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
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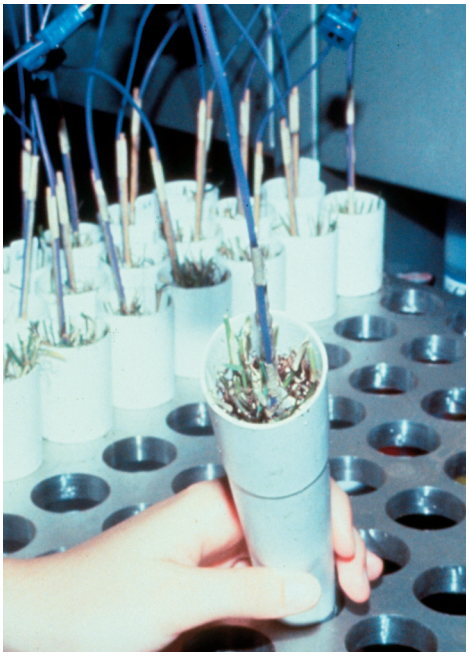
Injury to bermudagrass turf caused by freezing temperatures during winter is a persistent problem over much of its geographic area of use in the USA. This research seeks to reduce risk of freeze injury to bermudagrass grown in temperate regions. The research focuses on accurately assessing the freeze tolerance of bermudagrass varieties, 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.

Initially, the seeded, and putting green categories, was determined. The T_{mid} values (C; midpoint of survival vs. temperature curves determined by nonlinear regression) for the varieties were: Fairway; 'GN-1' (-5.9), 'Baby' (- 6.7), 'Tifway' (-6.7), 'TifSport' (-7.2), 'Quickstand' (-8.0), 'Midlawn' (-8.4), Seeded; 'Arizona Common' (-5.6), 'Mirage' (6.1), 'Jackpot' (-6.3), 'Guymon' (-7.4), 'Yukon' (-7.6), Putting Green 'Champion' (-4.8), 'Floradwarf'(-4.9), 'MS-Supreme' (-5.2), 'MiniVerde' (-5.8), 'Tifeagle' (-6.0), 'Tifdwarf' (-6.5), and 'Tifgreen' (-6.5).



At Oklahoma State University, the freeze tolerance of 18 standard and recently released turf bermudagrass varieties are being evaluated.

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The molecular genetic mechanisms regulating freeze tolerance in plants are complex and incompletely understood though the knowledge base of these mechanisms is steadily increasing. The ability to enhance freeze tolerance via recombinant DNA technology would have enormous beneficial impact.

We have determined that freeze-tolerant bermudagrass plants synthesize some chitinase proteins in larger amounts in response to cold acclimation than do less hardy plants. Two class II chitinase genes, designated CynCHT1 and CynCHT2, were isolated from 'Midiron' turf bermudagrass and sequenced. Subsequent research demonstrated that CynCHT1 is expressed at higher levels in crown tissues of more freeze-tolerant 'MSU' and 'Midiron' bermudagrasses than in freeze-sensitive 'Zebra' bermudagrass in response to cold acclimation, drought, or abscisic acid. *Arabidopis thaliana* ecotype RLD was transformed with CynCHT1.

Selfing and screening procedures applied over the past year have produced plants homozygous for the gene. Current research seeks to determine if over-expression of the CynCHT1 gene will enhance freeze tolerance, and if the CynCHT1 protein has antifreeze properties. 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 commercial turf bermudagrass varieties.
- Isolated and sequenced two genes, CynCht1, CynCht2, coding for class II chitinase proteins.
- Established that CynCHT1 is expressed in response to cold acclimating temperatures, drought, and abscisic acid and at greater levels in freeze-tolerant bermudagrass plants than in freeze-susceptible plants.
- Overexpression of the CynCHT1 gene in transformed *Arabidopsis thaliana is* currently being studied to determine the effects on freeze tolerance.