

# Low Temperature and Drought Regulated Gene Expression in Bermudagrass

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## Goals:

- *Isolate cDNA clones of genes preferentially transcribed under conditions of low temperature or related water stress.*
- *Characterize their stress-specific expression.*
- *Determine the primary molecular structure of these clones.*
- *Isolate the corresponding genomic clones that contain the inducible response elements(s).*

Cellular membranes have been considered a primary site of freezing injury, and alterations of membrane composition correlate with cold acclimation processes that allow plants to tolerate freezing temperatures. As major components in membrane bilayers, the polar lipid fatty acids could directly regulate membrane structure, and therefore membrane function, through the alterations of acyl chain length (number of carbon atoms) and/or unsaturation (number of double bonds). Alterations in plant membrane lipid fatty acids can be induced by many physiological and environmental factors, and these changes could play an important role in adaptation to low temperature.

Bermudagrass, *Cynodon dactylon*, shows an increased tolerance to cold after a period of exposure to moderately low temperatures. However, whether this cold acclimation correlates with cell membrane alterations, and how the membrane lipid fatty acids respond to low temperature, are unknown for bermudagrass.

MIDIRON and U3 bermudagrass were exposed to conditions known to induce cold acclimation [e.g., 8/3°C (day/night) temperature, 10 hr photoperiod, 250  $\mu\text{mol}/\text{m}^2/\text{s}$  photosynthetic photon flux density] for one or four week time periods. Tissues (leaves, crowns and roots) were harvested and total lipids isolated by organic extraction. The polar fraction was purified by thin layer chromatography. The fatty acids (FA) were released from the polar lipids by saponification, and then converted to FA-methyl esters. Separation and

identification of individual FAs within the mixture was accomplished by gas chromatography, with the aid of an automated, computer-based identification system.

Different organs of the same plant responded differentially to low temperature. Crowns showed the most dramatic and sustained increase in total FA content. Overall, significantly greater than 95% of the polar FA content was accounted for by four FA species: palmitic acid (16:0), stearic acid (18:0), linoleic acid (18:2) and linolenic acid (18:3).

MIDIRON (relatively cold-tolerant) responded more rapidly and to a greater extent than did U3 (relatively cold-sensitive) for the changes in FA composition documented in this study. This was illustrated by the nearly four-fold increase of unsaturated:saturated FA ratio for MIDIRON over U3, and by the significant difference between the double bond index of the two genotypes, at the end of the cold acclimation treatment. Our results point to specific desaturase enzymes (e.g., W-3 and A-12) as being of primary importance in controlling membrane lipid-FA composition in response to low temperature, and ultimately in avoiding the winter damage suffered by bermudagrass along its northern zone of adaptation.

Differential display/RNA profiling has been used to identify genes expressed in bermudagrass in response to low temperature (i.e., during the process of cold acclimation). This procedure uses total or poly(A) + RNA as a substrate for the synthesis of cDNA

molecules (i.e., partial gene clones) expressed in the particular organ, developmental stage or treatment from which the RNA was isolated. Additionally, this method uses a set of *random* anchor and 10-mer oligonucleotide primers to initiate clone synthesis.

By comparing gel *displays* from 0, 24 and 48 hrs post-low temperature exposure to that from control non-acclimating tissue over the same time frame, four sequences putatively expressed in response to low temperature were identified. These sequences have been subcloned for further analysis and characterization; such as DNA sequence determination and their use as probes on Northern blots to confirm their differential, cold-specific expression.

In addition to continuing this avenue of investigation, we are designing degenerate (sequence variable) oligonucleotide primers to conserved sequence regions of fatty acid biosynthesis genes for use in differential display experiments and in reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.