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

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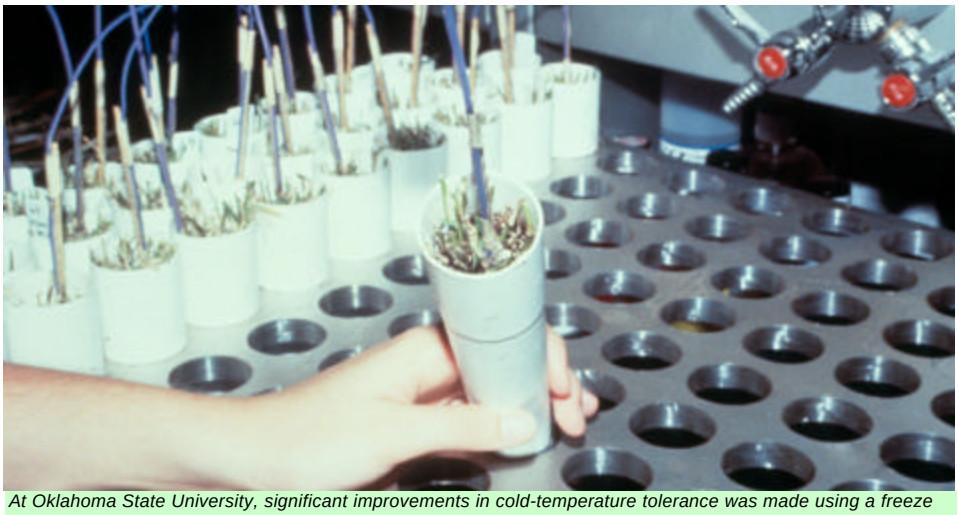
Objectives:

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.

Start Date: 1998
Project Duration: 5 years
Total Funding: $125,000



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

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 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 for clonal varieties were: GN-1 =-5.8 C, Baby = -6.1 C, TifWay = -6.6 C, Quickstand = -8.0 C, and Midlawn = -8.4 C. LT_{50} values for seeded varieties were: Arizona Common = -5.6 C, Mirage = -6.1 C, Jackpot = -6.3 C, Guymon = -7.4 C, and OKS 91-11 = -7.6 C. 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.

The primary structure of the pre-protein encoded by the bermudagrass chitinase genes (CynChtl, CynCht2) were analyzed. Both chitinase genes encode low molecular weight hydrophilic (secreted) proteins, which can be structurally classified as Class II chitinases. The mature polypeptide of CynChtl is composed of 227 amino acid residues with a molecular weight of 25 kDa and calculated pI of 8.10. CynCht2 mature polypeptide, on the other hand, consists of 229 amino acids with a molecular weight of 25.5 kDa and calculated pI of 8.82.

Alignment of the amino acid sequences of the mature polypeptides encoded by the two genes revealed significant homology with a number of known chitinases from higher plants. Both chitinases are most closely related to the Class H chitinases from peanut and tomato.

Functional analysis of the products of the low-temperature inducible CynChtl gene via *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* is underway. Selection of a suitable *Arabidopsis* ecotype has been conducted. Results suggested that the T_{mid} values (using electrolyte leakage test) slightly differ between the non-acclimated and cold acclimated plants, and there is no significant difference in the cold hardiness of the ten ecotypes evaluated so far.

The plasmid that will be used for *Agrobacterium*-mediated transformation (via the binary system) is being constructed. The coding region (1.2 kb) of the CynChtl from the main clone Stul 456-1 was PCR-amplified using forward and reverse primers.

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