*EST Analysis and Gene Discovery in Perennial Ryegrass (*Lolium perenne *L.) in Response to Cold*

Shui-zhang Fei
Iowa State University

Objectives:

1. To identify genes that play critical roles in cold hardening and freezing tolerance in perennial ryegrass by analyzing the frequencies of various expressed sequence tags (ESTs) from cold-acclimated (CA) and non-cold acclimated (NA) cDNA libraries and by probing the mRNA populations from CA and NA perennial ryegrass using the Affymetrix barley gene chip.
2. To isolate and functionally characterize genes that are most responsive to cold acclimation.

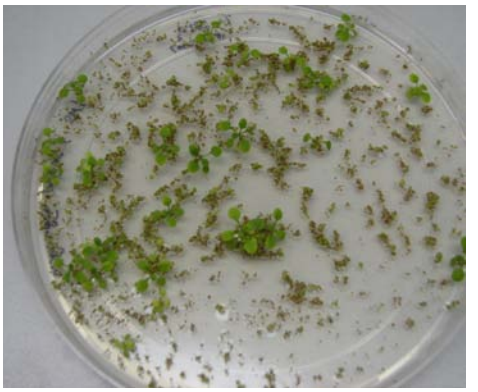
Start Date: 2006
Project Duration: three years
Total Funding: $89,784

It is important to understand the underlining molecular mechanisms that regulate stress tolerance. The development of sequencing technology has made it possible to take a systems approach to study the roles each gene plays and their *in vivo* interactions. This can be done by using either the analysis of expressed sequence tags (ESTs) or microarray, which generate the gene expression profiles in stressed or non-stressed plants by examining what genes are turned on and off and to what degrees in response to a particular environment stimulus.

Two cDNA libraries, one from leaf tissues of a cold acclimated (CA) perennial ryegrass plant ('Caddyshack') and the other from a non-acclimated (NA) plant of the same genotype were constructed. A total of 4,159 bacterial clones containing cDNA inserts were randomly selected and ESTs of the cDNA inserts were sequenced. A total of 1,912 ESTs found at least one match based on the set criteria against the NCBI database. A large number of genes are shown to be significantly up-regulated or down-regulated in cold-acclimated perennial ryegrass compared to the non-acclimated perennial ryegrass.

We used reverse transcriptase PCR (RT-PCR) to test a subset of 24 selected genes that have been shown to differentially express in cold-treated perennial ryegrass. RT-PCR results showed that 20 out of the 24 selected genes had similar expression trends as revealed by the EST analysis; therefore, suggesting that small-scale EST analysis can be an effective tool for gene expression profiling.

Because of the lack of Affymetrix chips specifically designed for perennial ryegrass, we used the barley Affymetrix chips to probe the gene expression profiling in perennial ryegrass. We optimized the microarray procedure prior to the initiation of the experiment. Total RNA was isolated from the control (0 hour cold treatment), 1- and 7 - day cold treated leaf tissues. Purified RNA was used for cDNA synthesis and subsequent labeling and hybridization to the 22K barley gene chip. Preliminary analysis indicated that many differentially expressed genes showed similar expression trend as observed in the EST analysis.



T2 generation of the Arabidopsis *overexpressing either IRI-1 or IRI-2 gene was obtained and will be subjected to analysis including the measurement of ion leakage and survival rate under low temperatures.*

ESTs encoding two ice recrystallization inhibition proteins (IRIPs), also called antifreeze proteins (AFPs) were present at high frequencies in the CA library but not in the NA library, similar to microarray and RT-PCR results. One of the functions of plant AFPs is to inhibit ice recrystallization by binding to the small crystals, thereby preventing it from forming larger ice crystals which can be very injurious to plant cells.

The full-length coding regions of two IRI genes, designated LpIRI-1 and LpIRI-2 were obtained by sequencing the corresponding cDNA clones from both 5' and 3' ends. Both IRI-1 and IRI-2 genes have high similarities with the published partial LpIRI gene obtained from perennial ryegrass and TaIRI-1 and TaIRI-2 obtained from wheat. Part of the IRIP (from amino acid 139 to 256) showed 99% identity and 100% positives to the published partial LpIRIP (from amino acid 1 to118) with only one substitution of aspartic acid by asparagine at amino acid 33.

The cDNAs of the LpIRI-1 and LpIRI-2 were introduced into the pMDC 32 vector and into the *Agrobacterium tumefaciens* strain GV 3101. The genes are under the control of a double 35S promoter. *Arabidopsis* plants reaching the reproductive stage were infected by the *Agrobacterium* and transgenic plants were selected with hygromycin. T2 generation of the *Arabidopsis* overexpressing either IRI-1 or IRI-2 gene was obtained and will be subjected to analysis including the measurement of ion leakage and survival rate under low temperatures.

Summary Points

- A total of 1,912 expressed sequence tags (ESTs) from the cold-acclimated and non -acclimated libraries found at least one match in the NCBI database; analysis of the expression profiles revealed that many stress-related genes are differentially expressed in cold-acclimated perennial ryegrass; RT-PCR of a set of selected genes suggested that EST analysis is an effective way of gene profiling.
- Microarray was performed on RNA isolated from non- acclimated control, 1- and 7-day cold-acclimated perennial ryegrass by using the barley Affymetrix gene chips; array data is currently being analyzed.
- Two antifreeze protein genes LpIRI 1 and LpIRI 2 were isolated from perennial ryegrass; T2 generation of the transgenic *Arabidopsis* overexpressing the cDNAs of these two genes was obtained and the functions of these two genes are currently being assessed.