

# 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 (MLFA) respond to low temperature are unknown for bermudagrass.

Bermudagrass total MLFA (ug), per unit of total lipids (mg), increased in crown tissues, but not in roots or leaves, over the four week exposure to moderately low temperatures (46°F day /37°F night, 14 hour photoperiod). The major fatty acids in bermudagrass were determined to be palmitic acid, stearic acid, linoleic acid, and linolenic acid. These four made up 95% of the total MLFA.

In bermudagrass crown tissues, the concentration of shorter chain and saturated fatty acids declined significantly during the cold treatment, while the concentration of the longer chain, unsaturated fatty acids increased. As a result, the double bond index increased in crown tissues over this same four-week period. These changes increase

the fluidity of membranes, and, therefore, could reduce cold-induced membrane leakage and freezing injury.

Messenger mRNA profiling and differential display techniques are being refined and employed in our efforts to characterize genetic polymorphisms between bermudagrass cultivars differing in levels of cold tolerance. These PCR-based methods allow for the relatively rapid identification and cloning of gene sequences expressed in response to a particular environmental stimulus such as low temperature, drought or chemical applications. The reproducibility of this technique and the identification of the appropriate mRNA or DNA primers has been the focus of our current efforts.