Molecular Approaches to Understanding Stress Tolerance in Perennial Ryegrass

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Objectives:

1. Determine gene expression profiles in perennial ryegrass in response to abiotic stresses by sequencing of expressed sequence tags (ESTs) and by performing microarray analysis.

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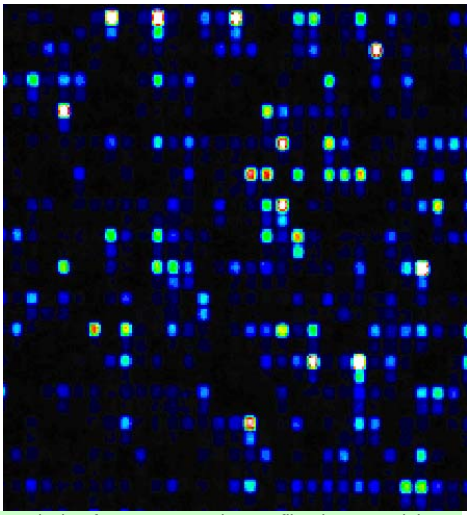
To develop stress tolerant perennial ryegrass germplasm, it is important to understand the underlining mechanisms responsible for stress tolerance at the molecular level. The development of high-throughput sequencing has made this possible by using either microarray or analysis of expressed sequence tags (ESTs) to probe the global 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 stress. This systems approach will provide important insights into the roles that certain genes may play in stress responses and will facilitate dissecting complex genetic mechanisms that regulate developmental and physiological responses.

Two cDNA libraries, one from leaf tissues of a cold acclimated (CA) perennial ryegrass plant (cultivar 'Caddyshack') and the other from leaf tissues of a non-acclimated (NA) plant of the same genotype were constructed. The insertion size of the libraries was examined by PCR of 24 random clones. The libraries were amplified using semi-solid amplification methods and stored at a -80 °C freezer for long-term use. Isolation of plasmid DNA and subsequent sequencing of the cDNAs were performed. In collaboration with Dr. Scott Warnke at USDA-Beltsville, we have sequenced ~1,450 ESTs from the NA control library and 1,500 ESTs from the cold-treated library. Sequencing of additional ESTs is in progress. EST sequences are searched against GenBank, the leading DNA sequence database to find out what genes are turned on or off in perennial ryegrass in response to cold.

Cold responsive gene family and other stress responsive genes were highly expressed in the CA library. Other genes encoding for proteins such as leucine rich repeat family protein, ice recrystallization



Analysis of gene expression profiles in perennial ryegrass using a 22K barley Affymetrix GeneChip microarray

inhibition protein 1, thioredoxin family protein, ribulose 1, 5- bisphosphate carboxylase activase, protein kinase protein were represented two to five times more frequent in the EST sample of the CA library than in that of the control library. On the other hand, genes encoding for the lectin/ high light proteins was represented only once in the EST sample of the CA library, but was represented 43 times in the EST sample of the control library. The genes encoding for phosphoribulokinase, protein kinase Xa21, Drm3 [*Pisum sativum*]/auxin-repressed protein, and serine/threonine kinase were observed six to eight times more frequently in the EST sample of the control library than in that of the CA library. Other genes encoding for proteins such as jasmonate induced protein, oxygen-evolving enhancer protein 2 or receptor kinase LRK14 were also observed three to five times more in the EST sample of the control library than in that of the CA library.

Some genes appear equally expressed in both libraries. For example, photosystem II 10 KD polypeptide gene is the most abundant and equally expressed gene in both libraries. Genes encoding for metallothionein, ribosomal and zinc finger proteins and chlorophyll A-B binding protein- pfam00504 were also observed with near equal frequency in both libraries.

A preliminary microarray experiment was done using the perennial ryegrass cDNA to hybridize with a 22K barley Affymetrix GeneChip. The results showed that 1,306 genes (6.0 %) are differentially expressed in the one-day cold-treated material. Many interesting genes are highly turned on by the one-day cold acclimation treatment. These include the genes encoding for proteins of dehydrin 4, zinc finger transcription factor, chloroplast inner envelope protein, or auxin binding protein which were all up-regulated by more than 15 times compared with those in the non-cold treated tissues. A total of 396 genes were up-regulated by cold acclimation by at least 2 folds.

Meanwhile expression of many other genes was down-regulated by the 1-day cold acclimation treatment. For example, thaumatin-like protein, UDP-N_acetylmuramoylananyl-D-glutamate-2, 6-diminppimelatese, or RNA-binding protein, among others were down-regulated by more than 20 folds. A total of 501 genes were down-regulated by cold acclimation by at least two folds.

Summary Points

- Two cDNA libraries, one constructed from a non-cold treated plant, the other from cold-treated plant are constructed. A total of close to 3,000 expressed sequence tags are sequenced and will be deposited to the GenBank.
- Some genes that are differentially expressed in the cold-treated plant will be characterized.
- A preliminary microarray experiment with a 22K barley Affymetrix GeneChip has been performed with RNAs from cold treated and non-treated tissues of perennial ryegrass. About 1'300 genes (6.0 %) are differentially expressed in the 1- day cold-treated material. Optimization of the washing conditions will be conducted to increase the hybridization rate.