Bermudagrass Cold Hardiness: Characterization of Plants for Freeze Tolerance and Character of Low Temperature-Induced Genes

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

Objectives:

1. *Quantify cold hardiness of advanced breeding lines, recently released varieties, and established standard varieties using laboratory-based methodology.*
2. *Isolate and characterize genes corresponding to low temperature-induced and antifreeze proteins by constructing and screening a representative genomic library from MIDIRON with both homologous and heterologous gene probes.*
3. *Characterize the low temperature induced expression of the cloned genes by northern blot analysis.*
4. *Sequence the cloned genes and characterize gene structure and function based on nucleotide sequence data.*

Injury to bermudagrass turf caused by freezing temperatures during winter is a persistent problem throughout its geographic area of use in the United States. This research seeks to reduce risk of freeze injury to bermudagrass grown in temperate regions. The research focuses on accurately assessing the freeze tolerance of bermudagrass cultivars, isolating genes responsible for enhanced freeze tolerance, and enhancing knowledge of the fundamental mechanisms associated with cold hardiness. Specific objectives are to: 1) quantify cold-hardiness of advanced breeding lines, recently released varieties, and established standard varieties and 2) isolate and characterize cold regulated (*Cor*) genes responsible for conferring freeze tolerance.

Experiments were initiated to determine the low temperature tolerance (LT_{50}) of turf bermudagrasses using laboratory-based methodology. The LT_{50} values will be determined sequentially for selected bermudagrasses in each of four groups. The groups are: 1) vegetatively-propagated fairway types, 2) seeded fairway types, 3) vegetatively-propagated putting green types, and 4) experimental fairway breeding lines. Experiments with the vegetatively propagated fairway types are underway.

Substantial progress has been made toward the goal of isolating and characterizing cold regulated (*Cor)* genes. A *Cynodon* genomic library was constructed from MIDIRON (*C. dactylon* x *C. transvaalensis*) turf bermudagrass. Screening the library using a 300-bp cDNA bermudagrass clone provided by Mr. Stephen McMaugh from the University of Sydney, Australia, identified nine putative chitinase genes. Sequencing and homology studies completed for three of the clones provided strong evidence that they are indeed chitinase genes, which we designate as *CynCht-1, CynCht-2,* and *CynCht-3.* We expect all of the clones to also be chitinase genes.

Northern blot analyses indicated chitinase gene expression in *MIDIRON, UGANDA*, and MSU turf bermudagrasses to be strongly affected by acclimation temperatures (4-8 °C). Substantial increases (75-100%) in gene activity in crown and root tissues occurred after 24 hours of cold acclimation. Increases of gene activity in crown and root tissues were proportional to the LT_{50}'s and ploidy levels of the three cultivars. Cold acclimation for 28 days caused an approximate three-fold increase in chitinase gene activity in leaves of *MIDIRON* and *UGANDA*, but had little effect on MSU. Leaves of MSU remained relatively green during the 28 day acclimation, while those of *MIDIRON* and *UGANDA* strongly senesced. Different *Cor* gene regulatory mechanisms may be involved in leaf and crown/root tissues.

Determining the Heritability of Salt Gland Density: A Salinity Tolerance Mechanism of Chloridoid Warm Season Turfgrasses

University of Arizona
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Start Date: 1998
Number of Years: 3
Total Funding: $55,815

Objectives:

Determine the broad and narrow sense heritabilities of salt gland density in zoysiagrass. Zoysiagrass is the ideal model system to determine salt gland heritability, as salt gland densities and relative salt tolerances of a large number of genotypes have already been determined. As salt gland density had been found to be an important salt tolerance mechanism in other turfgrasses in the Chloridoideae subfamily (i.e. bermudagrass, buffalograss) results should be applicable to these breeding programs as well.

For this project, Greg Wess was selected for the M.S. student position in the Department of Plant Sciences, having received his B.S. from our department last spring. After consulting with turfgrass breeders, and review of the literature, it was decided to limit the main polycross study to a wide selection of *Zoysia japonica* types. This was decided, based on recent findings from Sharon Anderson (Ph.D. student, Texas A&M University). First, interspecific crossing between *Z. matrella* and *Z. japonica* types in zoysiagrass is possible, but often difficult and unpredictable. Second, flowering requirements differ between species: *japonicas* behave as long day plants, while *Z. matrellas* and *Z. tenuifolias* tend to be day-neutral.

Because a polycross nursery will be utilized to determine heritability, uniform cross-compatibility and simultaneous flowering among genotypes is necessary. However, in addition to the *Z. japonica* polycross nursery, individual crosses will be attempted between several *Z. matrella* accessions and the *Z. japonicas*. The progeny resulting from these crosses will be evaluated for salt gland density.

Fifteen *japonica* genotypes have been selected, representing a broad range of salinity tolerance and salt gland density (previously determined from work at Texas A&M University). These are: *CROWNE, K162, J2-1, K157, PALISADES, BELAIR, EL TORO, J3-2, J94-5, KOREAN COMMON, JS23, P58, SUNRISE, MEYER*, and *K12*. These genotypes have been increased from single sprigs in a greenhouse (a slow process), and the replicated polycross nursery will be established next month, with 10 replications per genotype. Growth is being accelerated by supplemental lighting and liquid fertilization. Flowering of the *Z. japonicas* is expected in the late spring at which time polycrosses will be made.

Identification of Genetic Insect and Mite Pest Resistance in Turfgrasses

Texas A&M University

James Reinert

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

Objectives:

1. *Establish a Regional Center to identify genotypes of Cynodon, Zoysia, Buchloe, Paspalum, Agrostis, and Poa with genetic resistance to insects and mites (fall armyworms, black cutworm, sod webworms, greenbug and host specific eriophyid mites) for use in cooperating turf breeding programs.*
2. *Bioassay resistant line with insect diets to characterize the mechanisms of resistance and determine their biochemical nature.*
3. *Develop effective and efficient procedures to accommodate screening and identify typical breeding populations-heretofore unavailable to the plant breeder.*

The Project has established a Regional Center to screen and evaluate turfgrass germplasm for resistance to insect and mite pests. The primary goal of the project is to identify genetic lines of bermudagrass, zoysiagrass, buffalograss, seashore paspalum, bentgrass, and bluegrass with resistance to the primary pests; caterpillars (fall armyworms, black cutworms, sod webworms) and host specific eriophyid mites, and characterize the mechanisms of resistance.

Work was initiated on elite bermudagrass germplasm *(Cynodon spp.)* from Dr. Charles Taliaferro's breeding program at Oklahoma State University, and with commercial cultivars under culture at the TAMU-Dallas Center.

Thirty-two bermudagrass hybrids and nine commercial cultivars were evaluated by feeding four day-old larvae on them in no-choice feeding studies. Among the hybrids, *4200W 49-17, 4200W 53-1*, and *4200W 55-5* (Table 9) produced the highest mortality with from 42 to 52 percent mortality, identified as failure of the individuals to emerge as adults from the pupa stage. Also, *3200W 70-18* provided 37 percent mortality at adult emergence with *3200W 94-2, 4200W 38-2, 3200W 18-11* and *3200W 30-20* each producing 33 percent mortality. These same grasses with the exceptions of *4200W55-5* and *4200W 38-2*, usually produced the smaller larvae when weighed at 10 days. At the other end on the gradient, *CCB 24-4* and *3200W 6-12* were the most susceptible hosts and produced only 4 percent mortality of the fall armyworm larvae.

Among the commercial bermudagrass cultivars, mortality was 8.25 percent or less at 17 days for all of the cultivars and 20.6 percent or less at adult emergence. Fall armyworm development was slowest with the smallest larvae and pupa on Common, but this experiment supports previous experiments that Common is not resistant and is a relatively good host for this insect pest. None of the cultivars of bermudagrass in this experiment or in the above experiments exhibits an acceptable level of resistance to the fall armyworm. These experiments support the hypotheses that new cultivars may be developed that are superior to existing cultivars in pest resistance.

Residential landscapes are frequently invaded by large populations of grasshoppers that develop in adjacent landscapes or in agricultural lands. These invasions occur annually in late summer to autumn in some areas, but high populations tend to cycle every three to five years across the southern or southwestern states. Representative cultivars of cool and warm season turfgrasses (Tall fescue, *REVEILLE* hybrid bluegrass, Syn1 Texas bluegrass, *TIFWAY* bermudagrass, *COMMON* bermudagrass, *RALEIGH* St. Augustinegrass, *MEYER* zoysiagrass, *CAVALIER* zoysiagrass, *PRAIRIE* Buffalograss and Johnsongrass) were evaluated for feeding preference or resistance to adult feeding by the differential grasshopper *(Melanoplus differentialis)*. The degree of feeding was ranked (rank = 0 – 5, 0 = no feeding during the test period, 5 = near complete consumption of ration) and measured by the number and weight of fecal pellets produced during the feeding period.

Based upon ranked feeding and the number and weight of fecal pellets after 2-days of feeding, tall fescue was the most preferred host evaluated. *REVEILLE* hybrid bluegrass, *TIFWAY* and *COMMON* bermudagrass, Syn1 Texas bluegrass and *MEYER* zoysiagrass were also highly preferred hosts based upon fecal pellet weights. *PRAIRIE* buffalograss and *CAVALIER* zoysiagrass were resistant to the grasshoppers and exhibited very low feeding damage, and fecal pellets. These trends held true throughout the 8-day feeding period of the test.