L^{-1} of *PPM* in the medium for bermudagrass provided better results.

Clean cultures of clones of buffalograss, bermudagrass, and saltgrass have been established and are being propagated for use in the cryopreservation studies.

Germplasm Development for Buffalograss Varieties

University of Nebraska

Terrance Riordan

Start Date: 1998
Number of Years: 5
Total Funding: $125,000

Objectives:

1. *Acquire additional germplasm through collection and recombination of germplasm already in our collection.*
2. *Evaluate germplasm with superior turfgrass characteristics including mowing tolerance, color, length of growing season, insect resistance, establishment and recovery of vigor, sod strength, combining ability, and seed production.*
3. *Obtain inheritance data on important traits, conduct genome size and molecular marker analyses, and evaluate the impact of inbreeding and genetic diversity on variety development.*

Seeded Releases. Native Turf Group (NTG) is considering the possibility of selling *NTG-5*, which was included in the 1991 National Turfgrass Evaluation Trial, and they are looking at *NTG-7* and *FW-3* (a low mowing tolerant experimental) for future release and production.

Vegetative Releases. Patents were filed for new releases *NE 86-61, NE 86-120* and *NE 91-118*, but have not been granted. Official UNL release statements have been completed and these cultivars are included in the 1996 National Turfgrass Evaluation Program Buffalograss Trial. *NE 91-118* has been vegetatively increased at Crenshaw Turf (CT) located at Poteet, Texas. Todd Valley Farms located at Mead, NE, bought a new farm and planted 35 acres of *NE 86-61*.

Sod Production. Crenshaw Turf (CT) has purchased Ellsberry Sod in Florida and Milberger Sod in Bay City, Texas. They continue to grow and have positive growth plans for production of buffalograss and other southern turf species. Todd Valley Farms (TVF) continue to increase sales of *378*, but TVF now has a greater role in developing the buffalograss market in the Northern United States. UNL, CT and TVF are working cooperatively on the development of new releases.

Summary of Breeding Work. Overall, the levels of performance continue to improve with each generation of selection. Newly released cultivars continue to show their superiority over older varieties with improved sod strength, color, quality, and density. Accessions from fairway maintained areas look very promising and show continuing improvements towards a high quality, low maintenance fairway turf. The top performers in the Nebraska National Buffalograss trial were *91-118* and *86-61*, which are being commercialized. The seeded varieties *CODY* and *TATANKA* showed little differentiation during the first year of this study. However, in 1998 the advance-seeded types began to show better performance than the common types like *TEXOKA*.

Evaluation for Low-mowing and Wear tolerance. Under low mowing and no wear the female clone *92-135*, which outperformed all other entries in 1997, performed very well again in 1998 along with the female clone *92-31*. However, two male clones, *92-141* and *92-116*, had the best overall performance in 1998. All seed established experimentals exhibited average color and quality characteristics. The trial had a number of promising male and female clones. Wear results indicated that male and monoecious clones exhibited the most damage, while wear tolerance of female cultivars was significantly better than males, but not as good as for mixed seeded types.

Fertility and Mowing Effects on Buffalograss. At the Nebraska site, *NE 91-118* and *378* had the highest quality ratings at the 2.5 cm mowing heights for years 1996-1998. *CODY* and *TEXOKA* had poor quality ratings at the 2.5 cm mowing height for all years. In 1998, *NE 91-118*, *378*, and *CODY* had the highest quality ratings at the 5.1 cm mowing height. At the 7.6 cm mowing height, *CODY* and *TEXOKA* had the highest quality rating in 1997 but *CODY* and 378 had the highest quality ratings in 1998.

From 1997 to 1998, several trends were evident. First, turfgrass quality decreased from 1997 to 1998 for all cultivars at the 0, 2.4, and 5.0 g N m^{-2} rates. At 10 g N m^{-2}, *NE 91-118* and *378* had higher quality in 1998 than in 1997. All cultivars had improved quality ratings in 1998 at the 20 g N m^{-2} rate. Quality ratings in 1998 were poor (< 6) for all cultivars at 0, 2.4, and 5.0 g N m^{-2} rates. At 10 g N m^{-2} *NE 91-118, 378*, and *CODY* had good turfgrass quality. Management recommendations for 378 and *NE 91-118* are 2.5 or 5.1 cm mowing heights and a nitrogen rate of 10 g N m^{-2} $year^{-1}$. Recommendations for *CODY* and *TEXOKA* are 5.1 or 7.6 cm mowing heights and a nitrogen rate of 10 g N m^{-2} $year^{-1}$.

Nitrogen Partitioning in Turfgrasses. Field experiments to determine the fate of nitrogen fertilizer applied to three turfgrass species were initiated in 1997 at the John Seaton Anderson Turfgrass Research Facility near Mead, Nebraska. Fate of fertilizer nitrogen will be followed in buffalograss, Kentucky bluegrass, and tall fescue. Established turfgrass plots of two cultivars of buffalograss, *NE 91-118* and *NE 86-120*, a blend of Kentucky bluegrass, and a blend of tall fescue. The total amount of actual nitrogen that will be applied each year to a 9 m^2 plot is 0, 10, and 20 g N m^{-2}. For Kentucky bluegrass and tall fescue 80 percent of evapotranspiration will be returned every four days and for buffalograss 60 percent of evapotranspiration will be returned weekly. Plots will be randomly sampled prior to each fertilizer application to analyze for nitrogen content in plant and soil fractions. A Giddeon Soil Probe will be used to extract six cores (5 cm diameter) to a

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.