Initially, elite lines from the University of Arizona collection and the CSU-USGA lines were established in both Arizona and Colorado. This initial year was a grow-in year with data on turf quality and seed production to be observed in future years. The material in Arizona will be used for drought studies in the field as well. CSU-USGA elite lines previously established in Colorado were observed for flowering and seed set. Seed production was evident but shattering was a problem. An extensive nursery consisting of the Arizona lines as well as additional lines from a collecting trip to Utah, Nevada, South Dakota and Nebraska with approximately 200 accessions was established at the CSU Horticulture Research Center outside of Fort Collins.

In order to understand seed fertility of inland saltgrass, a study of chromosome numbers for genotypes found throughout the region was initiated. Variation in chromosome number can lead to low pollen or egg viability resulting in poor seed set. Root tip smears of 40 genotypes were observed with the most common chromosome number being 2n = 38. However, 39, 42, 40, and 74 chromosome counts were observed. Coastal saltgrass was determined to have 40 chromosomes, as previously published and in our observations as well. This would indicate that our most commonly observed chromosome number of 38 is likely an aneuploid, probably a nullisomic.

Studies to determine pollen viability via examination of pistils demonstrated that pollen readily germinated in those clones examined. This was seen via microscopic examination of pistil structure. Furthermore, pollen tube growth reached the egg sac as well. Therefore, pollen viability is not apparently a problem in those clones observed. However, crossing among clones of different chromosome number may still influence successful seed production. In crosses among plants with 38 chromosomes and between 38 and 42 chromosomes, successful seed set was apparent. These seed have been germinated and will be examined in future studies. Due to high pollen viability and observable seed set, we believe that poor fertility as reported elsewhere is probably due to pollen availability rather than pollen quality or genetic deficiencies in most cases.

Three seed lots of inland saltgrass were examined for viability and germination. Seed viability for these lots was 15, 62 and 92 percent as determined by the tetrazolium (TZ) test. This low viability, in some cases, was probably due to a combination of extreme age, early harvest and poor storage conditions.

Inland saltgrass seed at maturity appears to be dormant and this dormancy is apparently due to the seed coat. Recently harvested seed lots that were scarified readily germinated in excess of 90 percent viable seed. For unscarified seed, alternating extremes of temperature prompted greater germination than moderate temperatures. Old seed exhibited low viability but seed that were determined to be alive via the TZ test germinated without scarification.

A Multigene-Transfer Strategy to Improve Disease and Environmental Stress Resistance in Creeping Bentgrass

Michigan State University

Mariam B. Sticklen

Start Date: 1998
Number of Years: 3
Total Funding: $75,000

Objectives:

1. *Enhance the expression (increase level of pest resistance) of the American elm chitinase gene in creeping bentgrass.*
2. *Transfer two drought-resistance genes controlled by either a constitutive or an ABA-inducible promoter into creeping bentgrass.*
3. *Determine disease resistance of transgenic plants expressing different levels of the chitinase gene and transgenic plants containing single-versus multiple-inserted genes under green house and field conditions.*
4. *Determine environmental stress resistance of transgenic plants containing single-versus multiple-inseted genes grown under greenhouse and field condiditons.*
5. *Evaluate transgenic creeping bentgrass clones for turf quality characteristics under field conditions.*
6. *Release transgenic creeping bentgrass germplasm with combined improvements in turf quality and pest and stress resistance to Pure Seed Testing, Inc. and/or other sectors for use in their field testing and commercial breeding program.*

Major biotic and abiotic problems associated in the management of creeping bentgrass turf include several pathogenic disorders and certain environmental extremes such as drought, heat, and cold stress. In addition, environmental extremes such as drought can influence the health of the plant and its ability to resist infection by biotic agents.

Resistance to biotic and abiotic stress in plants has been reported to be associated with relatively complex genetic factors. Most biotechnological approaches of the last two decades, especially those related to the control of insects and diseases, have concentrated on transferring a single gene into plants. The single gene approach may sound attractive over a short period; however, this approach may result in more serious problems over a longer period as populations of biotic agents (i.e., insects or pathogens) develop resistance the single gene. Our long-term goals include development of transgenic turfgrasses with improved resistance to pathogens and drought tolerance.

Previously, the research team developed creeping bentgrass clones that contain the glufosinate ammonia resistant herbicide, a chitinase gene, a proteinase inhibitor gene, and a drought and salt tolerance mannitol dehydrogenase (mt1D) gene. So far, it

glufosinate ammonia was confirmed to have fungicidal as well as herbicidal properties. Therefore, we have been able to simultaneously control weeds and turfgrass pathogens (mainly *Sclerotinia ulnocarpal* and *Rhizoctonia solani*) by spraying this herbicide on transgenic creeping bentgrass expressing the gene under greenhouse conditions.

Studies have shown that the chitinase genes can make transgenic plants resistant to pathogenic fungi such as *R. solani*, etc. Research in Dr. Vargas' laboratory has shown that our transgenic creeping bentgrass clone 711, transcribing the elm chitinase gene controlled by the cauliflower mosaic virus 35S promoter, has improved resistance of plants to *R. solani* under controlled environmental conditions. Recently, Dr. Sticklen's laboratory has constructed a plasmid containing the elm chitinase gene controlled by rice actin promoter (shown to provide greater gene expression in grass family than the 35S promoter) and transformed creeping bentgrass with this construct. Theoretically, using this grass-specific promoter, we could improve the level of expression of the chitinase gene, and the degree of resistance to *R. solani* in transgenic creeping bentgrass plants.

The mannitol 1-phosphate dehydrogenase (mt1D, known for its drought tolerance) gene that we have used to transform creeping bentgrass is also confers salt tolerance, A preliminary experiment performed by Dr. Baird's laboratory has not shown any drought tolerance of transgenic plants. More studies are needed to confirm whether these plants have any tolerance to drought and/or salt.

Determining the Genetic Stability of Triploid Bermudagrasses

Mississippi State University

Michael Goatley, Jr.

Start Date: 1998
Number of Years: 3
Total Funding: $72,790

Objectives:

Determine the origin of the off-types that arise in hybrid (triploid) bermudagrass golf greens through a combination of cytological and molecular analyses.

Off-types in hybrid bermudagrass (*Cynodon dactylon* x *C. transvaalensis* Burtt-Davy) putting green varieties are a persistent problem in southeastern golf courses. They disrupt green uniformity and interfere with ball roll; their effects sometimes necessitate green replacement. Our current goal at Mississippi State is to learn if their formation has a genetic and/or cytological basis. To minimize contamination to the smallest practical extent, we are conducting a greenhouse study using sterilized growth medium.

The genetic detection phase involves cross-species hybridization between bermudagrass DNA and RFLP clones from maize (*Zea mays* L.), and chromosome counts of bermudagrass root-tip cells for the cytology. We chose to use maize clones for two reasons. First, maize has a well characterized genetic map with many markers to choose from, so we know the chromosomal location of the clones we selected. Second, there is considerable evidence that gene order among the grasses is strongly conserved. Therefore, we can select clones from maize with reasonable assurance that we are monitoring a large portion of the bermudagrass genome, rather than using markers that are potentially biased towards small regions of the genome. Additionally, we are attempting to learn if there is a relationship between off-type formation in bermudagrass green varieties and chronic application of mitotic inhibitor herbicides such as pendimethalin and oryzalin.

Off-types in other grasses, most notably the cereal grain species, are frequently due to absence of one or more chromosomes, a condition known as aneuploidy. This chromosome loss may occur spontaneously, but it also may be induced with the application of mitotic inhibiting compounds. Oryzalin is now frequently used in place of colchicine to induce chromosome doubling for production of doubled haploids in lab experiments, mainly because it is much less toxic to humans than colchicine. When a plant's exposure to oryzalin is inadequate, chromosome doubling is incomplete, and aneuploidy sometimes results. If this occurs in bermudagrass putting green varieties, we hope to correlate it with the formation of off-types.

To meet this goal, six varieties are being subjected for one month to weekly drench applications of a 0.5X rate of oryzalin or pendimethalin in a replicated greenhouse experiment, designed as a randomized complete block. This is intended to expose the plants to a cumulative 2X rate application. The varieties are *TIFGREEN, TIFDWARF, TIFEAGLE, MS-SUPREME, CHAMPION,* and *FLORADWARF*. The grasses were established from small stolon pieces (2 nodes in length) in horticultural flats containing an approximate 75:25 masonry sand:peat moss mix, and are maintained at about 0.25-inch mowing height to encourage lateral growth in the flats. As a safeguard against latent contamination, the flats are irrigated for 10 days prior to stolon planting to encourage germination of seed or other dormant propagules so that they can be eliminated.

When the grass reaches 75 percent coverage of the flat, herbicide treatment is commenced. At the conclusion of the herbicide applications, the flats are left unmowed to detect any morphological off-types that might arise. Presence of differences will be determined by comparing leaf blade length and width, as well as internode length and width, between untreated checks and the treated clones using a two-tailed Dunnett's test (following a significant ANOVA). Stolons from these treated flats are then sampled to establish new flats. To date we have completed one cycle of the experiment and are initiating a new cycle with stolons from the previous round of treatment.

To test the efficacy of the herbicides, leachate from the final herbicide application at the end of the first treatment cycle