# Low Temperature and Drought Regulated Gene Expression in Bermudagrass

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**Goals:**

- *Characterize membrane-specific changes in fatty acid and lipid composition during cold-acclimation.*
- *Characterize the expression of genes involved in fatty acid and lipid biosynthesis during cold acclimation, using heterologous gene probes.*
- *Clone fatty acid biosynthesis genes (e.g., desaturase) expressed in bermudagrass in response to low temperature*

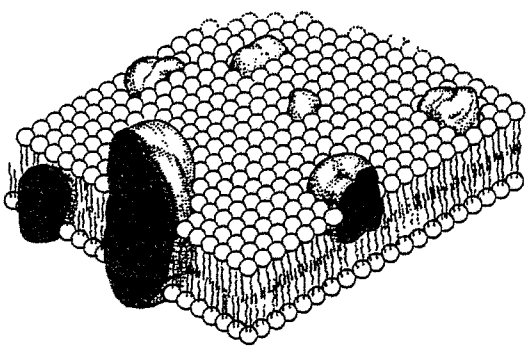

**Figure 3. The lipid bilayer provides the necessary environment for proper functioning of membrane proteins.**

The physical and biochemical changes, which occur in chilling sensitive plants exposed to reduced temperature, together with the subsequent expression of low temperature stress symptoms, are collectively referred to as cold or chilling injury. The physiological change includes alterations in cytoplasmic streaming, enzyme activity, respiration and photosynthesis as well as effects on membrane permeability, structure and composition. Which, if any, of these changes are responsible for the primary low temperature induced injury, remains uncertain.

The primary goal of this project is to gain a more complete understanding of the process of cold acclimation in bermudagrass by characterizing the changes in membrane composition and gene expression that accompany exposure to low temperature.

Disruption of cellular membrane integrity, as a result of low temperature-induced water removal, is a primary cause of cold injury. The lipid bilayer (Figure 3) provides the necessary environment for proper functioning of membrane proteins. Membrane lipid *fluidity* is thought to be one of the prerequisites for unimpaired survival at low temperature. Membrane fluidity is affected by the degree of unsaturation (i.e., number of double bonds) in the constituent fatty acid (FA) side-chains of membrane lipids. These double bonds induce *kinks* in the molecules (Figure 4), thus resisting molecular compaction, and maintaining fluidity. Therefore, increase in FA unsaturation

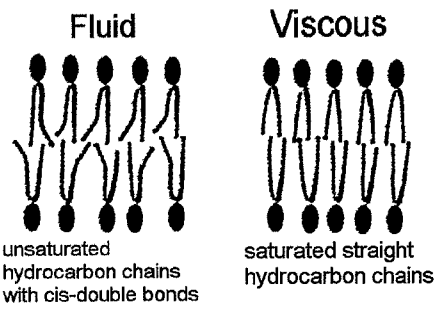


**Figure 4. Double bonds in unsaturated hydrocarbon chains increase the fluidity of a phospholipid bilayer by making it more difficult to pack the chains together.**

reduces the temperature at which membranes undergo damaging, dehydration-induced, phase transitions from a flexible to a more rigid, gel-like, state.

MIDIRON and U3 bermudagrass were exposed to conditions that induce cold acclimation, and crown tissue from rhizomes was harvested for total membrane lipid isolation. Fatty acid analysis of total lipids revealed a significant increase in tri-unsaturated species (i.e., linolenic acid; C18:3), over shorter and saturated species, and an overall increase in the double-bond index. These changes were more pronounced in MIDIRON (relatively cold tolerant) than in U3 (relatively cold sensitive) bermudagrass, during cold acclimation.

Preliminary biochemical analysis of the isolated membrane lipids identified neutral lipids, glycolipids and phospholipids. At least four species of neutral lipids were detected, two of which were in abundance. Three glycolipids species were detected, in equlmolar amounts. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyl glycerol (PG) and phosphatidylserine (PS) were the four phospholipids detected. Their relative abundance is PC >> PE = PG = PS.

Differential display is used to identify genes expressed in bermudagrass during cold acclimation. Comparisons of *displays* from 0, 12, 24, 48 and 72 hours post-low temperature exposure to that from non-acclimating tissue over the same time period, allows for the identification of genes differentially expressed between treatments, time points and/or cultivars. The cDNA clones obtained represent both RAPD-type fragments, those with 0-mer/complimentary sequences on the ends, as well as gene fragments derived from amplifications utilizing both the anchor (e.g., $T_{11}$AG or $T_{11}$AC) and a specific 10-mer. The latter have an easily recognizable poly(A) addition signal, just 5' of the anchor primer sequence. The clones range in size from 300 to 450 base pairs. All were chosen as *up-regulated* genes from differential display gels.

Searching DNA and protein databases with translations of these sequences failed to show significant homology to any previously cloned gene or protein/peptide sequence. This is to be expected for at least two reasons: 1) since the clones are biased toward the 3' untranslated end of a gene transcript, protein databases or those containing gene sequences from genomic or random-primed cDNA libraries may not have sequence data for this region of any of their clones; and 2) since, to date, few genes whose expression is regulated during cold acclimation have been cloned from any organism, such genes would be under-represented in current databases (i.e., we are cloning new, undiscovered genes).