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

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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 temperatue 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.

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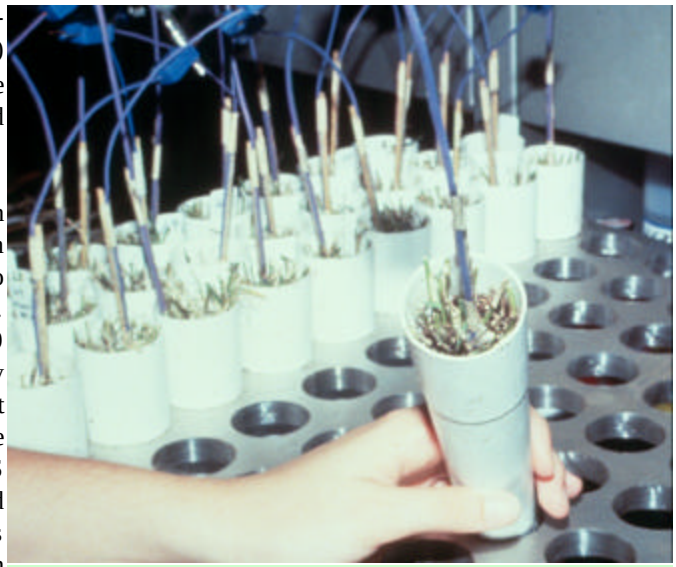
Project Duration: 5 years
Total Funding: $125,000

Injury to bermudagrass turf caused by freezing temperatures during winter is a persistent problem over much of its geographic area of use in the USA. 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.

To overcome the unpredictable occurrence of test winters and to expand evaluations year-round, a number of laboratory-based approaches to measure freeze tolerance have been developed. Our objective was to determine relative freeze-tolerance levels of recently released and standard varieties using laboratory-based methodology.

Bermudagrass plants were vegetatively established and acclimated in growth chambers. Experiments characterizing freeze tolerance of Midlawn, OKS 18-4, Princess, Riviera, Tifway, Tifsport, and U-3 (obtained from Ken Diesburg, Southern Illinois Univ.) were conducted in a convection freeze chamber. Following an overnight soak at -2.5 (C) the chamber was cooled linearly at one degree (C) per hour. Four samples from each genotype were removed when they reached the target temperature.

Target temperatures (C) intervals covered a range anticipated to span complete survival to complete mortality. The midpoints (T_{mid}) of survival vs temperature curves were determined by nonlinear regression .



At Oklahoma State University, significant improvements in cold-temperature tolerance was made using a freeze chamber selection method.

Bermudagrasses ranged in freeze tolerance from Princess (-6.9 C) to Midlawn (-10.3 C). Tifway and Tifsport (-7.9 C) were significantly hardier than Princess, but had less freeze tolerance than U-3 (-8.9 C) OKS 18-4 (-9.7 C) and Midlawn. Riviera was significantly hardier than Princess, but less freeze tolerant than OKS 18-4 and Midlawn. Midlawn was significantly hardier than all varieties except OKS 18-4.

We have evidence showing that some chitinases are synthesized in larger amounts in response to cold acclimation in the freezing tolerant cultivar Midiron than in the moderately freeze tolerant Tifgreen bermudagrass. It is now hypothesized that the biochemical basis for the involvement of PR proteins in freezing tolerance is by virtue of their structural ability to bind to growing extracellular ice crystals, thereby preventing further crystallization.

In our previous progress reports, we have reported the isolation and sequencing of two chitinase genes in bermudagrass, CynCHT1 and CynCHT2. The salient features of these two PR genes encoding chitinases are consistent with the possibility that they may be involved in freeze-tolerance mechanisms. A major question that needs to be answered concerns the magnitude of increase in cold hardiness if a chitinase gene is over-expressed without cold acclimation. To address this question, we will over-express the bermudagrass chitinase gene (CynCHT1) in a suitable *Arabidopsis* ecotype. Chitinase overproducing transgenic plants will be evaluated at the phenotypic, biochemical and genetic levels in order to determine the role of chitinases in freeze tolerance.

Summary Points

- Established cold hardiness rankings for several commercial varieties.
- Discovered bermudagrass chitinase genes (CynCht1, CynCht2) and established their role in winter hardiness and resistance to spring dead spot.
- Developed a CynCht1 plasmid for conducting *Agrobacterium* transformation.
- Performing functional analysis on the products of low-temperature inducible CynCht1 gene.