depth of 62 cm. Cores will be divided into thatch, verdure, roots, and soil components. The soil cores will be partitioned to four depths: 0 to 8, 8 to 16, 16 to 32, and 32 to 62 cm. After partitioning the cores by depth, the six samples will be composited, mixed thoroughly, and analyzed for total N, NH_4^+-N, NO_3^--N, and N-isotope ratio.

Buffalograss Resistance to Chinch Bugs. The initial phase of this research involved developing screening methods and evaluating selected buffalograss germplasm for resistance to *Blissus occiduus*. Eleven buffalograss cultivars/selections (*CODY, BONNIE BRAE, TATANKA, TEXOKA, NE 91-118, NE 86-120, NE 86-61, 315, 378, 609*, and *NE 84-45-3*) were screened for resistance to *B. occiduus* in two greenhouse trials. Using chinch bug numbers and plant damage ratings to assess levels of resistance, the 11 buffalograss cultivars/selections were separated into categories of resistance. *CODY* and *TATANKA* consistently exhibited high levels of resistance to chinch bug feeding, while *BONNIE BRAE* and *NE 91-118* showed high to moderate levels of resistance. Other cultivars/selections, including *378, 315, NE 84-45-3*, and *NE 86-61*, were moderately to highly susceptible. *CODY* and *TATANKA* maintained acceptable turf quality although both became heavily infested with chinch bugs. This suggests tolerance may be a mechanism of the resistance. Studies designed to characterize the mechanisms of resistance are currently underway. Antixenosis experiments have revealed chinch bug preference for *TEXOKA, NE 86-120*, and *BONNIE BRAE*. Other cultivars/selections such as, *609* and *NE 91-118* are rarely preferred.

Hybrid Bermudagrass Improvement by Genetic Transformation

North Carolina State University

Rongda Qu

Start Date: 1998
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 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 procedure to transform the embryogenic calli by the biolistic method and to recover transgenic plants, and to obtain transgenic plants of bermudagrass that express potential nematode resistant genes. Bermudagrass is a recalcitrant species for plant tissue culture. Thus, most of the efforts have been concentrated on optimizing tissue culture conditions, especially at the callus induction stage to improve the callus quality and the regeneration ability.

Various tissues, culture media and supplements to the media have been tested in order to optimize tissue culture conditions of bermudagrass. Approximately 20 percent of the calli induced from young inflorescence (0.5 to 1 cm) of *TIFGREEN* and *SAVANNAH* (a common bermudagrass cultivar) had an embryogenic structure when cultured in MS medium (1 mg L^{-1} 2,4D) supplemented with 0.01 mg/L 6-benzylarninopurine (BAP). No such structures were found in media without BAP. The calli were slow growing, compact, pale or off-white in color, and highly regenerable. The regeneration rate of the calli with embryogenic structure was higher than 50 percent while calli without this structure had the regeneration rate about 1 to 5 percent. In addition, the callus induction rate was raised from 21 to 33 percent to over 60 percent by excising young inflorescence into pieces before the culture inoculation.

It was very difficult to induce callus from the young inflorescence of *TIFWAY* due to the quick browning of the explants in culture medium. The situation can be improved by pretreatment of explants with 0.2 percent ascorbic acid, an anti-oxidant.

Pilot experiments were performed to determine the pressure parameter of the biolistic apparatus. It was found by transient assay of GUS reporter gene that bombardments at 1550 psi on osmotically treated calli were the best for genetic transformation experiment.

Callus growth inhibition assays were performed with three potential selection agents at various levels. Effective selection was found at 250 mg L^{-1} of kanamycin and hygromycin B, while 5 mg L^{-1} was appropriate for selection with bialaphos, an herbicide.