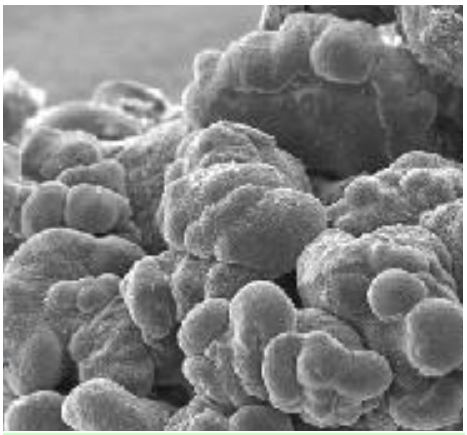
1. Develop and optimize tissue culture conditions in order to obtain embryogenic callus and regeneration of hybrid bermudagrass plantlets.
2. Develop a procedure to transform the embryogenic callus by the biolistic method (particle bombardment) and to recover transgenic plants.
3. Obtain transgenic plants of hybrid bermudagrass that express nematode-reistance 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 this research is to improve bermudagrass cultivars for the golf courses through biotechnology.

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. We have spent a great deal of effort and have successfully improved bermudagrass tissue culture by adjusting the composition and levels of phytohormones in the culture medium.

Experiments completed in 2001 confirmed importance of benzylaminopurine (BAP) and abscissic acid (ABA) in the callus induction medium and giberellic acid (GA) in the regeneration medium. We have been able to track a full course of secondary somatic embryogenesis in bermudagrass callus culture and recorded it by microphotography. We also initiated tissue culture experiments using nodal segments as explants.



Globular structures of early development of secondary somatic embryogenesis

We have developed transformation procedures using the biolistic method and the *Agrobacterium* approach for bermudagrass. A total of 19 putative transgenic callus lines were obtained by selection with hygromycin B (250 mg/L) or bialaphos (5 mg/L). Some callus lines also showed GUS reporter gene activity. Transgenic nature of some lines have been confirmed by Southern Hybridization Analysis.

Callus resistant to bialaphos selection (5 mg/L) after being transformed with an *Agrobacterium* strain.

In this project, we have so far drastically improved bermudagrass tissue culture responses, particularly the regeneration ability, by adjusting the levels of various phytohormones in the culture media. We have mostly used young inflorescence tissue and the derived callus for transformation efforts, but have initiated efforts to expand to nodal segments which are available year round. We have been able to stably transform bermudagrass with both the biolistic and the *Agrobacterium* approaches, but have not obtained the transgenic plants yet.

Our research focus in next year will be to obtain stably transformed bermudagrass plants by the following approaches: (1) obtain regenerable calli which are also transformation competent; (2) concentrate on *Agrobacterium* transformation and further improve the transformation efficiency by optimizing the transformation procedure; and (3) try to directly transform meristematic cells using *Agrobacterium.*

Summary Points

- Bermudagrass is recalcitrant in tissue culture and genetic transformation.
- Regeneration of bermudagrass plants through tissue culture has been further improved by adjusting plant hormones in the culture medium.
- A full course of secondary somatic embryogenesis has been photographed under a stereo microscope by tracking an embryogenic callus for over 70 days.
- Transformation procedures using 'gene gun' or *Agrobacterium* have been developed. A total of 19 putative transgenic callus lines were obtained by selection with hygromycin B (250 mg/L) or bialaphos (5 mg/L). Some callus lines also showed GUS reporter gene activity.
- Transgenic nature of some lines have been confirmed by Southern Hybridization Analysis.