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FY 97 FINAL REPORT

1. PROJECT TITLE: Protecting the Fiber Supply Through Genetic Engineering of Drought Tolerance, (PP97-FS-5)

2. PRINCIPAL INVESTIGATOR: Dr. John Cairney

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3. EXECUTIVE SUMMARY OF WORK COMPLETED

- Mechanisms of gene regulation under drought-stress have been investigated in the drought-hardy desert shrub *Atriplex canescens* with a view to transferring understanding (and gene sequences) into a Forest Species of commercial importance to Georgia
- Several truncated versions of the 3' untranslated regions from genomic clones, thought to be important in stability and/or translatability of mRNA under drought-stress, have been created and cloned into plant transformation vector (replacing NOS 3'UTR).. This has been done for two different genes bearing or lacking sequences thought to influence mRNA stability and/or translation.
- In similar fashion, constructs containing different lengths of two different promoter sequence have been constructed.
- Coding regions of the Proteinase Inhibitor gene have been cloned into an expression vector
- Preparations have been made for transformation in woody species and model plants
- The extensive and meticulous work described above will allow us, in future months, to determine features important in the accurate and timely expression of a drought inducible gene, and by application of this knowledge allow us to design vectors which will optimize expression of genes of choice.
- Evidence of a novel gene regulatory mechanism has been discovered in Loblolly Pine (*Pinus taeda*)
- PCR-based methods are being used to isolate the genes for more detailed analysis
- Two Research Papers are in preparation, one Poster Abstract has been Published (see attached)
- One Seminar and Three Poster presentations will be made at International Meetings within the next three months
- A Post-Doctoral Scientist with several year experience in International Research laboratories has been recruited to work on the project and a Ph.D. student has been supported by IPST
- DNA Vectors have been constructed which will be of general use to other scientists

Protecting the Fiber Supply Through Genetic Engineering of Drought Tolerance

Introduction

Drought is a persistent problem in Plantation Forestry and the remedies of continual irrigation can be costly. Several advances have been made in the understanding of how plants respond to water stress by activating defense and repair genes and success has been achieved with agronomic crops in enhancing salt tolerance in lab trials, by specific gene transfer. When designing tree improvement programs and introducing novel approaches such as gene transfer to tree improvement, one must recognize that trees are growing under continual and fluctuating stress and make allowances for this factor. Many disappointments have occurred in transfer experiments when successfully integrated genes failed to produce mRNA or protein as expected. The events subsequent to transcription are now beginning to be analyzed.

In this project we are analyzing sequences which may optimize the expression of a particulare mRNA under drought stress. Once their effect is demonstrated, these sequences can be introduced into gene expression vectors to allow genes of choice to be expressed with greater accuracy.

Background

The desert shrub *Atriplex canescens* (saltbush) is know to be extremely drought-resistant and may possess unique genes or novel mechanisms of drought tolerance. To understand the molecular basis of this tolerance a number of cDNA clones were isolated by differential screening of cDNA libraries. Members of a drought-induced multigene family were chosen for further study. These cDNAs were divided into two classes based on sequence data. The clones differed in the length of their 3'UTR: the longer class contained a long AU-rich motif which was absent from the shorter class - a possible consequence of alternative sites of pre-mRNA cleavage/polyadenylation. Under severe desiccation, more than one transcript was observed in Northerns. RNaseH experiments suggest that differences in transcript size may be attributable to differences in poly(A) tail length. The exact role of the AU-rich region in poly(A) tail differences merits further investigation. To gain insight into the function of this motif, genomic clones corresponding to each class were isolated and characterized in detail.

Summary of Accomplished Goals

Construction of 3'UTR deletion derivatives

Using basic procedures of gene manipulation fragments of the 3' UTR region of the AU-rich class gene (clone 18-1) have been spliced downstream of a 35S-GUS cassette to result in a total of six constucts. Sequences immediately downstream of the GUS coding region from pGUS101, approx. 60 bp, were first deleted and convenient sites were engineered to splice the corresponding 3'UTR *Atriplex* fragments. An initial 1.5 kb fragment was progressively deleted, in the 3' - 5' direction, using convenient sites to create five deletion derivatives. Their effect both on GUS mRNA stability and translatability will be examined in transgenic plants. The cassettes have been transferred into the pBIN19 binary vectors and the resulting constructs are being introduced into *Arabidopsis* plants. Also, transient expression assays using *Atriplex* and *Arabidopsis* cell suspensions-derived protoplasts are being started. Conditions necessary for electroporation are being adjusted.

Construction of Promoter deletion derivatives

Another part of this project is the promoter analysis of two genes induced during the plant response to drought-stress in the desert shrub *Atriplex canescens*. Genomic clones 18-1 and 12-95 contain 1.3 and 2.4 kb promoter sequences respectively. Several constructs including full-length and deletion mutants of these promters haven been made and fused to the GUS reporter gene. In both cases the GUS gene was fused in-frame a few codons downstream from the AUG initiator codon. Similarly, two extra constructs contain the full length promoters all the way to the first few codons of the second exon fused also in-frame upstream of the reporter gene. These cassettes have been transferred to the binary vector pBIN19 and are currently being introduced into Arabidopsis plants. Also, work on gain-of-function constructs is being conducted by placing 150-200 bp overlapping promoter fragments in an optimized CaMV35S minimal promoter obtained from Bernd Weisshaar (Max Planck, Cologne, Germany).

These sets of constructs will give us information about the tissue and organ expression of these two genes during drought-stress. However, in order to gain a more complete understanding of the mechanisms involved in the regulation of these two genes we will also use cell suspension-derived protoplats from *Atriplex canescens* that will allow us to test the promoter fusions using optimized transient assays.

Preliminary work to approximate the total number of these genes in the genome of *Atriplex canescens* has been started by designing primers flanking the unique intron. A preparatory amplification resulted in two bands that correponded to the introns present separately in 12-95 and 18-1 genomic clones. We think that all genomic isoforms fall into these two classes and after cloning and sequencing a determinate number of independent clones containing these two bands we will have a good approximation to the total number of these isoforms.

Establishment of Arabidopsis and Atriplex cell suspensions

Following protocols described in the literature we have been able to establish Arabidopsis cell suspensions obtained from leaves, stems and roots of Arabidopsis seedlings (var. Columbia). Similarly, we have also established cell suspensions from Atriplex canescens seedlings germinated in vitro. After several weeks in callus induction media (WNA plus hormones) friable calli were obtained and transferred to liquid media. Succesive transfers are being made to obtain finer cell aggregates.

Preliminary examination into a gene down-regulated in response to drought stress from loblolly pine (*Pinus taeda*)

Previous experiments in the group showed that a cDNA, LP6, isolated by differential hybridization from loblolly pine, consisted of a very long 5' UTR and that the longest open reading frame (ORF) does not start until nucleotide 721. LP6, was found to be strongly expressed in roots and stems of well watered plants, but mRNA levels decline rapidly as plants dehydrate. Different experiments confirmed that LP6 is derived from a single mRNA molecule. The protein shows strong homology to a number of Class I chitinases from bean, tobacco, and poplar; however the similarity is only with the carboxy half of these proteins. A number of inverted repeats (IR's) have been identified in the putative 5'-UTR of LP6 mRNA. Secondary structure energies suggest that four stem-loops formed by these IR's would be of moderate to high stability. Also, up to 6 ORF's have been recognized in the 5'-UTR, two in each reading frame (Plant Mol. Biol. 31: 693-699, 1996). A series of chimeric constructs, placing the full length 5'-UTR and deletion derivatives upstream of the GUS reporter gene, have been made. The use of these CaMV35S/5'UTR/GUS/NOS3' constructs in transient expression assays and in *in planta* experiments will provide information as to the role of this unusual long 5'UTR in the post-transcriptional (if any) regulation of this gene.

A preliminary look into the gene structure has rendered some intriguing results. When primers designed to amplify the second third of the cDNA are used in genomic PCR's no products are observed even when LD-PCR conditions are used (up to 10 kb). These results are consistent either with the presence of a rather long intron (very unsusual in plants) or with transplicing. When total RNA from different stages of development of loblolly pine somatic embryos are used in RT-PCR experiments only the presence of the first third of the message is detected suggesting that the long 5'UTR might be spliced upstream of different messages. Experiments to confirm the gene structure and the expression pattern of this fascinating gene are currently underway. Promoter libraries of loblolly pine DNA are being made and will be used to characterize the promoter and the "intervening sequence". Also, to test the role of the chitinase homolog during drought stress an overexpression construct is being introduced into tobacco plants. Likewise, different expression vectors are being tested to obtain enough protein to generate antibodies. Non-embryonic loblolly pine cell suspension cultures are also being generated to complement the molecular approach. The long term goal is to study the 5'UTR in detail and the characterization of any protein factor(s) involved in its regulation. If the presence of transplicing is confirmed it would be interesting to characterize the mechanisms involved in its control and test if this can be study in an heterologous system such as Arabidopsis. Efforts to isolate LP6-like sequences in Arabidopsis are also underway.

4. DELIVERABLES

Major Milestones & Dates	Original Proposal	Actual
1. 3'UTR Constructs. Isolate 3' untranslated regions from genomic clones, truncate these and clone into plant transformation vector (replacing NOS 3'UTR). Sequence constructs to confirm structure.	Months 1-3	COMPLETED
2. Protein coding constructs. Isolate protein coding region and clone into plant transformation vectors for transfer of protein coding sequences into plants. Sequence constructs to confirm structure.	Mo. 1-3	COMPLETED
3. Promoter Constructs. Isolate promoters from genomic clones and clone into plant transformation vectors (e.g. GUS vectors) for subsequent transfer into plants. Sequence constructs to confirm structure.	Mo. 2-5	COMPLETED
4. Begin Transformation of 3'UTR Constructs into Arabidopsis and Cottonwood	Mo. 3-6 &12-15	ONGOING
5. Begin Transformation of Protein coding constructs into Arabidopsis and Cottonwood	Mo. 3-6	ONGOING
6. Begin Transformation of Promoter Constructs into Arabidopsis and Cottonwood	Mo. 6-8 &12-15	ONGOING
7. Assay size and quantity of Proteinase Inhibitor RNA in various trees and cell cultures. Conduct RNaseH assays	Mo. 6-9	ONGOING

5. BUDGET

	Total FY 97	6-Month Expended
State Funds	$NA	$NA

	Original Proposal	Actual
Matching Funds	$NA	$NA

6. Additional Information/Results/Graphics can be attached if available

Time Table for the Tasks Proposed for FY 1997 and Potential for Future Work

Title: "Protecting the Fiber Supply Through Genetic Engineering of Drought Tolerance", John Cairney (IPST), Gerald S. Pullman (IPST) (PP97-FS-5)

1. 3'UTR Constructs. Isolate 3' untranslated regions from genomic clones, truncate these and clone into plant transformation vector (replacing NOS 3'UTR). Sequence constructs to confirm structure.
2. Protein coding constructs. Isolate protein coding region and clone into plant transformation vectors for transfer of protein coding sequences into plants. Sequence constructs to confirm structure.
3. Promoter Constructs. Isolate promoters from genomic clones and clone into plant transformation vectors (e.g. GUS vectors) for subsequent transfer into plants. Sequence constructs to confirm structure.
4. Begin Transformation of 3'UTR Constructs into Arabidopsis and Cottonwood
5. Begin Transformation of Protein coding constructs into Arabidopsis and Cottonwood
6. Begin Transformation of Promoter Constructs into Arabidopsis and Cottonwood
7. Assay size and quantity of Proteinase Inhibitor RNA in various trees and cell cultures. Conduct RNaseH assays.
8. Continue to cultivate transgenic plants
9. Expose transgenic plants to various levels of drought stress (and additional stresses as time allows)
10. Assay GUS protein levels and GUS RNA levels and transcript size for transgenic 3'UTR Construct plants
11. Assay GUS protein levels and GUS RNA levels and transcript size for Promoter Construct transgenic plants

Months 1 3 6 9 12 15 18 21 24	Estimated Cost in $
Tasks	
1 xxxxxxx	NA
2 xxxxxxx	NA
3 xxxxxxxx	NA
4 xxxxxx xxxxx	NA
5 xxxxxx	NA
6 xxxxx xxxxx	NA
7 xxxxxxx	NA
8 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx	NA
9 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxx	NA
10 xxxxxxxxxxxxxxxxxxxxx	NA
11 xxxxxxxxxxxxxxx	NA

Potential for Future Work: Objectives 4-11

Sucrose is well known now as an inducer/repressor of several genes, and may have an important role in signal transduction pathway(s) related to perception of stress conditions. Sugar-mediated gene regulation likely represents a central control mechanism mediating energy homeostasis in a wide range of plants. We have used four gene-specific cDNA probes corresponding to ADP-glucose pyrophosphorylase (AGPase), a key enzyme of starch biosynthesis, to study sugar-mediated expression of AGPase transcripts in *Arabidopsis*. Detached leaves were fed with various sugars and sugar alcohols, followed by isolation of total RNA and, subsequently, Northern blot analyses. Two of the genes were found to be dramatically induced by 0.3 M sucrose. Glucose at 0.1 or 0.3 M was much less effective when compared to sucrose. Expression of the third AGPase gene was down-regulated by sucrose levels as low as 0.1 M, whereas the fourth gene appeared not to be affected by various sugar treatments. The presence of several AGPase genes that are differentially regulated by sugars may represent an excellent model system to study sugar-mediated signal transduction pathways in *Arabidopsis*. We are currently using plants expressing antisense hexokinase gene to test the possible involvement of hexokinase in sugar-mediated AGPase-regulation. *This research has been supported by the Swedish Foundation For Strategic Research.*

1295 Session 58, Regulation of Gene Expression

GluB gene expression is differentially suppressed in LGC-1 mutant. Miyahara, K. *National institute of agrobiological resources, M.A.F.F* Takano, T. *National institute of agrobiological resources, M.A.F.F* Kusaba, M. *National institute of agrobiological resources, M.A.F.F* Iida, S. *Chugoku national experimental station, M.A.F.F* Sassa, H. *Yokohama city university* Takaiwa, F. *National institute of agrobiological resources, M.A.F.F* Nishio, T. *National institute of agrobiological resources, M.A.F.F.*

Glutelin forms about 60% of the total protein of rice seed. 57kDa precursor form, undergoing post-translational cleavage, yields acidic(37-39kDa) and basic(22-23kDa) subunits. Glutelin genes consist of multi-gene family and are divided into two classes. We have found a rice mutant, LGC-1, that has a low glutelin and a high prolamin content and is controlled by a single dominant gene. We examined glutelin gene expression in this mutant. Southern blot analysis using GluA and GluB cDNA clone as a probe showed no difference of band pattern between LGC-1and original cultiver 'Nihonmasari'. It is suggested that there is not a large deletion or duplication in the coding region of glutelin genes in LGC-1. Tatal RNA is prepared from developing seed 15 to 18 days after flowering. Northern hybridization pattern showed that, in LGC-1, mRNA level of GluA genes decreased slightly, on the other hand, that of GluB genes became lower than one tenth in 'Nihonmasari'. In 2-D gel electrophoresis, the deletion of two spots was observed among several spots of the glutelin acidic subunits. These two spots are controlled by different genes. Amino acid sequence of these two spots showed that both spots are product of GluB gene. These result suggested that GluB gene expression is differentially suppressed in LGC-1. To examine whether this mutant gene affects glutelin promoter activity, the transformats that have GUS gene controlled by GluB gene promoter were crossed to LGC-1 as pollen parent. The F_1endosperm has the same activity as transformats, suggesting that LGC-1 mutant gene dose not affect GluB gene promoter activity. To clarify the mechanism of the gene suppression in this mutant, we are now doing run-on assay and trying to isolate the mutant gene from the genomic library of LGC-1

1296 Session 58, Regulation of Gene Expression

Abscisic acid -dependent and -independent expression of carrot *Lea* class gene *Dc3* in transgenic tobacco seedlings. Siddiqui, Najeeb U. *Dept. of Horticultural Science, Texas A&M University, College Station, TX 77843* Thomas, Terry L. *Dept. of Biology, Texas A&M University, College Station, TX 77843* Drew, Malcolm C. *Dept. of Horticultural Science, Texas A&M University, College Station, TX 77843*

Plants respond to environmental stresses by specific physiological and biochemical changes. Several plant hormones like abscisic acid (ABA), ethylene, and jasmonic acid (JA) are involved in the transduction of stress signals to bring about cellular changes. We studied the expression of three promoter deletion mutants (-218, -599, and -1312) of *L* ate *E* mryo *A* bundant (*LEA*) class gene *Dc3* fused to GUS in 14 d old transgenic tobacco seedlings in response to dehydration, hypoxia, salinity, exogenous ethylene, and exogenous methyl jasmonate (MeJa). GUS activity was quantified fluorometrically and expression was observed by histochemical staining of the seedlings. An increase in GUS activity was observed in -599 and -1312 in response to dehydration and salinity within 6 h of stress, and at 12 h in response to hypoxia . No increase in endogenous ABA was detected in any of the three mutants even after 24 h of hypoxia. ABA-independent increase in GUS activity was observed when endogenous ABA biosynthesis was blocked by fluridone and plants were exposed to 5 ppm ethylene or 100 uM MeJa. No expression was observed in -218 in response to dehydration, salinity, or MeJa. A mild response was observed mainly in leaves of this mutant in response to ethylene or hypoxia. This suggests that region between -218 and -599 is absolutely necessary for ABA (dehydration adn salinity) and MeJa -dependent expression whereas ethylene mediated expression does not require this region of the promoter. This study shows that although *Dc3* which is an ABA responsive gene can also be expressed by stress signals other than ABA.

1297 mcSession 58, Regulation of Gene Expression

An alfalfa (*Medicago sativa* L.) Beta-amylase gene is down-regulated by cold acclimation and defoliation stress. Gana, Joyce A. *Purdue University* Cunningham, Suzanne M. *Purdue University* Volenec, Jeffrey J. *Purdue University*

Alfalfa (*Medicago sativa* L.) taproots accumulate high concentrations (~8% of soluble protein) of Beta-amylase. To begin understanding its function we have isolated and sequenced a cDNA clone for alfalfa Beta-amylase. The deduced amino acid sequence from the cDNA shows 85 to 95% homology to plant Beta-amylases. The deduced protein contains the three conserved catalytic domains characteristic of Beta-amylases. Northern blot hybridization analysis revealed that transcript levels of Beta-amylase were high in September and October as plants acclimated for winter and declined approximately ten-fold in November and December after shoot tissues were killed by frost. High transcript levels also were observed in taproots of greenhouse-grown alfalfa. Defoliation resulted in taproot starch degradation, but decreased Beta-amylase mRNA levels as early as 6 h after defoliation. Transcript levels continued to decline until they were barely detectable on Days 2, 4 and 8, but transcripts for Beta-amylase began to re-accumulate on Day 12. Patterns of mRNA accumulation and Beta-amylase activity are not consistent with trends in taproot starch degradation. The high abundance of Beta-amylase in taproots, along with decline during shoot regrowth during spring and after defoliation, suggests that Beta-amylase may serve as a vegetative storage protein in alfalfa taproots.

1298 Session 58, Regulation of Gene Expression

Is annexin involved in the low temperature signal transduction in plants? Vazquez-Tello, Alejandro *Université du Québec à Montréal. Département des Sciences Biologiques. C.P. 8888 Succ. "Centre-Ville", Montréal, Québec, Canada. H3C 3P8* Breton, Ghislain *Université du Québec à Montréal. Département des Sciences Biologiques. C.P. 8888 Succ. "Centre-Ville", Montréal, Québec, Canada. H3C* Uozumi, Takeshi *University of Tokyo, Department of Biotechnology, Faculty of Agriculture. Bunkyo-Ku, Yayoi 1-1-1, Tokyo 113* Sarhan, Fathey *Université du Québec à Montréal. Département des Sciences Biologiques. C.P. 8888 Succ. "Centre-Ville", Montréal, Québec, Canada. H3C 3P8*

In our efforts to elucidate the molecular mechanisms regulating gene expression by low temperature (LT) which lead to the development of freezing tolerance, a cDNA library from a cold-acclimated (CA) *Lavatera thuringiaca* was screened by subtractive hybridization. Several cold-regulated cDNAs were isolated. One of them is 1112 bp in length and encodes for a protein that shows high homology with both plant and animal annexins. The annexins are a family of proteins sharing the common property to bind acidic phospholipids in a Ca2+-dependent manner but their precise physiological role is still unknown. The expression of the annexin cDNA was analyzed by Northern blot. The transcript accumulated 3-fold during CA with respect to the plants kept at normal temperature. This increase was observed from 2 to 8 days of CA, then declined at 22 days of CA to the control level. The annexin protein, produced in *E. coli* and antibodies were raised for use in western blots. The antibody cross-reacted with a 35-kDa protein in extracts prepared from *La* callus and pistil. In alfalfa, the annexin was undetected in soluble extracts of leaves and roots, but accumulated preferentially in the crown. Preliminary results using crown tissues showed that the protein levels increased transiently after 2 days of CA then declined to the levels of control plants within 9 days of CA. In wheat, the annexin was detected in the microsomal fraction but not in total soluble fractions. Based on recent evidence showing that Ca2+ may act as second messenger in LT signal transduction, we are currently investigating the possibility that annexins could be involved in the modulation of cytosolic calcium concentration or in signal transduction.

1299 Session 58, Regulation of Gene Expression

Molecular analysis of the promoters and 3' UTR sequences of two stress related genes in Atriplex Canescens. Destefano-Beltran, Luis *Institute of Paper Science and Technology* Villalon, Debbie *International Paper* Castillo, Cielo *Institute of Paper Science and Technology* Cairney, John *Institute of Paper Science and Technology*

The desert shrub *Atriplex canescens* is known to be extremely drought-resistant and may possess unique genes or novel mechanisms of drought tolerance. To understand the molecular basis of this tolerance a number of cDNA clones were isolated by differential screening. Members of a drought-induced multigene family were chosen for further study. These were divided into two classes based on sequence analysis. The clones differed in the length of their 3'UTRs the longer class contained a long AU-rich motif which was absent from the shorter class - a possible consequence of alternative sites of pre-mRNA cleavage/polyadenylation. Under severe dessication more than one transcript

was observed in Northerns. RNAseH experiments suggest that differences in transcript size may be attributable to differences in poly(A) tail length. To better understand the function of this AU-rich motif, genomic clones corresponding to each class have been characterized. Next, (3'UTR) fragments of different length of the AU-rich class gene have been spliced downstream of a 35S-GUS cassette and their effect both in mRNA stability and translatability are being evaluated in transgenic plants. Further, a series of truncated promoter fragments of both genes have been fused to a GUS-NOS reporter gene and are currently introduced into plants. The results of transient assays using cell suspensions of Atriplex and Arabidopsis will be presented.

1300 Session 58, Regulation of Gene Expression

Cloning of anthocyanin biosynthetic genes from japanese parsley (*Oenanthe stolonifera*, DC.), and stimulation of their expression by low temperature. TOMOKO FUKASAWA-AKADA *Aomori Green BioCenter* HAJIME HASEGAWA *Aomori Green BioCenter* MASAHIKO SUZUKI *Aomori Green BioCenter*

Japanese parsley (*Oenanthe stolonifera*, DC.), called Seri in Japanese, changes leaf colors to red in late autumn. We have learned that the pigmentation is due to an accumulation of anthocyanins in response to low temperature, as observed in many deciduous tree species. To investigate molecular mechanisms of the induction of the pigmentation, we have cloned three genes involved in anthocyanin biosynthesis: phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and dihydroflavonol reductase (DFR), from Japanese Parsley. Partial sequences of these genes are obtained through PCR amplification of genomic DNA, with primers designed based on sequences of these genes from other species. DNA sequences of the isolated PAL, CHS, and DFR clones consist 428, 721, and 713 bps, respectively. The PAL and CHS sequences correspond to a part of the exon II sequence of respected genes. The DFR sequence corresponds to parts of exon II and VI, and entire exon III, IV, V sequences. Deduced amino acid sequences of these genes have significant similarity to corresponding regions of the sequences reported from other species. These clones were used as hybridization probes of RNA-gel blot analyses to study expression of these genes under low temperature. In the course of 7-day treatment, low temperature (2°C) caused a significant increase in the DFR transcript level, some fluctuations in the CHS transcript level, and no obvious change in the PAL transcript level in leaves. This changes in the CHS and DFR transcript levels became apparent after five to six days upon an exposure to low temperature. We are studying a cultivated variety of japanese parsley, which accumulate little anthocyanins, in order to understand regulations of the expression of these genes.

1301 Session 58, Regulation of Gene Expression

Differential regulation of structural and osmoregulatory components during rapid cell expansion of developing cotton fibers. Vojdani, Fakhrieh *Dept. of Agronomy and Range Science, University of California, Davis, CA 95616* Smart, Lawrence B. *Dept. of Vegetable Crops; University of California, Davis, CA 95616; Faculty of Environmental and Forest Biology, SUNY College of Environmental Science and Forestry, Syracuse, NY 13210* Maeshima, Masayoshi *School of Agricultural Sciences, Nagoya University, Nagoya, Japan* Wilkins, Thea A. *Dept. of Agronomy and Range Science, University of California, Davis, CA 95616*

Cotton fibers are single-celled seed trichomes which undergo a period of very rapid cell expansion, resulting in highly tapered cells 32 mm in length. At present, very little is known about the regulation of key osmoregulatory proteins during turgor-driven cell expansion or how the expression of such a diverse group of genes may be coordinated. As a first step to addressing this question, we investigated the regulation of a major intrinsic protein, PEP Carboxylase (PEPCase), alpha-tubulin, and three proton-pumps, including the plasma membrane H^+-ATPase, vacuolar H^+-ATPase, and vacuolar H^+-PP_iase genes at the levels of RNA accumulation, protein abundance, and/or enzymatic activity. Ribonuclease protection assays showed that expression of the genes encoding turgor-related proteins are induced during specific stages of expansion, with the exception of PP_iase, which is constitutively expressed in developing fibers. However, the relative abundance of proteins corresponding to these genes does not necessarily follow the same pattern as the RNA. Likewise, enzyme activity for all proton pumps and PEPCase increased parallel to the rate of fiber expansion, regardless of protein or RNA levels. Results demonstrated that the genes studied are regulated at the transcriptional and/or post-translational levels, thereby providing further insight into cellular processes during rapid cell expansion.

1302 Session 58, Regulation of Gene Expression

Promoter analysis of the low temperature-induced *wcs120* gene from wheat. Ouellet, François *Université du Québec à Montréal, Département des Sciences biologiques* Vazquez-Tello, Alejandro *Université du Québec à Montréal, Département des Sciences biologiques* Sarhan, Fathey *Université du Québec à Montréal, Département des Sciences biologiques*

The *wcs120* gene encodes a protein that is thought to play an important role in the cold acclimation process in wheat. Its expression is specifically induced by low temperature (LT) and is regulated at the transcriptional level. To identify the *cis* and *trans* -acting elements involved in its LT responsiveness, the activity of the promoter region was assessed by transient expression analysis. Analysis of promoter deletion series fused to the luciferase (LUC) reporter gene revealed a potential enhancer element, a putative negative regulatory region and a region important for constitutive expression. The major cold inducibility region was found between -487 and -287. Transient expression experiments revealed that the activity of the full length promoter is similar in both cultivars at 21 and 49 days of cold treatment, even though the level of *wcs120* transcript was shown to decline in the spring but not the winter variety after 21 days of LT treatment. This suggests that the difference in the level of expression of *wcs120* between the spring and winter cultivars is not due to the promoter strength or to the activating factors. The LT inducibility of the *wcs120* promoter was assayed in different freezing-tolerant monocots, in a sensitive monocot and in tolerant dicot species. The results show that the promoter activity is not correlated with the capacity of the species to develop freezing tolerance, since good promoter activity was obtained at LT in species that do not express the endogenous *wcs120* homologs, such as rice, alfalfa and *Brassica*. Taken together, the results suggest that universal transcription factors responsive to LT are present in all plants and that other mechanisms of regulation are likely involved in the expression of LT-responsive genes.

1303 Session 58, Regulation of Gene Expression

Analyses of light-induced expression of the gene for phospho*enol*pyruvate carboxylase in maize. Kurotani, Ken-ichi *Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01 Japan* Hata, Shingo *Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01 Japan* Izui, Katsura *Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01 Japan*

In *Zea mays* the expression of the phospho *enol* pyruvate carboxylase gene (*Ppc1*) is known to be induced during greening of etiolated leaves in response to light signals. However, little is known about the mechanisms of light regulation. Here we investigated whether or not the phosphorylation/dephosphorylation are involved in the light induced expression of *Ppc1*.(1) Seedlings of 12-day old *Z. mays* H84 grown in the dark were used. The etiolated leaves were cut and put in the aqueous solutions of protein kinase- and protein phosphatase inhibitors, and then exposed to light for 12 hr. Total RNA was isolated from detached leaves, and subjected to Northern blot analysis with the 3'-non coding region of *Z. mays Ppc1* as a probe. The transcripts of *Ppc1* were markedly increased by light treatment (20 fold). However, the induction was completely inhibited with 1 microM okadaic acid (OA), a protein phosphatase 1 (PP1) and PP2A inhibitor. In contrast, staurosporine and K252a, protein kinase inhibitors showed no effects.(2) Seedlings of 7-day old maize leaves grown in the continuous light were cut and put into the solution of 1 microM OA. After 6 hr the transcripts of *Ppc1* were decreased (1/7 fold), which suggests that the transcripts of *Ppc1* became unstable, and/or the transcription of *Ppc1* was arrested by OA.(3) The nuclei were isolated from detached greening leaves treated with OA as (1), and measured the transcriptional rate of *Ppc1* by run-on assay. The transcriptional rate of *Ppc1* increased by exposing to light. And 1 microM okadaic acid showed no effect to the transcription of *Ppc1*. These results suggest that protein phosphatases are involved in the post-transcriptional regulation, but not in the transcriptional regulation, for the light-induced expression of *Ppc1*.

1304 Session 58, Regulation of Gene Expression

Examination of the expression patterns of genes involved in carbon metabolism in soybean seedlings grown in space. Johnson, Kathleen M. *NRC Resident Research Associate, NASA, Kennedy Space Center, FL* Brown, Christopher S. *Dynamac Corporation, Durham, NC* Piastuch, William C. *Space Biology Laboratory, Dynamac Corporation, Kennedy Space Center, FL*

One of the most consistent observations regarding plant tissue from spaceflight experiments is the decrease in starch content relative to control plants grown on Earth. It has been speculated that altered growth conditions, such as microgravity and elevated CO2 levels, may be responsible for the observed changes. To date, however, the molecular basis for the effects of the space flight environment on plants has not been thoroughly investigated. The research presented here was conducted in order to elucidate changes in gene expression presumed to underlie the altered quantitative and spatial accumulation of starch in plants. Soybean was chosen as the model system for this research because seeds germinate and young seedlings accumulate starch normally in a light-independent manner. This was an important consideration because our plant culture aboard the NASA Space Shuttle was conducted in light-tight BRICs (biological research in canister). A novel experimental approach was developed to allow gene expression analysis of an entire panel of cDNAs using the very small quantities of plant tissue available. This procedure employed RT-PCR to label cDNA pools synthesized from mRNA isolated from various plant tissues and experimental treatments. The digoxigenin-11-dUTP labeled