## 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. I

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

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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