SAUNDERS

Thirteenth Annual Meeting
Beltsville, Maryland
1996

CONTENTS

INTRODUCTION

Welcome to the thirteenth annual meeting of the Mid-Atlantic Plant Molecular Biology Society. The goal of this society and these meetings is to provide a forum for area scientists to exchange ideas and information relating to plant molecular biology. These meetings are designed to bring some of the best scientific minds to our area and to introduce some of the most interesting advances in plant molecular biology to our area scientists at a reasonable price and at an accessible location. We hope to entice a large number of students, postdocs and senior scientists to attend and actively participate in presentations and discussions. In addition, the meeting is designed to encourage mixing of scientists in an informal atmosphere during on-site lunches and breaks to provide each participant the opportunity to meet invited speakers and other members.

The meeting encompasses a large range of important research areas, which we hope will stimulate participants by informing them about advances outside of their own immediate interests. Please contact members of the organizing committee if you have thoughts or comments for consideration in the planning of future meetings; or join next year's organizing team and volunteer your services to improve upon what we did this year. All are welcome at every stage of planning and organizing each meeting.

Many people were involved in the organization and planning of the meeting and we give them our hearty thanks. We wish to thank our sponsors and exhibitors who furnish us with up-to-date product advances and help defray costs. Please visit our sponsors' and exhibitors' displays which are located with the posters. The level of interest you show in their products is a critical factor in their willingness to support future meetings.

We thank you for your continued support of and participation in the Mid-Atlantic Plant Molecular Biology Society. Enjoy the meeting!

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1996 MAPMBS MEETING SCHEDULE

THURSDAY, JULY 18

9:00 a.m. Registration and Poster set-up

9:20 a.m. Opening remarks - Ben Matthews

GENE REGULATION I
(Moderator: Gordon Snyder, USDA-ARS)

9:25 am John C. Walker (University of Missouri). Transmembrane signaling in plants.

9:55 am Carole L. Bassett, Reuben A. Cohen, Michael L. Nickerson, and M.S. Rajeevan (USDA-ARS, AFRS, Kearneysville; Genetic Therapy, Gaithersburg; and University of South Florida, Tampa). Characterization of a genomic clone encoding a receptor-like protein kinase from morning glory.

10:15 am Lisa J. Rowland, Amnon Levi, Mubarack Muthalif, Ganesh Panta, and Rajeev Arora (USDA-ARS, Beltsville; University of Tennessee; University of Georgia; and West Virginia University). Isolation and expression of dehydrin genes in blueberry: their putative role in determination of freezing tolerance.

10.35 am COFFEE BREAK/POSTERS (Set-up and viewing)/EXHIBITORS

GENE REGULATION I (continued)

11.05 am Judith Bender (Johns Hopkins University). Epigenetic control of endogenous genes in *Arabidopsis*.

11:35 am Hema Bandaranayake and Asim Esen (Virginia Polytechnic Institute and State University). Beta-glucosidase activity is encoded by at least two genes in maize (*Zea mays* L.).

11:55 am Mohammad Shahid and Asim Esen (Virginia Polytechnic Institute and State University). Temporal and spatial expression of *GLU1* and *GLU2* genes in maize (*Zea mays* L.).

12:15 pm LUNCH, EXHIBITORS/ POSTERS

PLANT-MICROBE INTERACTIONS
(Moderator: Rose Hammond, USDA-ARS)

1:15 pm Jane Glazebrook (University of Maryland, College Park). Genetic dissection of defense responses in *Arabidopsis*.

1:45 pm Fumiaki Katagiri (University of Maryland, Baltimore County). Functional analysis of a "gene for gene" disease resistance gene.

2:15 pm Yi Hu, P.J. Bottino, and R.A. Owens (University of Maryland, College park; and USDA-ARS, Beltsville). Analysis of the long term stability of a "lethal" mutant of potato spindle tuber viroid in transgenic plants.

2:35 pm COFFEE BREAK/POSTER VIEWING/EXHIBITORS

PLANT-MICROBE INTERACTIONS (continued)

3:10 pm Margaret Pooler and John Hartung (USDA-ARS, USNA, Washington D.C.; and USDA-ARS, Beltsville). Genetic relationships among strains of *Xylella fastidiosa* and application of PCR-based detection assays.

3:40 pm John Hammond, H. Pühringer, A. da Câmara Machado, and M. Laimer da Câmara Machado (USDA-ARS, USNA, Beltsville; and University of Agriculture, Vienna, Austria). Combined RT-PCR and RFLP analysis to detect and differentiate field isolates of plum pox potyvirus.

KEYNOTE ADDRESS
(Introduction: John Hammond, USDA-ARS)

4:00 pm Barbara Baker, S.P. Dinesh-Kumar, Wai-Hong Tham, and Steve Whitham (University of California, Berkeley, and USDA-ARS Plant Gene Expression Center, Albany). Molecular characterization of the tobacco mosaic virus resistance gene *N*.

FRIDAY, JULY 19

NEW TECHNOLOGIES
(Moderator: Kathy Kamo, USDA-ARS)

9:10 am David McElroy (University of California, Berkeley). What's brewing in barley biotechnology?

9:40 am Jhy-Jhu Lin and Nacyra Garcia-Assad (Life Technologies, Inc.) A novel medium for the improvement of regeneration efficiency of rice plants using mature seeds.

10:00 am Mukesh K. Malik, Janet P. Slovin, and J. Lynn Zimmerman (University of Maryland, Baltimore County; and USDA-ARS, Beltsville). Antisense to a LMW heat shock protein (HSP17.7) reduces acquired thermotolerance in carrot.

10:20 am COFFEE BREAK/POSTER VIEWING/EXHIBITORS

NEW TECHNOLOGIES (continued)

11:15 am Lorin R. DeBonte, Z.-Z. Chen, W.D. Hitz, and K.G. Ripp (Cargill Research, Fort Collins; and E.I. Du Pont Co., Wilmington). Genetic engineering of canola oils.

11:45 am Gordon W. Snyder, John C. Ingersoll, and Lowell D. Owens (USDA-ARS, Beltsville). Genetic transformation of sugarbeet using particle bombardment and novel plant pathogen defense genes.

12:05 am BUSINESS MEETING

12:15 pm LUNCH

GENE REGULATION II
(Moderator: James Saunders, USDA-ARS)

1:30 pm Zhongchi Liu, Mark P. Running, and Elliot M. Meyerowitz (University of Maryland, College Park; and Caltech, Pasadena). *TSO1* and its role in cell division and organ morphogenesis in *Arabidopsis* flowers.

2:00 pm Muzaffer Cicek and Asim Esen (Virginia Polytechnic Institute and State University). Molecular cloning and sequencing of a cDNA encoding the enzyme β-glucosidase from *Sorghum bicolor* (L.) Moench and expression analysis in seedlings.

2:20 pm Ben Matthews (USDA-ARS, Beltsville). Gene mapping of soybean (*Glycine max*) and genes conferring resistance to soybean cyst nematode, *Heterodera glycines,* Race 3.

2:40 pm Frank J. Turano, Sona S. Thakkar, Tung Fang, and Jane M. Weisemann (USDA-ARS, Beltsville; and National Center for Biotechnology Information, Bethesda). Characterization and expression of NADH-dependent glutamate dehydrogenase genes in *Arabidopsis thaliana*.

3:00 pm CLOSING REMARKS

1996 MAPMBS POSTER SESSIONS
Thursday 10:35 am - 11:05 am and 2:35 pm - 3:10 pm
Friday 10:20 am - 11:15 am

POSTER	(Page)	
1	(31)	Maw-Shenq Chern and Mauricio M. Bustos (University of Maryland, Baltimore County). Negative regulation of maturation-specific gene expression in bean embryos by transcription factors ROM1 and ROM2.
2	(32)	Martha S. Wright and Martha Hill (Ciba Biotechnology, Research Triangle). Expression of the *aph* IV gene in the T3 generations of protoplast-derived transgenic maize.
3	(33)	Amy J. Stephens and Gregory J. Wadsworth (Buffalo State College). Partial purification of soybean glyoxisomal aspartate aminotransferase.
4	(34)	Stephen Wilhite and David Straney (University of Maryland, College Park). Cloning and disruption of a putative peptide synthetase gene in the biocontrol fungus *Gliocladium virens*.
5	(35)	Sharon J. Keeler, Janine G. Haynes, Marisia M. Johnson, John J. Frett, and Sherry L. Kitto (University of Delaware). 100kD heat shock protein gene expression and the acquired thermotolerance response in *Phaseolus lunatus*.
6	(36)	Gwo-Jiunn H. Hwang and J. Lynn Zimmerman (University of Maryland, Baltimore County). Post-transcriptional regulation of a homolog of ribosomal protein L36 during carrot somatic embryogenesis.

7 (37) Craig R. Dubois and Mauricio Bustos (University of Maryland, Baltimore County). Cloning and characterization of allantoate permease cDNAs from *Arabidopsis thaliana* by functional complementation in *Saccharomyces cerevisiae*.

8 (38) Jeffrey S. Skinner and Michael P. Timko (University of Virginia). Two distinct classes of the NADPH:protochlorophyllide oxidoreductase (*por*) gene family are present in pines.

9 (39) A. Bruce Cahoon and Michael P. Timko (University of Virginia). Characterization of nuclear genes required for light-independent protochlorophyllide reduction in *C. reinhardtii*.

10 (40) Jhy-Jhu Lin, Jin Ma, Jonathan Kuo, Wanyin Deng, and Eugene Nester (Life Technologies Inc.; and University of Washington). Use of AFLP DNA fingerprinting techniques to identify species of *Agrobacterium tumefaciens*.

11 (41) Jhy-Jhu Lin, Jin Ma, and Nacyra Garcia-Assad (Life Technologies, Inc.). Improvement of the establishment of transgenic plants using membrane-based liquid culture.

12 (42) Joan Gebhardt,Gregory Wadsworth, and Benjamin Matthews. (USDA ARS, Plant Molecular Biology Laboratory). Characterization of a soybean aspartate aminotransferase cDNA endocing isozymes that are differentially targeted to two subcellular compartments.

TRANSMEMBRANE SIGNALING IN PLANTS

John C. Walker, Division of Biological Sciences, University of Missouri, Columbia MO 65211

Recent evidence strongly suggests that plant cells have plasma membrane receptors with intrinsic protein kinase activity. The plant receptor-like protein kinases (RLK) are structurally related to the animal polypeptide growth factor receptors; however, the plant RLKs all appear to be serine/threonine protein kinases. Diversity among plant RLKs, reflected in their structural and functional properties, has opened up a broad new area of investigation into cellular signaling in plants with far-reaching implications for the mechanisms by which plant cells perceive and respond to extracellular stimuli. Part of our ongoing research is to characterize the biochemical properties, both in vitro and in vivo, of one receptor-like protein kinase (RLK5) from Arabidopsis thaliana. We have shown that a recombinant fusion protein of this transmembrane protein kinase will autophosphorylate several sites and that autophosphorylation is critical for interaction with cellular targets. One of these targets, KAPP (kinase associated protein phosphatase) contains three functional domains: an amino-terminal membrane insertion signal, a kinase interaction domain and a phosphoprotein phosphatase domain. Interaction of KAPP with RLK5 requires RLK5 phosphorylation. We hypothesize that KAPP association with RLK5 may regulate its protein phosphatase catalytic activity toward as yet unidentified downstream targets.

CHARACTERIZATION OF A GENOMIC CLONE ENCODING A RECEPTOR-LIKE PROTEIN KINASE FROM MORNING GLORY

Carole L. Bassett[1], Reuben A. Cohen[2], Michael L.Nickerson[1] and M.S. Rajeevan[3]
[1]USDA, ARS, AFRS, Kearneysville, WV; [2]Genetic Therapy, Gaithersburg, MD;
[3]University of South Florida, Tampa, FL

A clone (λ2C) isolated from a genomic library prepared from the Japanese morning glory (*Ipomoea* [*Pharbitis*] *nil* Roth.) cultivar Violet, was identified as encoding a potential protein kinase by hybridization to a maize probe containing the conserved domains VII, VIII and IX characteristic of protein kinase catalytic regions. High resolution endonuclease restriction mapping located the gene to a 5.3 kbp *Eco*RI fragment which was subsequently cloned into pBluescript II. Sequence analysis revealed that the fragment contains approximately 75% of a gene encoding a receptor-like protein kinase (RLK) which includes the carboxy terminus of the translated polypeptide and continues into the 'receptor-like' region. The putative full length of the gene was determined from sequence analysis of a PCR-generated 5' extension product using λ2C as the template. Sequence analysis suggested that a small (92 bp) intron was present in the region encoding the catalytic portion of the kinase; this was confirmed by PCR analysis of cDNAs obtained from polyA$^+$RNAs isolated from roots and cotyledons. A single potential membrane-spanning region was identified between the catalytic and receptor regions and 26 direct leucine-rich repeats (LRRs) in a single continuous block were identified in the receptor region. Comparison with other plant LRR RLKs demonstrates a closer relationship between MGRLK1 and ERECTA from *Arabidopsis*. The intron position is virtually identical with that of TMK1 of *Arabidopsis*. Northern analysis indicates highest RNA abundance in roots compared to cotyledon, leaf and shoot tips, although, even in the root the mRNA is very low in abundance. In roots, the size of the single transcript is estimated to be ca. 5.3 kb, whereas in leaves and cotyledons multiple transcripts are observed ranging in size from around 1.7-4.2 kb.

ISOLATION AND EXPRESSION OF DEHYDRIN GENES IN BLUEBERRY: THEIR PUTATIVE ROLE IN DETERMINATION OF FREEZING TOLERANCE.

Lisa J. Rowland[1], Amnon Levi[1], Mubarack Muthalif[2], Ganesh Panta[3], and Rajeev Arora[4]. [1]USDA/ARS, Fruit Lab, Bldg 010A, Beltsville Agricultural Research Center-West, Beltsville, MD 20705. [2]Dept. of Pharmacology, College of Medicine, University of Tennessee, Memphis, TN 38163. [3]Dept. of Horticulture, University of Georgia, Athens, GA 30602. [4]Div. of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506. Previous studies identified three major chilling-responsive proteins of 65, 60, and 14 kD whose levels increase in floral buds of blueberry during cold acclimation and decrease during deacclimation and resumption of growth. Characterization of these proteins found them to be members of a family of proteins responsive to drought and low temperature stress called dehydrins. The 65 and 60 kD proteins were purified, digested into peptides, and several peptides from each were sequenced. The sequence information was used to synthesize degenerate DNA primers for amplification of a part of the gene(s) encoding these proteins. One pair of primers amplified a 200 bp fragment which was cloned and sequenced. As expected, the 200 bp sequence had homology to several dehydrins from other plants. The 200 bp fragment was used to screen a cDNA library (prepared from RNA from cold acclimated blueberry floral buds) and resulted in the isolation of a cDNA clone with a 1.9 kb insert. Northern blots revealed hybridization of the 1.9 kb probe to two cold-responsive messages of 1.9 and 0.5 kb. Using 1 kb RNA:~36 kD protein as a conversion factor, these messages could encode proteins of ~68 and 18 kD, respectively. A comparison of their expression patterns during the winter in two blueberry cultivars, 'Bluecrop' and 'Tifblue', with different freezing tolerances ('Bluecrop' being more hardy than 'Tifblue') revealed that both the 1.9 and 0.5 kb messages increased to relatively higher levels more quickly in 'Bluecrop' than in 'Tifblue'; and, in addition, the 0.5 kb message declined relatively faster in 'Tifblue' than in 'Bluecrop'. Currently, efforts are underway to isolate a cDNA clone encoding the 14 kD dehydrin protein, to sequence the 1.9 kb clone, and to map the genes encoding the dehydrin proteins.

EPIGENETIC CONTROL OF ENDOGENOUS GENES IN ARABIDOPSIS.

Dr. Judith Bender, Department of Biochemistry, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

We are elucidating the establishment and maintenance of epigenetic control using the Arabidopsis Phosphoribosylanthranilate Isomerase (*PAI*) gene family as a model system. The Wassilewskia (WS) strain of Arabidopsis has four *PAI* genes at three unlinked sites. These four genes are methylated over their regions of DNA sequence homology. When *PAI* copy number is reduced by deletion of two tandemly arrayed genes, a mutant with fluorescent, tryptophan-deficient phenotypes results because the two remaining methylated *PAI* genes supply insufficient PAI activity for normal development. However, the mutant phenotypes conferred by the methylated *PAI* genes are unstable, and mutant plants yield occasional non-fluorescent revertant progeny with hypomethylated *PAI* loci. We are investigating the signals that initiate *PAI* methylation with genetic and transgenic approaches. We have found that the tandem *PAI* genes from WS can stimulate *de novo* methylation of an unlinked unmethylated *PAI* gene crossed in from another strain background. This result suggests that the tandem *PAI* genes provide a primary signal for methylation of homologous sequences. We are further investigating this possibility by transforming constructs based on the tandem *PAI* genes into an Arabidopsis strain with unmethylated resident *PAI* genes and monitoring methylation and gene silencing in the resulting transgenic lines.

BETA-GLUCOSIDASE ACTIVITY IS ENCODED BY AT LEAST TWO GENES IN MAIZE (*Zea mays* L.).

Hema Bandaranayake and Asim Esen, Biology Department, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061-0406

Genomic sequence of β-glucosidase gene *glu1* was determined by doing PCR on genomic DNA with primers specific for its cDNA and by cycle sequencing the PCR products. The genomic sequence consists of 12 exons ranging from 35 to 334 bp in length and 11 introns which ranges in length from 78 to 1041 bp. Partial sequencing of a second β-glucosidase gene *glu2* show that locations of introns 10 and 11 are identical in both genes but the length of intron 10 is reduced from 1041 bp in glu1 to 429 bp in *glu2*. The sequence identity of the two genes between introns 10 and 11 are 45% and 57% respectively while it is 88% for their 1.9 kb long cDNAs. Although Beta-glucosidases are encoded by a small multigene family *glu1* and *glu2* appears to be single copy genes.

Northern analysis of various tissues from etiolated seedlings indicated that *glu2* is expressed only in the leaf while *glu1* expression is found in roots, mesocotyl and node regions with less expression in the coleoptile and the least in leaf. When the level of these messages were checked on 5, 8, 11, 14 day old dark grown and light grown leaves; *glu1* mRNA was seen both on light and dark grown leaves but only on the 5 day olds while *glu2* message is present up to 14 days in the dark grown but only up to 11 days in the light.

TEMPORAL AND PALATIAL EXPRESSION OF GLU1 AND GLU2 GENES IN MAIZE (*Zea mays L.*)

Mohammad Shahid and Asim Esen
Department of Biology, Virginia Polytechnic Institute and State University
Blacksburg, VA, 24061-0406

Maize (*Zea mays* L.) β-glucosidase has an important role in the defense of plant against different organisms. It cleaves DIMBOA-G, releasing DIMBOA, a hydroxamic acid, which is toxic to bacteria, fungi and insects. It also acts on inactive cytokinin conjugates, releasing hormones, which are important to the growth of plant .We have found two forms of β-glucosidase in maize (*Zea mays L.*) plants. We isolated both isozymes and found they differ in their kinetics of various synthetic and natural substrates. Both proteins have a molecular weight of 60 kd. β-glucosidase1 which is the product of Glu1 gene is present in the roots, mesocotyles, nodes, coleoptiles and leaves of 5 day old seedling. In the leaves β-glucosidase1 disappears during development and is replaced by β-glucosidase2, the product of Glu2 gene. By day 11 only β-glucosidase2 is present in maize leaves. In the adult maize β-glucosidase2 is found only in leaves whiles β-glucosidase1 is present in the roots, stems, nodes and female inflorescence. Seedlings grown in the dark exhibit the same β-glucosidase expression pattern as plants exposed to regular light.

Genetic dissection of defense responses in Arabidopsis

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glazebro@umbi.umd.edu

To discover which components of plant defense responses make significant contributions to limiting pathogen attack, we screened a mutagenized population of *Arabidopsis thaliana* for individuals that exhibit increased susceptibility to the moderately virulent bacterial pathogen *Pseudomonas syringae* pv. maculicola ES4326 (PsmES4326). The twelve enhanced disease susceptibility (eds) mutants isolated included alleles of two genes involved in phytoalexin biosynthesis (pad2, which had been identified previously, and pad4, which had not been identified previously), two alleles of the previously identified npr1 gene, which affects expression of other defense genes, and alleles of seven previously unidentified genes of unknown function. The npr1 mutations caused greatly reduced expression of the PR1 gene in response to PsmES4326 infection, but had little effect on expression of two other defense genes, BGL2 and PR5, suggesting that PR1 expression may be important for limiting growth of PsmES4326. While direct screens for mutants with quantitative pathogen-susceptibility phenotypes have not been reported previously, our finding that mutants isolated in this way include those affected in known defense responses supports the notion that this type of screening strategy allows genetic dissection of the roles of various plant defense responses in disease resistance. Preliminary results suggest that the EDS4 gene may function in regulation of defense gene expression in response to PsmES4326 infection, but not in response to salicylic acid treatment.

Functional analysis of a "gene-for-gene" disease resistance gene

Fumiaki Katagiri, Dept. of Biological Sciences, Univ. of Maryland Baltimore County

Strong and specific disease resistance of plants often conforms to the "gene-for-gene" relationship: presence of both an avirulence gene in a pathogen and the corresponding resistance gene in a plant is required for resistance. To study the "gene-for-gene" type disease resistance, we use the Arabidopsis thaliana (plant host)-Pseudomonas syringae (bacterial pathogen) system as a model system because it has advantages for classical and molecular genetic studies. We isolated the A. thaliana resistance gene RPS2, which corresponds to the P. syringae avirulence gene avrRpt2. The RPS2 gene has defined a new class of resistance genes, the nucleotide binding site-leucine rich repeats (NBS-LRR) class. A growing number of NBS-LRR resistance genes include resistance genes against different types of pathogens, such as bacterial, fungal, and viral pathogens. Although this structural conservation strongly suggests the presence of common resistance mechanisms, direct demonstration of the molecular mechanisms by which the NBS-LRR resistance gene products function has not been achieved. To obtain insights into these molecular mechanisms, one of our approaches is to carry out functional analysis of mutant versions of the gene using a novel transient expression assay and transgenic plants. The recent results obtained using this approach and others will be discussed.

Analysis of the long term stability of a "lethal" mutant of potato spindle tuber viroid in transgenic plants

Y. Hu[1], P. J. Bottino[1] and R. A. Owens[2]

[1]Department of Plant Biology, University of Maryland, College Park, MD 20742;
[2]Molecular Plant Pathology Laboratory, USDA/ARS, Beltsville, MD 20705.

Viroids are the smallest known agents of infectious disease -- small, highly structured, single-strand RNA molecules which lack both a protein capsid and detectable mRNA activity, yet are able to replicate autonomously. The native structure of potato spindle tuber viroid (PSTVd) is organized into five structural domains, and introduction of three nucleotide substitutions into its left terminal domain abolished infectivity. Northern hybridization and RT-PCR were used to analyze RNA transcripts isolated from transgenic *Nicotiana benthamiana* expressing a precisely-full-length mutant PSTVd RNA via *in vivo* ribozyme cleavage. Most plants contained only non-replicating monomeric linear RNAs, but sequence analysis revealed that a spontaneous mutation at one of the these three positions could allow the mutant to resume replication. The noninfectious nature of this mutant seems to be due to its instability *in vivo* because we were unable to detect any circularized PSTVd RNA transcripts. This spontaneous mutation restores the native structure and results in a molecule that is able to replicate at wild-type (or near wild-type) rates. This study provides useful information about the design of PSTVd mutants to be used for construction of transgenic plants that are resistant to viroid infection.

GENETIC RELATIONSHIPS AMONG STRAINS OF *XYLELLA FASTIDIOISA* AND APPLICATION OF PCR-BASED DETECTION ASSAYS

Margaret Pooler[1] and John Hartung[2]
[1]US National Arboretum, 3501 NY Ave NE, Washington, DC 20002
[2]USDA Fruit Lab, Bldg. 010A, BARC-W, Beltsville, MD 20705

Genetic relationships among eleven *Xylella fastidiosa* strains isolated from mulberry, almond, ragweed, grape, plum, elm, and citrus were determined using random amplified polymorphic DNA (RAPD). Twenty-two 10-base primers amplified a total of 77 discrete polymorphic bands. Phenetic analysis based on a similarity matrix corresponded well with previous reports on *X. fastidiosa* RFLP-based similarity relationships, indicating that RAPD-PCR amplification products can be used as a reliable indicator of genetic distance in *X. fastidiosa*. By cloning and sequencing specific RAPD products, we have developed pairs of PCR primers that can be used to detect *Xylella fastidiosa* in general, and *X. fastidiosa* that cause citrus variegated chlorosis (CVC) specifically. We also identified a CVC-specific region of the *X. fastidiosa* genome that contains a 28-nucleotide insertion, and single base changes which distinguish CVC and grape *X. fastidiosa* strains. These primers are currently being used in conjunction with immunocapture and nested PCR to assess the host range of the CVC bacterium in citrus and to identify potential insect vectors in suburban shade trees.

COMBINED RT-PCR AND RFLP ANALYSIS TO DETECT AND DIFFERENTIATE FIELD ISOLATES OF PLUM POX POTYVIRUS.

J. Hammond[1], H. Pühringer[2], A. da Câmara Machado[2] and M. Laimer da Câmara Machado[2] .[1]USDA-ARS, USNA, FNPRU, Beltsville, MD 20705 and [2]IAM, University of Agriculture, A-1090 Vienna, Austria.

Plum pox potyvirus (PPV) is a destructive pathogen of stone fruit trees in Europe and other parts of the world, but is not yet present in the United States. Strict quarantine measures are used to prevent PPV being introduced to the U.S. in imported germplasm. Different serotypes of the virus have been detected in Europe and the Middle East, which differ in severity. Primers designed from conserved sequences in the NIb (replicase) gene of PPV in order to develop a sensitive assay for any serotype of PPV; these primers were also intended to differentiate between viral replication and the transgene transcript in transgenic trees expressing the PPV coat protein gene (introduced in order to obtain resistance to PPV). The primers were used in a reverse transcription-polymerase chain reaction (RT-PCR) assay to detect field isolates of PPV from leaf extracts of naturally-infected apricot trees. A PPV-specific PCR product of approximately 1040bp was obtained from each infected tree. Similar PCR products were also obtained from cDNA clones of several isolates of PPV that differ in serotype and/or symptom severity. The PCR products were further analysed by digestion with various restriction enzymes. PPV isolates from different trees and from a cloned cDNAs of different PPV isolates could be differentiated by the presence or absence of particular restriction sites within the PCR product. Evidence of heterogeneity in the viral population within individual trees was also obtained from the RFLP patterns. The combination of RT-PCR and restriction fragment length polymorphism should be useful for epidemiological studies and strain differentiation.

MOLECULAR CHARACTERIZATION OF THE TOBACCO MOSAIC VIRUS RESISTANCE GENE *N*.

Barbara Baker[ab], Dinesh-Kumar, S.P[a*], Wai-Hong Tham[a], and Steve Whitham[b], [a]Department of Plant Biology, University of California, Berkeley; [b]Plant Gene Expression Center, USDA-ARS, Albany, CA 94710.

R genes have been hypothesized to encode products that function as direct or indirect receptors for recognition of specific pathogen avirulence (*avr*) gene products and initiation of signal transduction pathways leading to expression of the resistance responses. To understand the molecular-genetic basis of disease resistance, we have isolated the resistance gene, *N*, of tobacco that mediates resistance to the viral pathogen, tobacco mosaic virus (TMV). The predicted N protein contains an amino-terminal domain similar to that of the cytoplasmic domains of the *Drosophila* Toll protein and the Interleukin 1 receptor (IL-1R) in mammals, a nucleotide binding site (NBS) and 14 imperfect leucine-rich repeats (LRR).

To understand the possible role of the N protein in TMV-*N*-mediated signal transduction pathway, we have created numerous deletion and point mutations in the *N* gene. The results of our structure-function analysis indicate that the Toll/IL-1R homology domain, the NBS, and LRR domains are required for N function. These results will be discussed in detail.

Sequence analysis of *N* cDNAs and genomic clones indicates that the *N* gene encodes both full-length (N) and truncated (N^{tr}) forms of proteins. The *N* gene contains five exons that are spliced together to form an open reading frame (ORF) of 3432 nt encoding a protein of 1144 amino acids. The N^{tr} form results from alternative splicing of a 70 bp exon to form a 1956 nt ORF encoding a protein of 652 amino acids. N^{tr} is identical to the amino terminus of N except for the 36 additional amino acids at the carboxyl terminus. The results of our analysis to discern the significance of alternative splicing for N-mediated resistance suggest that alternative splicing is required for N-mediated resistance. We will discuss the temporal and spatial regulation of N and N^{tr} and their functional role in N-mediated TMV resistance.

WHAT'S BREWING IN BARLEY BIOTECHNOLOGY?

David McElroy, Department of Plant Biology, 111 Koshland Hall, University of California at Berkeley, Berkeley, CA 94720

The primary aim of barley improvement for use in malting and brewing is to expand the germplasm available to breeders. The development of transformation systems for barley, along with an improved understanding of the biology underlying malting quality, has made it possible to consider improving barley by making planned genetic changes. In this talk I will outline recent advances in barley transformation technologies, discuss the opportunities for biotechnology in barley malting and brewing processes, describe the development of gene expression systems for use in malting and brewing biotechnology and present alternative systems for gene delivery in transgenic barley.

A Novel Medium For The Improvement Of Regeneration Efficiency Of Rice Plants Using Mature Seeds.

Jhy-Jhu Lin and Nacyra Garcia-Assad. Agricultural Biotechnology R&D, Life Technologies, Inc. (GIBCO BRL), 8717 Grovemont Circle, MD 20877.

A simple rice complete medium has been formulated for the regeneration of rice plants using yearly storage mature seeds. Callus formation was observed in 60% of seeds after three to four weeks incubation, and regeneration of shoots from these calli was obtained by further incubation for one month in the same complete rice medium. In addition, root formation was also obtained from the regenerated rice shoots after continuous incubation of the regenerated shoots in the rice complete medium without hormones. Only callus formation was shown to occur in the MS medium containing 2,4-D, and no regeneration of shoots was observed after a few months incubation. No difference in AFLP, (a PCR based DNA fingerprinting technique), patterns was observed between genomic DNA of the regenerated rice plants from rice complete medium and the rice plants from seed germination. Universal application of the rice complete medium in different rice ecotypes has been investigated. Mature seeds of three ecotypes of Indica , one hybrid of Japonica and Induce, Assamd, and six ecotypes of Japonica were incubated in the rice complete medium. The seeds from all these ecotypes were able to regenerate both the callus and shoots as in Orion. In addition, the expression of GUS activity was observed after bombardment of the plasmid pBI221 into regenerated rice callus cells. These results demonstrated that a simple procedure for the regeneration of rice plants has been achieved by incubating mature seeds in an innovative rice complete medium.

ANTISENSE TO A LMW HEAT SHOCK PROTEIN (HSP17.7) REDUCES ACQUIRED THERMOTOLERANCE IN CARROT

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Higher plants respond to increased temperatures by expressing a complex class of low molecular weight heat shock proteins, encoded by a few conserved gene families. We are investigating the roles of some of these LMW Hsps in the acquisition of thermotolerance in carrot and tomato. In the present study, we are investigating the function of one specific LMW heat shock protein gene, Hsp17.7, by analyzing the effects of a heat-inducible antisense version of the Hsp17.7 gene in transformed cells and regenerated plants. We have demonstrated that cells expressing this antisense gene have substantially reduced thermotolerance compared to control cells. There are no other obvious differences between the antisense-Hsp17.7 cells or regenerated plants and control cells and plants in terms of growth, regeneration or fertility.

Molecular analysis of the antisense-Hsp17.7 transformed cells revealed that these cells synthesized substantially reduced amounts of the entire spectrum of Hsps, including those in the high molecular weight range; however, the decrease is most dramatic in the low molecular weight class. Experiments are ongoing to characterize how antisense-Hsp17.7 is causing such a generalized decrease in Hsps production.

Genetic Engineering of Canola Oils

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The functionality and application of vegetable oils in the food industry is partly determined by the fatty acid composition of the oil. In high temperature applications oils with high oxidative stability are required. These oils are obtained through the reduction in polyunsaturated fatty acids. High melting point oils for pastry and margarine applications have higher contents of saturated fatty acids. To produce oil qualities required by the food industry domestic oils are processed to the desired fatty acid composition by hydrogenation and fractionation. Reducing the need for extensive oil processing and producing consistent fatty acid products for the food industry is highly desired. Genetic engineering provides the opportunity to alter the fatty acid biosynthetic pathway in canola to produce desired products on the farm in a consistent manner. Modification of enzymes in fatty acid biosynthesis pathway of developing canola seeds has been accomplished by seed specific expression of re-introduced cDNAs. Reduction in the expression of cytoplasmic oleate desaturase and the cytoplasmic linoleate desaturase by co-suppression has decreased the polyunsaturated fatty acid to less than 10% in the seed oil. Over-expression of the oleate acyl-ACP thioesterase increased the saturated fatty acid content to content to about 20%. Successful manipulation of the endogenous target genes was obtained using promoter regions from the napin, cruciferin, phaseolin and oleosin seed storage proteins. Seed specific regulation of the desaturase genes allows the plant breeder to overcome the agronomic deficiencies associated with plants producing very low polyunsaturate oils.

GENETIC TRANSFORMATION OF SUGARBEET USING PARTICLE BOMBARDMENT AND NOVEL PLANT PATHOGEN DEFENSE GENES

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Several transgenic sugarbeets have been produced each containing genes encoding pathogen-defense related proteins under transcriptional control of stress or wound-inducible promoters. Promoters used in this study include the CaMV 35S, and those derived from genes encoding osmotin and pathogenesis related protein-S (PR-S) from tobacco, and proteinase inhibitor II (Pin II) from potato. The promoters were fused 5' to coding regions of either β-glucuronidase (GUS), osmotin, PR-S, barley leaf thionin, or cecropin. A sugarbeet transformation method has been developed using embryogenic callus generated from seedling hypocotyls. To date plants have been recovered which carry the following chimeric genes: 35S-GUS, osmotin-GUS, osmotin-osmotin, osmotin-cecropin, PinII-thionin, PinII-cecropin, PrS-thionin, osmotin-osmotin/osmotin-cecropin. GUS activity in the osmotin-GUS plant, while in tissue culture, was found to be constitutive with expression 10 times the level found in the 35S-GUS plant and is not wound-inducible. When the plant was transferred to soil, the constitutive level of GUS expression in the leaf was approximately 50 nMol MU min^{-1} mg^{-1} protein. However, GUS activity was inducible by wounding of an excised leaf, with activity peaking (400 nMol MU min^{-1} mg^{-1} protein) at about 48 hours. Most of the plants have been transferred to soil and are being tested for their response to infection by known sugarbeet pathogens.

TSO1 AND ITS ROLE IN CELL DIVISION AND ORGAN MORPHOGENESIS IN _ARABIDOPSIS_ FLOWERS

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Several recessive mutations that cause callus-like green flowers were isolated in a genetic screen. These mutations were named *tso*, and they define two genetic loci: *TSO1* and *TSO2*. While *tso2* mutants exhibit defects in both flowers and cauline leaves, *tso1* primarily affects flowers.

Currently, we have focused our study in *tso1*. Scanning Electron Microscopy (SEM) analysis showed that *tso1* flowers produce 4 to 6 morphologically abnormal sepals and then produce callus-like cells in the center. These callus-like cells do not differentiate further, resulting in complete sterility. Using the Confocal Laser Scanning Microscopy (CLSM), we observed that *tso1* floral meristem sometimes lacks the properly organized L1, L2, and L3 layers. In addition, the nuclei of *tso1* flowers are irregular in size and shape. Microspectro- fluorometric measurements indicated polyploidy greater than 16C in some cells of *tso1-1* floral meristems, whereas the wild type floral meristems exhibited DNA contents of 2C and 4C. Furthermore, there are fewer cells in a young *tso1* floral meristem than in a wild type floral meristem, and the average cell size in *tso1-1* stage 3 floral meristems is five times that of wild type. Using Transmitting Electron Microscopy (TEM), we observed that *tso1* floral meristem cells have partially formed cell walls, and that the nuclear membranes frequently invaginate. These observations suggest that the primary defect of *tso1* likely resides in mitosis and cytokinesis. To further define the specific function of *TSO1*, a map-based cloning approach is being employed to isolate the *TSO1* gene. In addition, microtubule organizations in the *tso1* mutant cells are being examined using anti-tubulin antibody and GFP-tagged tubulin. Our finding that *TSO1* is essential for cell division in floral tissues but not in vegetative tissues suggests that the cell division machinery may be different in these two types of tissues.

MOLECULAR CLONING AND SEQUENCING OF A cDNA ENCODING THE ENZYME β-GLUCOSIDASE FROM *Sorghum bicolor* (L.) MOENCH AND EXPRESSION ANALYSIS IN SEEDLING

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(ABSTRACT)

A full-length cDNA encoding β–glucosidase (dhurrinase) was isolated and sequenced from *Sorghum bicolor* (L) Moench. The cDNA has a 1695-nucleotide-long open reading frame which codes for a 565-amino acid-long precursor and 514-amino acid-long mature protein. Deduce amino acid sequence comparisons show 72% identity between maize and sorghum β-glucosidase precursor proteins. Multiplicity of β-glucosidases and their expression in different tissues were studied using 5'- and 3'-end specific probes derived from the cDNA. Southern blotting data indicated that β–glucosidase is encoded by a small multigene family having at least two members. Northern blotting data indicated that the mRNA corresponding to the cloned cDNA is present in high levels in the node and upper portion of the mesocotyl, but at lower levels in the root and only in the zone of elongation and root tip region. Light-grown seedling parts had lower levels of mRNA than those of etiolated seedlings. The coleoptile, node, leaf and root regions of the ligt-grown seedlings all had detectable β-glucosidase mRNA levels under low stringency wash conditions but only the node and root blots retained the signal under high stringency wash conditions. Immunoblotting analysis performed using maize-anti-β-glucosidase sera supported the results of Hosel et al., (1987) with respect to the occurrence of two distinc dhurrinases in sorghum. The data indicated that the cloned cDNA corresponds to the dhurrinase I isozyme.

GENE MAPPING OF SOYBEAN (GLYCINE MAX) AND GENES CONFERRING RESISTANCE TO SOYBEAN CYST NEMATODE, *Heterodera glycines*, RACE 3

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A soybean genetic linkage map containing over 100 RFLP, AFLP, RAPD and phenotypic markers was constructed using SBML1 germplasm consisting of 146 recombinant inbred lines from a cross between PI290136 X BARC2(*Rj4*). Phenotypic traits such as resistance to soybean mosaic virus(smv), root fluorescence (Fr2), maturity (e), bacterial pustule resistance, seed coat color (i), and resistance to soybean cyst nematodes were integrated into the map. Many RFLPs placed on the map represent anonymous cDNAs while others are of known function, eg. GOGAT, asparagine synthetase, glutamine synthase, aspartate aminotransferase, aspartokinase-homoserine dehydrogenase, and chalcone synthase. Most RFLP markers were one-pass sequenced at each end to provide anchors on chromosomes.

One RFLP marker, pBLT65, was tightly linked to the *Rhg4* locus conferring resistance to soybean cyst nematodes, race 3. The RFLP assay for tracking the trait was replaced by a PCR assay, which was quicker and less labor intensive. The PCR assay will be used to screen a Bacterial Artificial Chromosome (BAC) library, currently being constructed in this laboratory, to identify large clones in the vicinity of pBLT65 and the *Rhg4* locus. Genes that are present on BAC clones in the region of *Rhg4* and involved in the resistance reponse will be identified. The expression of genes involved in the resistance response are being examined by differential display of mRNAs expressed in soybean roots inoculated and not inoculated with soybean cyst nematode (SCN) *Heterodera glycines*, race 3 using the resistant genotype, Peking and the sensitive genotype, Kent. Numerous bands were identified as present or much stronger in one but not the other. Some of these DNA bands were excised and eluted from the gel and cloned. Through these techniques we hope to better understand the resistance mechanism and identify the resistance gene at the *Rhg4* locus.

CHARACTERIZATION AND EXPRESSION OF NADH-DEPENDENT GLUTAMATE DEHYDROGENASE GENES IN *ARABIDOPSIS THALIANA*

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Two distinct cDNA clones encoding NADH-dependent glutamate dehydrogenase (NADH-GDH) have been identified and sequenced. The genes corresponding to these cDNA clones have been designated *GDH1* and *GDH2*. Southern blot analysis of genomic DNA suggest that *GDH1* is a single copy gene and *GDH2* may have more than one copy. The cDNA clones have high homology with each other and with other plant NADH-GDH cDNA sequences. Analysis of the deduced amino acid sequences suggest that both gene products contain mitochondrial transit polypeptides. Subcellular fractionation confirmed the mitochondrial location of the NADH-GDH isoenzymes. *GDH1* encodes a 42.5 kD polypeptide, designated α, and *GDH2* encodes a 43 kD polypeptide, β. The two subunits combine in different ratios to form seven NADH-GDH isoenzymes. The slowest migrating isoenzyme in a native gel, GDH1, is a homohexamer composed of the α-subunit, and the fastest migrating isoenzyme, GDH7, is a homohexamer composed of the β-subunit. GDH isoenzymes 2 through 6 are heterohexamers composed of different ratios of the α and β-subunits, e.g. GDH2 is composed of five α-subunits and one β subunit. NADH-GDH isoenzyme patterns varied among different plant organs. In addition, isoenzyme patterns changed in leaves treated with different nitrogen treatments or when exposed to darkness. Conversely, there were no measurable changes in isoenzyme patterns in roots treated with different nitrogen treatments or upon exposure to darkness. In most instances, the changes in isoenzyme patterns were correlated with relative differences in the amount of the α and β-subunits and the relative amounts of *GDH1* and *GDH2* transcripts.

Negative Regulation of Maturation-Specific Gene Expression in Bean Embryos by Transcription Factors ROM1 and ROM2. Maw-Shenq Chern and Mauricio M. Bustos. Department of Biological Sciences, UMBC. 1000 Hilltop Road, Baltimore, MD 21250

In French bean (*Phaseolus vulgaris*), the ABI3-like factor, PvALF, activates transcription from maturation-specific (*MAT*) promoters of *DLEC2* and *PHSβ* genes, encoding phytohemagglutinin (PHA) and phaseolin, respectively. This work reports cloning and characterization of the regulators of *MAT*1 (ROM1) and 2 (ROM2) that belong to the family of basic leucine zipper (bZIP) DNA binding protein. cDNA clones encoding ROM1 and ROM2 were isolated by the 3' and 5' RACE methods based on conservation among plant bZIP proteins. Recombinant ROM1 and ROM2 recognized DNA motifs with symmetric (ACGT) and asymmetric (ACCT) cores on both *MAT* promoters in gel mobility shift and DNase I footprinting assays. ROM1 and ROM2 repressed expression from the *DLEC2* promoter and antagonized *trans*-activation of the same promoter by PvALF in transient expression assays based on particle bombardments. Repression was abolished by mutations that prevented their binding to the promoter. Moreover, hybrid proteins composed of a PvALF activation domain and the DNA binding domain of ROM1 or ROM2 activated expression from promoters containing their binding sites, indicating that they recognize the same binding sites in vivo. Consequently, they function as DNA binding site-dependent transcriptional repressors. The expression profiles of ROM1 and ROM2 mRNAs are inversely correlated with those of phaseolin and PHA mRNAs. ROM1 mRNA expression preceded that of the two *MAT* genes and declined rapidly at the onset of maturation when *MAT* genes were highly induced. ROM2 mRNA level was low during the maturation stage but significantly increased during seed desiccation when both *MAT* mRNA levels declined. Supershift analysis of nuclear proteins, using a ROM2-specific antibody, revealed an increase in ROM2 DNA binding activity parallel to its mRNA level. Therefore, the data indicate that ROM1 and ROM2 may contribute to the temporal regulation of *MAT* gene expression by repressing PvALF-activated transcription.

EXPRESSION OF THE *aph* IV GENE IN THE T3 GENERATIONS OF PROTOPLAST-DERIVED TRANSGENIC MAIZE

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The *aph* IV gene confers resistance to the antibiotic, hygromycin B. The presence of the *aph* IV gene was confirmed in T_3 and F_3 maize lines derived via protoplast transformation and regeneration. T_0 plants regenerated from an A188-derived line of protoplasts were crossed in both directions with Ciba inbreds. The resulting T_1 and T_2 seed were grown to maturity in the greenhouse and pollinated or outcrossed to Ciba inbreds. The T_3 seed was embryo-rescued to condense maturation time and leaf tissues were analyzed by Polymerase Chain Reaction (PCR) for the *aph* IV gene and by leaf disk assay for expression of the gene.

Partial Purification of Soybean Glyoxisomal Aspartate Aminotransferase.

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Plants have multiple isozymes of aspartate aminotransferase, AAT, which are located in different subcellular compartments (i.e. the cytosol, mitochondria, plastids and glyoxisomes). In order to understand the biochemical and genetic relationship of the plant AAT isozymes, the glyoxisomal isozyme, AAT-1, was partially purified from soybean cotyledons. Cotyledons were harvested from dark grown seedlings (28°C) four days after imbibition. Crude extracts of this tissue were fractionated by a 40%-80% acetone precipitation and by several types of chromatography including DEAE ion exchange, phenyl sepharose hydrophobic and Sephacryl gel filtration. These steps provided substantial purification of aspartate aminotransferase, however electrophoretic analysis indicated that this preparation still contained a mixture of AAT-1 and AAT-2. To separate these AAT isozymes the sample was bound to a second DEAE cellulose column and eluted with a pH gradient (25 mM Tris-HCl, pH 7.0 to 25 mM Histidine, 25 mM Tris-HCl, pH 6.0). The chromatograph showed a major peak of AAT activity preceded by a broad shoulder of activity. Analysis of the activity by native gel electrophoresis demonstrated that the major peak contained AAT-2 and the broad shoulder contained AAT-1. To assess purity, the AAT-1 sample was analyzed by SDS electroporesis and only two polypeptide bands were detected. Molecular weights of the polypeptides were estimated to be 40Kd and 42Kd. Western blot analysis using anti-soybean AAT serum demonstrated that the 42Kd band was aspartate aminotransferase-1.

CLONING AND DISRUPTION OF A PUTATIVE PEPTIDE SYNTHETASE GENE IN THE BIOCONTROL FUNGUS *GLIOCLADIUM VIRENS*. Stephen Wilhite and David Straney. Department of Plant Biology, University of Maryland, College Park, MD 20742.

Gliocladium virens acts as an antagonist against *Pythium ultimum* and *Rhizoctonia solani*, and is used in the biocontrol agent to suppress damping-off diseases caused by these two plant pathogens. The synthesis of gliotoxin, an epidithiodiketopiperazine antibiotic, appears to contribute to this biocontrol activity. Since gliotoxin is a modified cyclic dipeptide, we have cloned a 5 kB partial cDNA *G. virens* gene based upon sequence similarity to known peptide synthetases. The cloned *G. virens* gene displays consensus sequences found in amino acid activation domains of other peptide synthetases. We have used the cloned cDNA to produce a gene replacement which disrupts this gene. Analysis of the gene disruption transformants demonstrates no change in gliotoxin production.

100 KD HEAT SHOCK PROTEIN GENE EXPRESSION AND THE ACQUIRED THERMOTOLERANCE RESPONSE IN *PHASEOLUS LUNATUS*.

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Phaseolus lunatus (lima bean) cultivars grown in Delaware typically yield less pounds per acre than the same cultivars grown in California. Part of this effect may be due to the extreme heat conditions or temperature fluctuations during Delaware's summers which can affect flower and pod set. "Acquired thermotolerance" is defined as the ability of an organism to become better able to withstand an extreme heat shock as a consequence of previous less extreme heat stress treatment. This phenomenon is linked to the induction of heat shock proteins, in particular the expression of the HSP100 Kd proteins. We are interested in correlating induction of the acquired thermotolerance response and the induction of expression of HSP100 Kd genes with yield under heat stress conditions in lima bean cultivars. We have assayed viability of leaf cells post heat stress (34°-37°) by measuring the release of ions (conductivity of external solution) during extreme heat shock (45°). Using this assay we have observed the induction of acquired thermotolerance as a function of temperature, length of treatment, age of plants and cultivar. There are distinct patterns of response among the cultivars assayed not necessarily correlating with known field performance under heat stress. Using the soybean HSP101 gene as a heterologous probe, we have screened DNA from 11 cultivars for RFLPs associated with HSP100 gene sequences. Using primers specific for conserved regions of the HSP100 sequence we have identified different patterns of PCR products linked to cultivar genotype. We are cloning the HSP 100 gene homologue from lima bean to use for sequence comparisons to other plant HSP 100 genes and for detection of expression of the HSP100 gene products on Northern and Western blots.

Post-transcriptional Regulation of a Homolog of Ribosomal Protein L36 during Carrot Somatic Embryogenesis

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We have identified a small collection of genes that are regulated at the post-transcriptional level in developing embryos of carrot and are now investigating the mechanisms of this regulation. One gene in this collection encodes a homolog of rat ribosomal protein L36. In the carrot genome, there are three different copies of this ribosomal protein homolog encoding the identical polypeptide. Some of the characteristic features of animal ribosomal protein message (e.g. polypyrimidine tract and GC-rich motifs), are also found in these carrot mRNAs although in a different configuration. Our studies revealed that the preferential accumulation of mRNAs from a class of genes exemplified by this ribosomal protein gene in the polysomal fractions is primarily due to a shift of these mRNAs from the subpolysomal fraction to the polysomal fraction. Interestingly, however, we have found that not all of the messages distributed in the polysomal fractions can be disrupted by high concentrations of EDTA. The degree of insensitivity to EDTA varies among the different genes in the class which have been analyzed at different developmental stages. In investigating the mechanisms responsible for the developmental shift of these mRNAs from the subpolysomal to polysomal pools, we have determined that modulation of the length of poly(A) tail does not seem to be a factor. Experiments to identify the cis-elements and the interacting factors involved in this regulatory pattern are under way using both biochemical assays and the generation of transgenic plants.

CLONING AND CHARACTERIZATION OF ALLANTOATE PERMEASE cDNAs FROM *Arabidopsis thaliana* BY FUNCTIONAL COMPLEMENTATION IN *Saccharomyces cerevisiae:* Craig R. Dubois and Mauricio Bustos; Dept. of Biological Sciences, Univ. of Maryland, Baltimore County, 1000 Hilltop Road, Baltimore, MD 21250

The regulation of nitrogen metabolism is central to plant growth and development. Many aspects of nitrogen uptake and utilization are similar between higher plants and the baker's yeast *Saccharomyces cerevisiae*. Consequently, we are using complementation of well characterized yeast mutants to isolate genes that coordinate plasma membrane transport and catabolism of nitrogenous compounds in *Arabidopsis thaliana* and soybean. So far, we are focusing on allantoate transport because of the important role that this ureide has in soybean and other tropical legumes. Mutant yeast strain 645-12 lacks a functional allantoate permease gene (*dal5*) and is deficient in allantoate uptake. Transformation of this strain with an *Arabidopsis* cDNA library constructed in the yeast expression vector pFL61 has yielded six clones encoding putative allantoate permeases or allantoicase activities. Further characterization of these clones will be presented. We also identified a mutant (645-11) displaying a deficient growth on minimal nitrogen media (*dgn*) phenotype in addition to the absence of allantoate uptake activity (*dal5*). Transformation of this strain with the *Arabidopsis* library yielded two clones (14.1 and 14.3) that restored growth on several nitrogen sources (allantoin, urea, ammonium, glutamate and glutamine) except proline and allantoate. Clone 14.1 encodes a putative protein that is 75-80% homologous to thiol-specific antioxidant genes from yeast and humans. Clone 14.3 has no significant homology to any known genes in the data base, but it is similar to the product of a yeast open reading frame identified by the yeast genome sequencing project. Because of their pleiotropic effects on nitrogen metabolism, the products of clones 14.1 and 14.3 are likely to function in a regulatory capacity, possibly as inducers of genes sensitive to nitrogen catabolite repression.

TWO DISTINCT CLASSES OF THE NADPH:PROTOCHLOROPHYLLIDE OXIDOREDUCTASE (*por*) GENE FAMILY ARE PRESENT IN PINES

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The light-dependent NADPH:protochlorophyllide oxidoreductase enzyme (POR) is encoded by a small gene family in the nuclear genome of angiosperms. We demonstrate that *por* occurs in the gymnosperm *Pinus taeda L.* as a large multigene family. Two distinct classes of the *por* gene family, designated *porA* and *porB*, are expressed in dark grown cotyledons. Gene specific probes to the two *por* classes showed similar expression patterns to each other and to a coding region probe that recognizes all *por* genes, suggesting that the *por* gene family is similarly expressed. Combined 5′ and 3′ RACE data demonstrated a minimum of three *por* genes were being expressed in this tissue. A probe to the *por* coding region that recognizes all *por* genes was found to hybridize to multiple bands in *P. taeda* by Southern analysis, indicating a large gene family was present. Gene-specific probes to the two *por* classes were found to hybridize to separate, distinct genomic fragment subsets. The *porA*-specific probe hybridized to a large *por* gene subfamily, while the *porB*-specific probe hybridized to a small *por* gene subfamily. The two *por* classes have duplicated at different rates, with the *porA* form having up to twelve potential members and the *porB* form having two potential members as determined by Southern analysis. In addition, pseudogenes appear to form a subset of the potential *por* gene family. Phylogenetic analysis supported two major classes of the *por* family occurring in pines. Contrary to the large multigene family observed for *por*, the three chloroplast-encoded genes (*chlB*, *chlL*, and *chlN*) making up the putative multisubunit, light-independent form of protochlorophyllide oxidoreductase occur as single gene copies with *chlL* and *chlN* lying tandem on the chloroplast genome.

CHARACTERIZATION OF NUCLEAR GENES REQUIRED FOR LIGHT-INDEPENDENT PROTOCHLOROPHYLLIDE REDUCTION IN *C. REINHARDTII*

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Protochlorophyllide (pchlide) reduction is a key step in chlorophyll (CHL) biosynthesis and chloroplast organogenesis. Two distinct mechanisms for pchlide reduction are present in the green alga *Chlamydomonas reinhardtii*. Like higher plants, *C. reinhardtii* cells reduce pchlide to chlorophyllide in a light-dependent manner catalyzed by the nuclear-encoded enzyme NADPH:protochloorphyllide oxidoreductase. *C. reinhardtii* cells also synthesize significant amounts of chlorophyll (CHL) in the dark by a light-independent pchlide reduction reaction. Although the catalytic mechanism and cofactor requirements for this latter process are unknown, the products of three chloroplast genes (designated *chlL, chlN, and chlB*) and at least seven nuclear loci (designated *y*) have been demonstrated to be required. Mutations in either the plastid or nuclear genes result in the same "yellow-in-the-dark" phenotype, that is, in the absence of light CHL is not synthesized and there is a buildup of the biosynthetic precursor pchlide. Both types of mutants are still able to reduce pchlide and synthesize CHL in the light. The three chloroplast encoded genes have been cloned and are believed to encode three subunits of the putative light-independent pchlide reductase enzyme. Our hypothesis is that the nuclear encoded y-loci somehow control the expression of the chloroplast encoded genes. Presented will be Northern and Western analyses demonstrating the effects of the various *y*-loci on *chlL*, *chlN*, and *chlB* expression.

Use of AFLP DNA Fingerprinting Techniques to Identify Species of *Agrobacterium tumefaciens*

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AFLP (Amplified Restriciton Fragment Polymorphism), a PCR based DNA fingerprinting techniques, was used to examine genomic DNA of several strains and species of *Agrobacterium*. In AFLP, genomic DNA is digested by restriction enzymes *Eco*R I and *Mse* I, ligated with *Eco*R I adapters and *Mse* I adapters, and selectively amplifed by PCR using selective primers which contain 16 adapter defined sequences and one to three arbitrary sequences. (Vos, et al, 1995, Nucl. Acids Res. 23, 4407-4414; Lin and Kuo, 1995, FOCUS, 17, 66-70). A 5-10% difference in AFLP patterns was observed between strains have the wild-type tumor inducing (Ti) plasmid and strains which have either no Ti plasmid or a different Ti plasimd. In *Agrobacterium tumefaciens*, AFLP patterns were different between nopaline strains like C58 and octopine strains like LBA4404. Quantitative analysis of AFLP patterns showed that similarities between C58 and a strain of *Agrobacterium rubi* are higher than the similarity between C58 and LBA4404. By comparing the sequences of 16S rRNA gene of C58, LBA4404, and *A. rubi*, we concluded that the strain of *Agrobacterium rubi* phylogenetically is located between C58 and LBA4404. These results indicate that (1) AFLP, in conjuction with 16S rRNA gene sequences, is a useful technique for identifying bacterial relationships and (2) at least two, and likely more, species exist within *Agrobacterium tumefaciens*.

Improvement of The Establishment of Transgenic Plants Using Membrane-Based Liquid Culture.

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Regeneration of both tobacco and potato plants was significantly improved, in fresh weight as well as the number of regenerated shoots, using membrane based liquid cultures with MS complete 50X concentrate medium. Toxic effects of antibiotics such as kanamycin, carbenicillin and hygromycin were shown when the plant tissues were grown in the membrane based liquid cultures or in agar based semi-solid cultures. Transgenic plants were achieved by using *Agrobacterium*-mediated transformation in tobacco as well as potato grown in the membrane based liquid cultures. The transformation efficiency was improved 6 fold in the number of regenerated tobacco shoots and 60% to 80% of fresh weight of regenerated shoots by growing in the membrane based liquid culture. Transgenic plants obtained from membrane based liquid culture were further analyzed by GUS activity and PCR. From 30 kanamycin resistant plants, 25 plants showed GUS activity, and all 30 plants contained the kanamycin resistance gene by PCR analysis. These results demonstrate that membrane based liquid culture is able to improve not only the regeneration of nontransgenic plants but also the transgenic plants.

CHARACTERIZATION OF A SOYBEAN ASPARTATE AMINOTRANSFERASE cDNA ENCODING ISOZYMES THAT ARE DIFFERENTIALLY TARGETED TO TWO SUBCELLULAR COMPARTMENTS

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A soybean leaf cDNA clone, pSAT1, which encodes two isozymes of aspartate aminotransferase (AAT) differentially targeted to two subcellular compartments was isolated. pSAT1 contained a large open reading frame with two in-frame start codons, designated ATG1 and ATG2, and 90% amino acid identity to other plant cytosolic AATs. Alignment of this protein with other plant cytosolic AAT isozymes revealed a 37 amino acid N-terminal extension resembling a peroxisomal targeting signal (PTS), designated PTS2. The second start codon aligns with previously reported start codons for plant cytosolic AAT cDNAs. Immune serum raised against the pSAT1-encoded protein expressed in E. coli reacted with three soybean AAT isozymes, glyoxysomal AAT1, cytosolic AAT2, and AAT3. We propose that the glyoxysomal isozyme AAT1 is produced using ATG1 and the cytosolic isozyme AAT2 is produced using ATG2. Further, AAT1 contains a PTS2 which is cleaved upon import to the glyoxysome. Processing of the N-terminus of AAT1 has been confirmed by amino acid sequencing of the purified protein. AAT2 lacks the PTS2 and remains cytosolic. Analysis of other plant cytosolic AAT genomic sequences suggests that they may utilize a second upstream start codon to generate a glyoxysomal or peroxisomal form of AAT.

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