Hybrid Bermudagrass Improvement by Genetic Transformation

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Number of Years: 5
Total Funding: $125,000

Objectives:

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

The ultimate goal of this research direction is to improve turf-type bermudagrass cultivars for the golf courses through biotechnology. The specific goals of the project include: to optimize tissue culture conditions for inducing embryogenic calli and regenerating plantlets of bermudagrass, to develop procedures to transform bermudagrass, and to obtain transgenic bermudagrass plants that express potential nematode resistant genes. The turf-type bermudagrass is particularly recalcitrant for plant tissue culture. We mainly use the young inflorescences of hybrid cultivar 'Tifgreen' and a common bermudagrass cultivar 'Savannah' as research materials. So far, we have spent substantial efforts to improve the tissue culture responses of bermudagrass. Last year, we showed that by adding 0.01 mg/L 6-benzylaminopurine (BAP) in the callus induction medium (MS with 1 mg/L 2,4-D), we were able to induce embryogenic structures from otherwise very fluffy callus and the bermudagrass regeneration was substantially improved. A manuscript dealing with this work has been accepted by a journal. This year, we further tested a total of 65 combinations of plant growth regulators at various concentrations in the culture medium in 52 batches of experiments. It was observed that a combination of BAP and abscisic acid (ABA) can further improve the callus quality and increase the occurrence of the embryogenic structures. It was observed that inclusion of gibberellic acid (GA) in the regeneration medium effectively improved the regeneration of the embryogenic calli.

Another hybrid cultivar, 'Tifway', was even more recalcitrant. By pre-treating the young inflorescences with 0.2% ascorbic acid, we have been able to induce calli from 'Tifway' tissue culture and to recover regenerated plants.

We have spent substantial efforts in the transformation experiments. By histological examination the callus structure, we have focused on the very young, undifferentiated calli as target tissue for transformation. We have performed 15 batches of transformation experiments using the biolistic method and bialaphos selection. Although some calli and plantlets survived the selection, we have no strong evidence to show they are transgenic. In searching for an alternative to the herbicide selection, we also identified G418 as a potential selection agent. In addition, we explored the possibility to transform bermudagrass with agrobacterium. Transient expression assays of an intron-GUS reporter gene suggested that agrobacterium can infect bermudagrass and thus may be used to transform bermudagrass.