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

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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 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. 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.

The low temperature tolerance (LT_{50}) of 11 turf bermudagrasses was evaluated. LT_{50} values (^{O}C) for clonal varieties were: GN-I =-5.8, Baby = -6. 1, Tifway = -6.6, Tifton 94, Quickstand = -8.0, and Midlawn = -8.4. LT_{50} values for seeded varieties were: Arizona Common = -5.6, Mirage = -6.1, Jackpot = -6.3', Guymon = -7.4, and OKS 91-11 = -7.6. These evaluations will continue with selected varieties from: 1) vegetatively-propagated fairway types, 2) seeded fairway types, 3) vegetatively-propagated putting, green types, and 4) experimental fairway breeding lines.

Cold-regulated (*Cor*) bermudagrass genes were identified. A *Cynodon* genomic library was constructed from Midiron (*Cynodon dactylon X Cynodon 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 chitinase genes, which we designated as *CynCht-1*, *CynCht-2* and *CynCht-3*. The nucleotide sequences of *CynCht- 1* and *CynCht-2* are in the European Molecular Biology Laboratory (EMBL),Genbank and DNA DataBase of Japan (DDBJ) databases as accession numbers AF 105425 and AF 105426, respectively.

The expression of bermudagrass chitinases during cold acclimation (CA), drought, and exogenous application of abscisic acid were determined. Expression was investigated in three cultivars that differ in cold hardiness (MSU > Midiron > Uganda). Northern blot analysis indicated that chitinase gene expression is regulated by cold acclimation (CA) at 8°C/2°C day/night temperature cycles. The level of chitinase gene expression varied positively with cold hardiness. The MSU cultivar exhibited the highest level of low temperature- induced accumulation of chitinase mRNA. The low temperature-induced transcript accumulation was reversed by deacclimation at ambient temperatures immediately following the acclimation period. Drought stress induced the expression of the chitinase genes in the three cultivars at levels significantly higher than the levels induced by CA. Exogenous application of abscisic acid also induced chitinase gene expression but only in Midiron and MSU. The ABA-induced expression occurred at much lower levels compared to those resulting from CA and drought. Midiron exhibited a slightly higher level of ABA-induced chitinase transcript accumulation than MSU. CA-, drought- and ABA-induced gene expressions were specific to the crown tissues.