Mid-Atlantic Plant Molecular Biology Society

Eighteenth Annual Meeting, 2001

CONTENTS

	Page
Introduction	2
Exhibitors and Sponsors	3
Organizing Committees	4
Meeting Schedule	5
Poster Sessions	8
Abstracts of Speakers	10
Abstracts of Posters	29
Directory of Participants	40

INTRODUCTION

Welcome to the eighteenth 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 number of important research areas, which we hope will stimulate participants by informing them of advances outside of their own immediate interests. Please contact members of the organizing committees if you have thoughts or comments for consideration in the planning of future meetings; or join next years 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 to 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 MidAtlantic Plant Molecular Biology Society. Enjoy the meeting!

Ben Matthews
Chair

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ABSTRACTS BOOK

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PROJECTIONISTS

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2001 MAPMBS MEETING SCHEDULE

Thursday August 2

9:00 am Registration and Poster set-up

9:25 am Introductions

Session I - **Gene Regulation**
Moderator: Frank Turano

9:30 am Elliot Herman, Ricki Helm, Rudolph Jung, and Anthony Kinney (USDA/ARS Beltsville, University of Arkansas, Pioneer Hi-Bred International Inc. DuPont)
Cosupression of an immunodominant allergen in transgenic soybean seeds.

10:00 am Steven W. Pechous, and Bruce Whitaker (USDA/ARS, Beltsville)
Cloning and expression of HMG-CoA reductase and α-farnesene synthase as a strategy for the elimination of superficial scald storage disorder in apple fruit

10:20 am Maryam Farzad, Rob Griesbach, John Hammond, Heidi Elmendorf, Martha Weiss (Georgetown University, USDA/ARS, Beltsville)
Regulation of anthocyanin production in viola cornuta under various environmental cues

10:40 am **Break - Coffee, Poster Set-up and Viewing, Exhibitors**

Session II - **Signalling & Gene Regulation**
Moderator: Janet Slovin

11:20 am Caren Chang, Chi-Kuang Wen, Jason Shockey, Paul Larsen, Ruth Stadler, and Zaiqun Pan (University of Maryland, College Park)
Ethylene receptor signaling in Arabidopsis

11:50 am Chi-Kuang Wen and Caren Chang (University of Maryland, College Park)
Arabidopsis RGL1 codes a negative regulator of gibberellin responses

12:10 pm **Lunch, Exhibitors and Poster Viewing**

Session III - **Targeted Transformation & Metabolic Profiling**
Moderator: Jim Saunders

1:20 pm Chris L. Baszczynski, Dave Peterson, Alex Lyznik, and Pramod Mahajan (Pioneer Hi-Bred International, Inc)
Approaches to targeted gene integration and modification in plants

1:50 pm Konstantina Karyotou and Robert P. Donaldson (George Washington University)
Antioxidant metabolism in peroxisomes

2:10 pm Lloyd W. Sumner (The Samuel Roberts Noble Foundation)
Proteomic and metabolic studies in Medicago truncatula

2:40 pm An Michiels, Liesbet Van Riet, Van wonterghem Niki, De Roover Joke, Andre Van Laere, Wim Van den Ende (KULeuven, Belgium)
Cloning and functional expression of fructan 1 exohydrolase I and II, key enzymes
of fructan breakdown in Cichorium intybus

3:00 pm **Break - Sodas, Exhibitors and Poster Viewing**

Keynote Address
Introduction: Ben Matthews

3:45 pm Andrew Bent (University of Wisconsin)
NBS-LRR R gene products: functional domains and divergent downstream transcript profiles

5:00 pm Close of day

Friday August 3

Session I – **Plant / Microbe Interactions**
Moderator: John Hammond

9:15 am James N Culver and Sheetal Golem(University of Maryland, College Park)
Tobamovirus-host interactions: remodeling cellular functions

9:45 am Sarah E Keates and Bryan A Bailey(USDA/ARS, Beltsville)
Differential gene expression in weed species treated with a necrosis-inducing peptide

10:05 am David Mackey, Aaron Wiig, Ben Holt, and Jeff Dangl
(University of North Carolina at Chapel Hill)
Function of rpm1 requires RIN4, a virulence target guarded by rpm1

10:35 am **Break - Coffee, Exhibitors and Poster Viewing**

Session II - **Bioinformatics, Genomics, & Gene Regulation**
Moderator: Kim Lewers

11:05 am George Michaels(Monsanto Co.)
Connecting function to phenotypes

11:35 am James A Saunders, Sue Mischke, Ricardo Goenaga, and Alaa Hemeida (USDA/ARS, Beltsville, Minufiya University, Egypt)
The use of DNA fingerprinting in chocolate trees to study genetic diversity

11:55 am Gromoslaw Smolen and Judith Bender(Johns Hopkins University)
Arabidopsis cytochrome P450 cyp83B1 mutations activate the tryptophan biosynthetic pathway

12:15 pm **Business Meeting**

12:25 pm **Lunch, Exhibitors and Poster Viewing**

Session III - **Bioinformatics & Genomics II**
Moderator: Rana Khan

1:35 pm Perry Cregan, Youlin Zhou, Charles Quigley, Qijian Song, Edward Fickus, Reynold Fernalld, Carolyn Smith (USDA/ARS,Beltsville)
Single nucleotide polymorphisms (snps) in soybean: discovery and detection

2:05 pm Kim Lewers, S D Nilmalgoda, A L Warner, H T Knap, B F Matthews (USDA/ARS, Beltsville, Clemson University)
Strategy for isolation of a soybean gene conferring resistance to soybean cyst nematode

2:25 pm Benjamin Matthews, Rana Khan, Nadim Alkharouf, Hunter Beard, Margaret MacDonald, Kim Lewers, Halina Knap
(USDA/ARS, Beltsville, Clemson University)
Microarray analysis of gene expression in soybean plants resistant or susceptible to soybean cyst nematode (SCN)

2:45 pm **Closing Remarks**

Poster Take-down

2001 MAPMBS Poster sessions

Poster	Page	
1	29	Smriti Mehra, Ganesh R Panta, Lisa Rowland(USDA/ARS, Beltsville,Univ. of Tennessee). cloning and sequencing dehydrin alleles from cold sensitive and cold tolerant parent plants of a blueberry mapping population
2	30	Catherine A. Whitelaw, Nicholas N. Lyssenko, Vanessa K. Thai and Mark L. Tucker (USDA/ARS, Beltsville) Functional analysis of regulatory sequences and isolation of protein factors controlling expression of an abscission-specific cellulase from bean (phaseolus vulgaris)
3	31	Rana Khan, Reynold Tan, Ever Ponciano, David Straney (Univ. of Maryland) Regulatory components of fungal recognition of the host plant through an isoflavonoid phytoalexin
4	32	Nadim Alkharouf, Benjamin Matthews (USDA/ARS, Beltsville) SMGD: the soybean genomics and microarray database
5	33	Rana Khan, Nadim A. Alkharouf , Hunter Beard, Margaret MacDonald, Halina Knap and Benjamin Matthews (USDA/ARS, Beltsville, Clemson University) Genomic expression profiling in soybean plants resistant or susceptible to soybean cyst nematode (SCN)
6	34	Qijian Song, Ed Fickus, Perry Cregan (USDA/ARS, Beltsville) Characterization and mapping of trinucleotide SSR motifs in Wheat
7	35	Stockett Tammy, Saunders Joseph, David Kuykendall (USDA/ARS, Beltsville, Michigan State University) Callus independent shoot regeneration in sugarbeet may be the key to routine transformation
8	36	C M Williams and D J Sherrier (University of Delaware) Proteomic analysis of the symbiosome membrane from nitrogen-fixing root nodules in Medicago truncatula
9	37	Ibrahim A Ibrahim and Adel E Hegazi (Minufya University, Egypt) In vitro cultivation of Date Palm; 1-micropropagation of adult and juvenile date palm

(continued)

10	**38**	Ibrahim A Ibrahim and Adel A Abo-El-Soud, El-Sayed I Baker, and Nabeil R El-Sherbeny (Minufya University, Egypt, Cairo University, Egypt) In vitro cultivation of Date Palm; 2-Factors affecting growth and development of in vitro date palm through embryogenesis
11	**39**	Ibrahim A Ibrahim and Adel E Hegazy (Minufya University, Egypt) In vitro cultivation of date palm; 3-Date Palm abnormalities during micropropagation via embryogenesis

COSUPPRESSION OF AN IMMUNODOMINANT ALLERGEN IN TRANSGENIC SOYBEAN SEEDS

Eliot Herman1, Ricki Helm2, Rudolf Jung3, and Anthony Kinney4. 1Soybean Genomics Improvement Laboratory, USDA/ARS Beltsville, MD; 2 Dept. of Pediatrics, Univ. of Arkansas, Little Rock, AR; 3 Pioneer Hi-Bred International Inc., Johnson, IA; 4 DuPont Nutrition and Health, Experimental Station, Wilmington DE

The wide use of soybean products in processed foods poses consumption difficulties for sensitive individuals. The clinical symptoms of soybean allergy can be manifested as gastric distress or atopic dermatitis and while usually not life threatening, suspected cases of anaphylaxis have been reported. In vitro assays on soybean seed proteins with sera from soy-sensitive individuals have shown that the major storage proteins as well as other minor seed proteins account for IgE-binding cross-reactivity. Among the immunodominant seed proteins of soybeans is P34, a member of the papain superfamily of cysteine proteases. The P34 epitopes recognized by human IgE from soy-sensitive individual's sera have been characterized. Biotechnology offers the prospect to alter immunoreactivity of plant products with the aim of eliminating allergens that pose difficulties for sensitive people. We have used sense cosuppression driven by a seed-specific b-conglycinin promoter in transgenic soybeans to eliminate the accumulation of P34. The resulting P34 knockout seeds were produced on plants that lack any obvious developmental or structural phenotype differences compared to the wild-type plants. Two dimensional protein gel analysis shows that P34 suppression produces collateral changes in polypeptide pattern that is not obvious in single dimension analysis. A goal for hypoallergencity to multiple allergens in food sources is to reduce/eliminate specific allergens without affecting the plant or nutrient source. The production of a P34 knockout eliminates one of the primary allergens present in soybean seeds.

CLONING OF AND EXPRESSION OF HMG-COA REDUCTASE AND α-FARNESENE SYNTHASE AS A STRATEGY FOR THE ELIMINATION OF SUPERFICIAL SCALD STORAGE DISORDER IN APPLE FRUIT

STEVEN W. PECHOUS and Bruce D. Whitaker – USDA-ARS Plant Sciences Institute, Produce Quality and Safety Laboratory, Beltsville, MD 20705

Superficial scald is a serious storage disorder of apples and pears, and is thought to be induced under oxidative stress. Susceptible cultivars can sustain up to 80 % crop loss when stored under ambient air at 0 – 5 °C for several months. Evidence to date suggests that the oxidation of the sesquiterpene α-farnesene in peel tissue is the principal cause of necrosis of the underlying cortical tissue that leads to scald development. The antioxidant diphenylamine is currently used to control the disorder, but this treatment is costly and poses serious risks to the environment and to consumer health. Therefore, we are developing a non-chemical strategy for control of scald, based on the idea of regulating expression of key enzymes in the α-farnesene biosynthetic pathway. Eventually, transgenic lines expressing greatly reduced amounts of these enzymes should provide new scald-resistant lines while offering definitive proof of the role of α-farnesene in scald development. We have targeted two enzymes for initial cloning and expression, 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) and α-farnesene synthase (FS). HMGR is the rate-limiting enzyme in isoprenoid biosynthesis, and FS is responsible for the direct conversion of farnesyl pyrophosphate to α-farnesene. Use of degenerate primers based on conserved gene sequences in other plants has enabled RT-PCR and cDNA library-based subcloning from apple peel RNA of two isozymes of HMGR. A similar strategy based on a small, loosely conserved region of plant terpene synthases has led to cloning of a gene that most likely encodes FS. If so, it will be the first α-FS to be described. RNA gel blot data indicate that the transcript levels of our putative FS in apple peel tissue decrease after a treatment of fruit that suppresses scald development. Expression of these cloned enzymes in *E. coli* and *Pichia pastoris* is now underway.

REGULATION OF ANTHOCYANIN PRODUCTION IN *VIOLA CORNUTA* UNDER VARIOUS ENVIRONMENTAL CUES

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A dramatic ontogenetic color change in *Viola cornuta* cv. Yesterday, Today and Tomorrow (YTT) flowers provides a unique opportunity to investigate regulation of anthocyanin biosynthesis. YTT flowers open white and turn purple within four to seven days, and the color change is based on a gradual accumulation of malvidin (an anthocyanidin) against a constant background level of myricetin. Unlike other anthocyanin model systems, in which color production follows a predictable trajectory, YTT color change can be brought on or prevented by manipulation of pollination status or exposure to light. Infection by virus can also affect anthocyanin production.

Because the concentration of myricetin remains constant during color development, we hypothesize that *Chs*, an early gene, will be expressed throughout YTT color change. On the other hand, leucoanthocyanidins, the products of a late step catalyzed by the enzyme DFR, accumulate over YTT development, suggesting that *Dfr* gene expression will increase over ontogeny. We are using northern analysis of Stage I (white), Stage II (lavender) and Stage III (purple) YTT flowers to determine how light, pollination and virus infection influence patterns of *Chs* and *Dfr* expression. We have successfully cloned and sequenced an 86 bp fragment of the *Chs* gene and a 245 bp fragment of the *Dfr* gene from YTT and are currently conducting northern analysis experiments.

ETHYLENE RECEPTOR SIGNALING IN *ARABIDOPSIS*

Caren Chang, Chi-Kuang Wen, Jason Shockey, Paul Larsen, Ruth Stadler and Zaiqun Pan; Department of Cell Biology and Molecular Genetics, HJ Patterson Hall, University of Maryland, College Park, MD 20742

Ethylene signal transduction in *Arabidopsis* involves five ethylene receptors, which are members of the two-component histidine protein kinase family. The ethylene receptors fall into two subfamilies; subfamily I contains all of the conserved motifs that characterize histidine protein kinases, whereas subfamily II lacks most or all of these motifs. We are interested in how the five receptors signal in the ethylene-response pathway to the downstream protein kinase CTR1. We previously found that the regulatory domain of CTR1 can physically associate with subfamily I receptors (ETR1 and ERS1). To examine the interaction between ETR1 and CTR1 in greater detail, we identified mutations in either ETR1 or CTR1 that disrupt or enhance their interaction, and the data suggests that the interaction may be dependent upon phosphorylation of an aspartic acid residue in ETR1. Such an association has not been detected between CTR1 and subfamily II receptors (ETR2, EIN4 and ERS2). Biochemical and genetic data suggest that ETR2 instead forms a complex with ETR1 (and/or ERS1) and CTR1, and that ETR1 is required for ETR2 signaling. Finally, we are investigating whether other proteins, such as small His-containing phosphotransfer domain proteins, might also be involved in ethylene receptor signaling.

ARABIDOPSIS RGL1 CODES FOR A NEGATIVE REGULATOR OF GIBBERELLIN RESPONSES

Chi-Kuang Wen and Caren Chang
Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

The *Arabidopsis* DELLA subfamily of the GRAS transcription factor family consists of five members encoded by *RGA*, *GAI*, *RGL1, RGL2* and *RGL3*. *RGA* and *GAI* are negative regulators of GA response. We investigated *RGL1* (*RGA-LIKE1*) function based on *rgl1* gain-of-function and loss-of-function phenotypes. A *35S-rgl1* transgene containing a DELLA domain deletion (as in the *gai1-1* mutation) conferred repression of GA responses. Like *gai1-1* and GA-deficient plants, the transformants were dark green dwarfs with poorly developed trichomes; unlike *gai1-1*, the flowers had protruding carpels with underdeveloped petals and stamens. In addition, transcript levels of the feedback-regulated gene *GA4* were increased. In contrast, an *rgl1* loss-of-function line had reduced *GA4* message levels, and exhibited GA-independent activation of seed germination, stem elongation and flowering time, as detected by resistance to the GA biosynthesis inhibitor paclobutrazol (PAC). Leaf size remained PAC-sensitive, however. Expression of *RGL1* differs from that of the DELLA homologs; *RGL1* transcript levels are high in flowers, but low in leaves and other parts of the plant. *in situ* hybridization to floral sections revealed that *RGL1* is specifically expressed in developing anthers. A GFP-RGL1 fusion protein was nuclear-localized in transformed *Arabidopsis*.

These findings indicate that RGL1 negatively regulates certain GA responses, playing a greater role in seed germination and flower development and a lesser role in leaf expansion, and suggesting that all DELLA subfamily members may have overlapping but distinct roles in GA signaling.

APPROACHES TO TARGETED GENE INTEGRATION AND MODIFICATION IN PLANTS.

Chris L. Baszczynski, Dave Peterson, Alex Lyznik and Pramod Mahajan, Pioneer Hi-Bred International, Inc., a DuPont Company, 7250 NW 62nd Ave., Johnston, IA, 50131, Email: chris.baszczynski@pioneer.com

Over the last several years, we have taken various research approaches towards developing methods to precisely target gene integration or to modify genes *in vivo* in plants to allow development of new or modified traits in agriculturally important crop plants. Site-specific recombination systems are proving to be useful for directed integration of genes into previously introduced and characterized target sites in plant systems. This opens up opportunities to more precisely target new genes in the genome, to characterize expression of different DNA sequences at the same chromosomal insertion site and to bring gene sequences adjacent to each other for applications such as trait stacking. We also reported on the successful application of the chimeric oligonucleotide technology for making targeted modifications to endogenous genes or introduced transgenes, thereby conferring new phenotypes (Zhu *et al.*, 1999, PNAS 96:8768-8773) or in the case of the AHAS gene, the creation of a commercially important trait (herbicide resistance; Zhu *et al.*, 2000, Nature Biotech. 18, 555-558). Additionally, we have been exploring the plant EST databases created at Pioneer and DuPont for plant orthologs of genes known to be associated with DNA metabolism, repair and recombination. Collectively the genes, technologies and approaches are helping us better understand mechanisms of DNA repair and recombination in plants towards development of efficient methods to carry out gene targeting or to study and modify gene function towards crop improvement.

ANTIOXIDANT METABOLISM IN PEROXISOMES

Konstantina Karyotou and Robert P. Donaldson

The George Washington University, Department of Biological Sciences, Washington, D.C. - Tel: (202) 994 6931 - Email: karyotou@gwu.edu

Efficient destruction of hydrogen peroxide (H_2O_2) in peroxisomes requires the action of an antioxidant defense system, which consists of low molecular weight antioxidant compounds, such as ascorbate, along with protective enzymes, such as catalase, ascorbate peroxidase (APX) and thioredoxin peroxidase (TPX). We investigated the contribution of the ascorbate system to the consumptions of H_2O_2, NADH and NADPH within glyoxysomes of germinating castor beans (*Ricinnus communis*). We purified the glyoxysomal membrane APX (gmAPX) using octyl-glucoside and demonstrated its association with a 34 kDa protein, as determined by SDS-gel electrophoresis and western blotting. The activity was further purified by either ion exchange chromatography on DEAE-Sepharose or by gel filtration using a Sephacryl 300-HR column. Our results show that the APX activity correlates with a 34 kDa protein recognized by a polyclonal antibody raised against a 67 kDa nondenatured peroxisomal membrane complex from cucumber (Corpas et al., 1994, European J. Cell Biology, 65: p280). This demonstrates for the first time that this glyoxysomal membrane protein is indeed APX. gmAPX was found to be highly effective at low concentrations of H_2O_2, with a Km of 60 μM. Thioredoxin was the other antioxidant detected in glyoxysomes. Glyoxysomal matrix was treated with dithiothreitol (DTT), heated to 60°C and run through a gel filtration column (Sephacryl 300-HR). Fractions were assayed for thioredoxin using the DTT-insulin reduction assay and a thioredoxin peak was detected corresponding to 10 kDa, the expected size of thioredoxin. Our data suggest that peroxisomal thioredoxin contributes to H_2O_2 scavenging through TPX and NADPH:thioredoxin reductase.

PROTEOMIC AND METABOLOMIC STUDIES IN *Medicago truncatula*

Lloyd W. Sumner, The Samuel Roberts Noble Foundation
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As many genome sequencing projects have been or are nearing completion, the scientific community is asking how these newly identified genes function. These questions are the foundation of the functional genomics. To answer these questions, functional genomics seeks to simultaneously profile and identify large numbers of expression products at the transcriptome, proteome, and metabolome levels to determine relationships and provide insight into cell biology and biochemistry. The Samuel Roberts Noble Foundation has recently initiated a functional genomics initiative to study the model legume, *Medicago truncatula*. This presentation will focus on initial proteomic and metabolomic studies of *Medicago truncatula* with an emphasis on modern mass spectrometric approaches and bioinformatic tools.

CLONING AND FUNCTIONAL EXPRESSION OF FRUCTAN 1-EXOHYDROLASE I AND II, KEY ENZYMES OF FRUCTAN BREAKDOWN IN CICHORIUM INTYBUS L.

An Michiels, Liesbet Van Riet, Van Wonterghem Niki, De Roover Joke, Andre Van Laere and Wim Van den Ende

Department of Biology, Laboratory for Developmental Biology, Botany Institute, KULeuven, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium

Fructans are highly water-soluble fructose polymers and the main polysaccharide reserve in vegetative tissues of many grasses, Asteraceae and Liliaceae. They accumulate in the vacuole and have important functions in the storage of assimilate, in osmo-regulation and in resistance to stress. The genes of Fructan 1-Exohydrolase I and II (1-FEH I and II) have been cloned from *Cichorium intybus*. To our knowledge, these are the first cloned plant FEH genes. Full Length cDNA clones were obtained by reverse transcriptase PCR (RT-PCR) and 5` and 3` rapid amplification of cDNA ends (RACE) using primers based on N-terminal and conserved amino acid sequences. Sequence analysis of different clones of 1-FEH II revealed two isoenzymes (1-FEH IIa and 1-FEH IIb) that share 94% homology at the amino acid level. To our surprise, the amino acid sequences of the FEH enzymes are more homologous to cell wall invertases (43%-41% identical) than to vacuolar invertases (40%-41% identical) and other fructan biosynthetic enzymes (33%-39% identical). Homologies to bacterial exohydrolase are only around 13 to 24%. The homology between 1-FEH I and 1-FEH II is only 50% although the two enzymes exhibit the same hydrolase activity. Genomic DNA fragments have been amplified and subcloned. Sequence analysis showed the presence of a 9bp mini-exon encoding the tripeptide DPN. This mini-exon could also be found in most invertases. Expression analysis showed that both Fructan Exohydrolases are differentially regulated. Northern blotting revealed a distinctive cold-induced expression of 1-FEH I in mature roots at the end of the growing season, while high expression levels of 1-FEH II were found in etiolated leaves. Defoliation or protecting leaves from light results in a sudden increase of the 1-FEH II expression in chicory roots. Also uprooting and wounding of mature roots will induce 1-FEHII. Although, 1-FEH II genes might be induced after stress induction or by a sudden drop of photosynthate entering the roots, a low level expression of 1-FEH I could be seen one week after 1-FEH II expression was detected. These results suggest that both fructan exohydrolase enzymes can be induced when large energy supplies are necessary or protection against environmental stress is required.

NBS-LRR R gene products: Functional domains and divergent downstream transcript profiles.

Andrew Bent, University of Wisconsin – Madison, WI

We are investigating the molecular basis of resistance gene (R gene) function and the signal transduction events that activate gene-for-gene resistance, HR cell death and other forms of disease resistance. Working with Arabidopsis RPS2, we have investigated the roles of the nucleotide binding site (NBS) and the leucine-rich repeat (LRR). Our evidence for nucleotide binding by recombinant RPS2 protein will be presented, along with a functional dissection of allowable amino acid changes in the NBS of RPS2. In separate studies, we have found that the LRR domain not only determines pathogen specificity, but can also control effective interaction with other host factors required for resistance. In a third set of studies, Arabidopsis responses mediated by four different R/avr pairings are being compared by expression profiling using Affymetrix GeneChips. Expression profiling experiments were replicated directly or with variation. For direct replication, essentially identical experiments were performed on two separate dates using P. syringae pv. tomato DC3000 to deliver Avr signal. For our first "variation" performed to date, the defense-eliciting Avr signal was delivered to plants by inoculation with non-virulent but hrp-secretion-competent P. syringae pv. glycinea. These experiments are revealing reliable and less reliable technical aspects of expression profiling experiments, but are also revealing common and distinctive aspects of the plant responses elicited during different R/avr interactions.

TOBAMOVIRUS-HOST INTERACTIONS: REMODELING CELLULAR FUNCTIONS

James N Culver and Sheetal Golem, Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute and Molecular Cell Biology Program, University of Maryland, College Park, MD 20742, jc216@umail.umd.edu.

Simple RNA viruses have evolved complex interactions with their host's cellular machinery that facilitate their replication, cell-to-cell movement, and systemic transport. These interactions often lead to a disruption in the development and homeostasis of the host's cellular processes, resulting in the expression of disease symptoms that impact the host's well being and/or its economic value. Underlying the development of symptoms are significant alterations in the transcriptional profiles of infected cells. Using cDNA microarray technologies we have begun to investigate the effects of virus replication on host gene expression using an *Arabidopsis-Tobacco mosaic virus* (TMV) model system. For these studies, mature *Arabidopsis* plants were mechanically inoculated with either purified TMV or mock-inoculated with buffer. Messenger RNA was obtained from inoculated and systemic leaves of infected and mock plants at either 4 or 14 days post inoculation. Labeled cDNA derived from these mRNA samples were hybridize to microarray slides spotted with more than 7,000 independent *Arabidopsis* cDNAs via the Arabidopsis Functional Genomics Consortium (http://AFGC.stanford.edu). Analysis from these experiments indicate that the majority of tested *Arabidopsis* cDNAs did not display significant changes in expression levels when comparing TMV infected to mock inoculated samples. However, a number of cDNAs whose expression levels were induced or repressed during infection were observed. In summary, 28 genes displayed significant alterations in their expression in inoculated leaf tissue at 4 days post-inoculation, while 152 genes were altered in systemically infected tissue at 14 days post-inoculation. Many of the altered cDNAs encode proteins that have been associated with stress responses, including pathogenesis and senescence. The predicted functions of these altered genes will be discussed in relation to the progression of virus disease development.

DIFFERENTIAL GENE EXPRESSION IN WEED SPECIES TREATED WITH A NECROSIS-INDUCING PEPTIDE Sarah E. Keates and Bryan A. Bailey; USDA, ARS, PSI, Alternate Crops and Systems Lab, Building 001, Room 322, BARC-W, Beltsville, MD, 20705, keatess@ba.ars.usda.gov, baileyb@ba.ars.usda.gov.

Determining how fungal peptides induce plant responses may further our understanding of the disease resistance mechanisms protecting agricultural crops, and the factors influencing the susceptibility of weed species to potential bioherbicidal agents occurring naturally in the environment. A 24-kDa peptide isolated from culture filtrates of *Fusarium oxysporum*, Nep1, elicits physiological responses in a broad range of plant species. Nep1 induces necrosis in a variety of weed species within 48 hours of foliar spray treatment. Dandelion (*Taraxacum officionale* Weber) and spotted knapweed (*Centaurea maculosa* Lam.) show extensive necrosis following treatment, and are being used to assess weed responses to the peptide. We are isolating RNA from treated and control plants and using differential display (DDRT-PCR) to identify genes that are differentially expressed in response to Nep1 treatment. Initial screening will focus on plant genes that are upregulated or downregulated within 4 hours of treatment with the necrosis-inducing peptide. Over 200 bands of interest have been isolated from differential display gels and will be confirmed based on replicate display profiles and Northern blots prior to cloning and sequencing. Parallel studies with *Arabidopsis thaliana* L., which also develops extensive necrosis following Nep1 application, may facilitate tentative identification of genes of interest based on sequence homology.

Function of RPM1 requires RIN4, a virulence target guarded by RPM1

David Mackey*, Aaron Wiig, Ben Holt, and Jeff Dangl
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RPM1 is an R-gene that confers resistance against Pseudomonas syringae carrying the avirulence (avr) genes avrB and avrRpm1. We have discovered an unknown Rpm1 interacting protein (Rin4) which interacts, in yeast and in vivo, with both Rpm1 and AvrB. Rin4 is required for function of Rpm1. Suppression of Rin4 protein-levels by antisense RNA inhibits the hypersensitive response (HR) normally induced by RPM1. In fact, suppression of Rin4 protein-levels causes Rpm1 protein-levels to decline. Significantly, suppression of Rin4 does not affect the HR induced by Rps2 in response to AvrRpt2. Rps2 is structurally very similar to Rpm1 and several mutants have been isolated which influence the function of both proteins. Rin4 functions prior to the convergence of these pathways and is specific to Rpm1. The guard hypothesis predicts that an R-protein exists in a complex with a partner protein that is the virulence target of an Avr-protein. Rpm1 is in a complex with Rin4 in uninfected tissue. When expressed in tissue lacking Rpm1, AvrB and AvrRpm1 enter into a complex with Rin4. AvrB and AvrRpm1 also induce a mobility shift in Rin4. Expression of AvrRpt2 induces rapid degradation of Rin4. These manipulations of Rin4 by Avr-proteins are likely to underlie the virulence activity of these proteins. Consistent with its manipulation by virulence factors, Rin4 is a negative regulator of defense. Reduced levels of Rin4 cause increased resistance against normally compatible isolates of Peronospora parasitica and Pseudomonas syringae. Rpm1 "guards" Rin4 from manipulation by AvrB and AvrRpm1.

CONNECTING FUNCTION TO PHENOTYPES

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The complexity of whole genome data sources and analysis of that data to enable the generation of models of genome operations in biological systems is the next grand challenge for Agricultural Research. The industrialization of high throughput laboratory methods is now generating more genetics, biochemical and phenotypic data on individual plants than ever before. This presentation will focus on the complexities of using expression profiling for functional genome analysis.

The use of DNA fingerprinting in chocolate trees to study genetic diversity

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DNA fingerprinting, a tool that has been widely used in forensic science, is also useful in plants to study genetic diversity within a breeding population. Two different procedures have been employed to establish DNA fingerprinting profiles with 180 accessions of *Theobroma cacao* grown in Puerto Rico that represent the USDA chocolate germplasm collection. Each of the two molecular techniques, Amplified restriction Fragment Length Polymorphic (AFLP) DNA analysis and Single Sequence Repeat (SSR) analysis, has its strengths and weaknesses. We analyzed the USDA Puerto Rico *T. cacao* germplasm collection with 6 AFLP primers and with 15 SSR primers to assess the genetic diversity of the collection on a molecular basis. Although the SSR DNA fragment analysis was easier to perform and easier to analyze than the AFLP techniques, SSR procedures do require substantial preliminary studies to define adequate PCR primers. AFLP techniques have the significant advantage of being suitable for virtually any population without extensive preliminary molecular analysis or sequencing procedure. Using the Puerto Rico collection of *T. cacao* germplasm, 15 international SSR primers were tested and the allele ranges for these primers were established. These primers can now be used in a routine manner to assess the genetic diversity of *T. cacao* collections from world populations.

Arabidopsis cytochrome P450 *cyp83B1* mutations activate the tryptophan biosynthetic pathway.

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In plants, the tryptophan biosynthetic pathway provides a number of important secondary metabolites including the growth regulator indole-3-acetic acid (IAA) and indole glucosinolate defense compounds. Genes encoding tryptophan pathway enzymes are transcriptionally induced by a variety of stress signals, presumably to increase the production of both tryptophan and secondary metabolites during defense responses. To understand the mechanism of transcriptional induction, we isolated altered tryptophan regulation (*atr*) mutants in *Arabidopsis thaliana* with activated transcription of tryptophan genes. One *atr* complementation group consisted of mutations in the cytochrome P450 gene *CYP83B1*. Mutant plants had constitutively activated expression of the *ATR1* Myb factor gene, which was previously identified as a positive regulator of tryptophan genes. *cyp83B1* mutants were initially characterized as having defects in IAA homeostasis due to perturbation of secondary tryptophan metabolism. Our findings indicate that the upregulation of tryptophan pathway genes might also contribute to the overaccumulation of IAA in mutant plants. Moreover, we have shown that *cyp83B1* mutants have lesion-mimic phenotypes, suggesting that multiple stress pathways are activated by the loss of CYP83B1 function.

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN SOYBEAN: DISCOVERY AND DETECTION

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SNPs are rapidly becoming the marker of choice for many applications in genome analysis. Information on the frequency and distribution of SNPs in plants is limited. Our laboratory is defining approaches for both the discovery and the high throughput detection of SNPs in soybean. **SNP Discovery:** Our objectives were to determine 1) SNP frequency in coding and non-coding soybean DNA sequence derived from complete genes, cDNAs, and random genomic sequence and 2) the proportion of genes and cDNAs in which SNPs could be discovered. These data address the feasibility 1) of mapping expressed sequence tags (ESTs) via the presence of an associated SNP in order to create a transcript map and 2) of developing a large set of SNPs from random genome sequence. Primers were successfully designed to amplify a locus-specific product from 65 of 90 complete genes and 52 of 90 cDNAs. In total, about 29 kbp of coding- and 38 kbp of non-coding (introns, 5' and 3' untranslated, and associated genomic region) DNA was sequenced in each of 25 diverse soybean genotypes and a mean of 1.69 and 3.94 SNPs per kbp was discovered in coding and non-coding regions, respectively. At least one SNP was found in 74 of the 180 genes to which primers were designed. In 9.8 kbp of sequence derived from bacterial artificial chromosomes and simple sequence repeat flanking regions, the frequency of SNPs was 6.70/kbp, indicating that SNPs could be readily discovered in random genomic sequence. Haplotype analysis of SNP-containing DNA fragments revealed fewer haplotypes than would be anticipated at linkage equilibrium. In 63 fragments with two or more SNPs, no more than four haplotypes were detected. The paucity of haplotypes suggests a genetic bottleneck or that the small number of haplotypes are derived from a few soybean domestication events. A cluster analysis indicated that the genome of any given soybean accession is a mosaic of three or four haplotypes. Subsets of four or five diverse genotypes were identified whose sequence analysis would permit the discovery of at least 75% of all SNPs present in the 25 genotypes. **SNP Detection:** Research is focusing on Single Base Extension (SBE) as the preferred system of SNP detection. SBE products are being analyzed using an Applied Biosystems 3100 capillary DNA Analyzer as well as a Luminex Flow Cytometer. Both systems promise high throughput multiplex SNP analysis. We are attempting to determine which has the greatest throughput at the lowest cost per data point.

STRATEGY FOR ISOLATION OF A SOYBEAN GENE CONFERRING RESISTANCE TO SOYBEAN CYST NEMATODE

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The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is the foremost pest of soybean (*Glycine max* L. Merr.) and is responsible for the loss of 10% of the annual U.S. soybean crop. The most practical management strategy has been the use of resistant cultivars. The rhg_1 resistance allele on Linkage Group G and the Rhg_4 resistance allele on Linkage Group A2 of the soybean genetic map are especially important. Markers closely linked to the Rhg_4 locus were used to screen a bacterial artificial chromosome (BAC) library of susceptible 'Williams 82' and identified a 150kb BAC. End-sequenced *Eco* R I subclones located to an *Apa* I restriction map provided landmarks used to identify the corresponding region in a BAC library of the soybean accession PI 437.654, with broad resistance to SCN. Restriction fragment analysis and sequence from BAC ends were used to develop a contig of PI 437.654 BACs representing the PI 437.654 counterpart of the Williams 82 BAC. Some of the markers on the PI 437.654 contig are separated by a greater physical distance (*ca.* 100 kb) than in the Williams 82 BAC, and some primers amplify bands from the mid-portion of the PI 437.654 BAC contig that are not amplified from the Williams 82 BAC. These observations suggest the presence of an insertion in the PI 437.654 genome relative to the Williams 82 genome. A high degree of multi-level duplication may be responsible for reported organizational instability in this part of the genome and supports the possibility of a deletion/insertion in this region. In addition, the degree of duplication in this region would likely complicate attempts at high-resolution mapping. Subclones of the Williams 82 BAC and subclones of two PI 437.654 BACs suspected to contain the Rhg_4 allele have been end-sequenced, and the sequences were compared with those in GenBank. These sequence similarity searches indicate the Rhg_4 region is densely populated with genes previously associated with stress or defense responses, so there is a low probability that Rhg_4 would be identified solely by sequence similarity. Because we cannot identify the Rhg_4 gene with high-resolution mapping and sequence similarity alone, carefully designed complementation tests will be required. Several transformation methods are being investigated for this purpose.

MICROARRAY ANALYSIS OF GENE EXPRESSION IN SOYBEAN PLANTS RESISTANT OR SUSCEPTIBLE TO SOYBEAN CYST NEMATODE (SCN).

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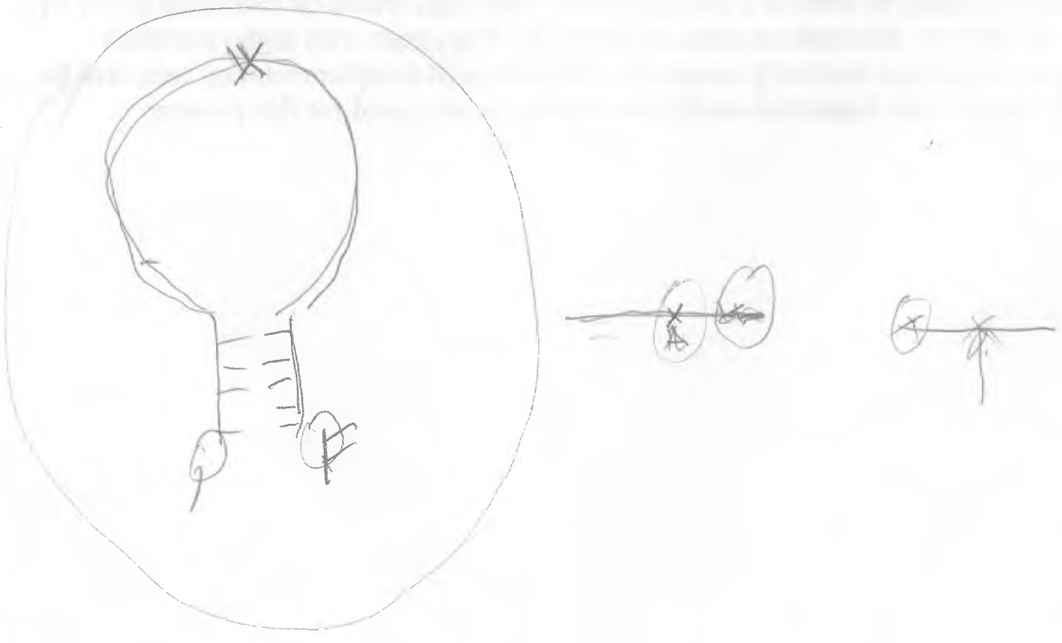
The soybean cyst nematode (SCN) is the major pest of soybean causing an estimated $1.5 billion in damage throughout the US each year, more than all other soybean pests combined. The defense response of soybean to SCN is a multigenic trait and varies depending upon the genotypes of soybean and SCN. The expression of approximately 1,500 soybean genes was monitored using microarrays to identify genes involved in the response of soybean to SCN. RNA was harvested from roots of soybean cv. Peking resistant to SCN race 3 and cv. Kent susceptible to SCN race 3, either two days after infection by SCN race 3 or not infected. Three independent biological samples were harvested and analyzed for each experiment. Microarrays were produced that display PCR amplified cDNA inserts from approximately 1500 root-specific genes. These genes are derived from cDNA libraries made from root tissue of resistant soybean cultivars infected or not infected by SCN. The arrays were hybridized with probes derived from root mRNA of Peking (resistant) or Kent (susceptible) cultivars 48 hr post invasion. The number of genes that were at least 2-fold induced in the presence of SCN was higher in the resistant cultivar as compared to the susceptible cultivar. Among the genes induced specifically in the resistant soybean variety are defense-related genes, potential regulatory factors such as phosphatases and transcription factors, and a number of genes encoding proteins of unknown function. These results provide some insights into the mechanisms used by soybean to respond to SCN attack.



CLONING AND SEQUENCING DEHYDRIN ALLELES FROM COLD SENSITIVE AND COLD TOLERANT PARENT PLANTS OF A BLUEBERRY MAPPING POPULATION

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Studies of herbaceous plants suggest that cold hardiness is a complex, quantitatively inherited trait. Although development of cold hardiness is an integral part of the life cycle of woody perennial plants, studies on its genetic control in woody perennials are scarce. A better understanding of its genetic control would be valuable for developing more effective strategies to increase cold hardiness and, hence, climatic adaptation of woody perennial crops. In blueberry, we have previously shown that three major dehydrins of 65, 60, and 14 kDa increase with cold acclimation and decrease with deacclimation. A comparison of these dehydrin levels among various blueberry cultivars and selections has revealed their level of accumulation to be closely associated with cold hardiness level. Efforts are underway to isolate and map the dehydrin genes of blueberry utilizing populations that segregate for cold hardiness in order to determine if the dehydrin genes map to or cosegregate with QTLs controlling cold hardiness. A 2.0 kb cDNA, which encodes the 60 kDa dehydrin from blueberry, has been cloned and sequenced. Here, we report using the DNA sequence of the cDNA clone to design primers to amplify and clone alleles of two dehydrin-related genes from the cold sensitive and cold tolerant parent plants of the blueberry mapping population. The alleles have been sequenced and primers based on the regions showing differences are being used to attempt amplifying polymorphic fragments for mapping purposes.

FUNCTIONAL ANALYSIS OF REGULATORY SEQUENCES AND ISOLATION OF PROTEIN FACTORS CONTROLLING EXPRESSION OF AN ABSCISSION-SPECIFIC CELLULASE FROM BEAN (*PHASEOLUS VULGARIS*).

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The bean abscission cellulase (BAC) gene is one of a number of genes induced coordinately with ethylene-promoted leaf abscission. Additionally, BAC gene expression is subject to complete repression by auxin. To investigate the molecular basis for ethylene and auxin regulation, we have examined the BAC promoter to identify *cis*-elements and *trans*-acting factors that are involved in BAC gene expression. Using deletion analysis and site-directed mutagenesis, we have characterized *cis*-acting regulatory elements in the BAC promoter involved in ethylene-induction and auxin-repression. A 10 bp mutation at –103 to -113 (with respect to the start of transcription) was sufficient to reduce reporter gene expression by 60%. Two different classes of transcription factor that bind this region of the BAC promoter were isolated by screening a one-hybrid library of bean leaf abscission zone cDNA in yeast. One class encode proteins of the NAC domain family, a novel class of plant transcription factors, while the other encode proteins belonging to the homeodomain leucine zipper family of DNA-binding proteins. Here we describe the identification and characterization of *cis*-elements and *trans*-acting factors from bean and discuss the implications of our results with respect to the regulation of abscission.

REGULATORY COMPONENTS OF FUNGAL RECOGNITION OF THE HOST PLANT THROUGH AN ISOFLAVONOID PHYTOALEXIN

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Plant-pathogens interactions are largely regulated through the ability of each organism to recognize each other. Although much is known about how plants recognize both specific and non-specific cues from potential pathogens, less is known about how the pathogens recognize cues from the host plant to trigger developmental or gene-specific traits needed for pathogenesis. We are studying *Nectria haematococca* MP VI (*Fusarium solani*) where development and specific genes are induced by the phytoalexin produced by the host, *Pisum sativum* (garden pea). Since this phytoalexin, the isoflavonoid pisatin, is uniquely produced by this one host plant, the positive responses to pisatin appear to be a means of host recognition.

One pisatin-induced virulence gene is the PDA1 gene, which encodes a cytochrome P450 that detoxifies pisatin. Pisatin appears to be the major inducer for this gene during pathogenesis as well. We are using the PDA1 gene to identify components of the signal pathway that allows this fungus to recognize and respond to host-specific cues to coordinate pathogenesis. We have defined a 40 bp region in the PDA1 promoter that mediates pisatin-responsive transcription in vivo. A DNA-binding factor was found to bind this 40 bp region in mycelial extracts. The yeast one-hybrid system was used to clone the gene encoding the protein that binds this 40 bp region. Sequence motifs from the cloned gene suggest that it is a Cys(6)-Zn(2) binuclear cluster factor, a class unique to the fungi. A short upstream ORF was found in the cDNA, suggesting a potential means of translational regulation. We are using targeted gene replacement to test the function of this regulator in other pisatin-induced traits. Further, expression of the protein in bacteria and yeast are allowing us to test its DNA-specificity and the potential ability of the protein to bind pisatin, and so function as a nuclear receptor similar to that found regulating many mammalian cytochrome P450 genes.

SMGD: The soybean genomics and microarray database

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The soybean genomics and microarray database was established in 1999 to serve as a sequence and microarray database for the Soybean Genomics and Improvement Laboratory (SGIL), Beltsville Agricultural Research Center (BARC) and collaborators. It serves both as a sequence repository, holding DNA sequences for numerous EST's, and also as a microarray experiment database. It allows scientists to explore the expression levels of the EST clones stored in the database and to correlate expression levels with function. SGMD is a relational database built on SQLServer2000 and incorporates the minimal information about a microarray experiment (MIAME) guidelines set forth by the microarray gene expression database (MGED) group. This ensures that the data can be interpreted and used following certain set standards. It also provides appropriate, complete information for other scientists to verify the data. Web interfaces have been developed for the database, whereby numerous statistical analyses, such as one-way analysis of variance (ANOVA) and T-tests can be performed on the fly from any web browser. Web interfaces also have been developed for complex comparison queries, allowing scientists to correlate and find similarities and differences across different experiments and probe combinations. This is all accomplished without the need to resort to third party software. Everything is handled by the use of SQL procedures within the database itself. Thus SGMD not only serves as a database, but as a data analysis tool as well, which makes it unique to most other microarray databases. Future improvements to the database will include the incorporation of clustering algorithms and data mining tools for the analysis of microarray time course data. SGMD can be accessed from http://bldg6.arsusda.gov/benlab.

GENOMIC EXPRESSION PROFILING IN SOYBEAN PLANTS RESISTANT OR SUSCEPTIBLE TO SOYBEAN CYST NEMATODE (SCN).

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The interaction between the soybean plant and soybean cyst nematode (SCN) may result in resistance or disease depending upon the variety of soybean and the race of SCN invading the plant. The economic loss caused by SCN, in the U.S, is significantly greater than any other known pathogen of soybean, and necessitates an understanding of the mechanisms underlying the basis of resistance to SCN, in order to develop more resistant varieties. Identification of plant host genes involved in defense responses is an important step towards elucidation of disease resistance mechanisms in plants. We have used microarray technology to look at the expression profile of approximately 1200 soybean root genes, in a resistant and susceptible soybean cultivar, two days after nematode invasion. The array was generated by spotting PCR amplified inserts from cDNA clones derived from the roots of SCN-infected resistant soybean variety, and were hybridized with fluorescently labeled probes prepared from total RNA isolated from SCN-infected roots of resistant or susceptible soybean variety. In each case the uninfected susceptible variety was used as a control. Data analysis reveals that approximately 10% of the 1200 genes are statistically induced in both cultivars. Among the genes that are uniquely induced in either the resistant or the susceptible cultivars, a large number are proteins of unknown functions. The others appear to be involved in general defense responses and fall into the categories of cell wall maintenance or development, synthesis of secondary metabolites, stress induced, and metabolic pathways. A few genes are likely components of signal transduction pathways. Similar to other pathogens, SCN invasion triggers the induction of some genes involved in systemic acquired resistance. Although the gene expression pattern is similar in both resistant and susceptible soybean cultivars, the results suggest that 2 days after nematode invasion, the overall induction levels are more enhanced in the resistant variety.

CHARACTERIZATION AND MAPPING OF TRINUCLEOTIDE SSR MOTIFS IN WHEAT

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As compared to dinucleotide-based microsatellite markers, length differences among trinucleotide-based microsatellite alleles can be more easily detected and frequently produce fewer "stutter bands". Our objective was to determine which trinucleotide motif(s) would be the most abundant source of polymorphic trinucleotide microsatellite markers in wheat. Four genomic libraries of cultivar 'Chinese Spring' were screened with nine trinucleotide probes. Based on the screening of 28550 clones, the occurrences of $(CTT)_n$, $(GGA)_n$, $(TAA)_n$, $(CAA)_n$, $(GGT)_n$, $(CAT)_n$, $(CGA)_n$, $(CTA)_n$, and $(CGT)_n$ repeats were estimated to be once every 293kbp, 456kbp, 500kbp, 1.3Mbp, 2.6Mbp, 3.2Mbp, 3.6Mbp, 3.6Mbp and 4.5Mbp in the wheat genome, respectively. Of 236 clones selected for sequencing, 38 (93%) $(TAA)_n$, 30 (43%) $(CTT)_n$, 16 (59%) $(CAA)_n$, 3 (27%) $(CAT)_n$ and 2 (4%) $(GGA)_n$ clones contained microsatellites with 8 or more perfect repeats. From these data, 29, 27, and 16 PCR primer sets were designed and tested to the $(TAA)_n$, $(CTT)_n$, and $(CAA)_n$ microsatellites, respectively. A total of 12 (41.4%) primers designed to $(TAA)_n$, 4 (14.8%) to $(CTT)_n$, and 2 (12.5%) to $(CAA)_n$ resulted in polymorphic markers. The results indicated that $(TAA)_n$ microsatellites would provide the most abundant and the most polymorphic source of trinucleotide microsatellite markers in wheat. Based on these finding, two hundred and forty mostly $(TAA)_n$ repeat-based markers have been developed, they were transferred to seventeen institutes in the USA, China, Egypt and Turkey and are being used for genetic and cytogenetic mapping, study of the association with *Fusarium* head blight and yellow rust disease resistance genes, and applications in barley genetic map development. One hundred and forty markers have been mapped in the Opata 85 and M6 population. Six markers were recently identified that flank the major scab resistance gene on the short arm of wheat chromosome 3.

CALLUS INDEPENDENT SHOOT REGENERATION IN SUGARBEET MAY BE THE KEY TO ROUTINE TRANSFORMATION

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Sugarbeet transformation technology is tedious and inefficient. However we recently obtained adventitious shoots in a one step procedure using leaf pieces of greenhouse grown REL-1 sugarbeet clones. Five of six fully expanded leaf pieces of one-to-two cm generated one or more shoots on 1 mg/L N^6-benzyladenine semi-solid Murashige-Skoog media maintained at 23.5°C in seven to twelve weeks under low light conditions. To determine whether leaf pieces from leaves not fully expanded have similar capabilities leaves 20% and 40% expanded were treated the same except that two leaf pieces were placed on each plate. Several shoots of the 20% expanded leaves where formed on 1mg/L N^6-benzyladenine semi-solid Murashige-Skoog media as well on media with two or three times that amount of N^6-benzyladenine, and nine of ten leaf pieces on 5 mg/L N^6-benzyladenine were formed. Similar results were observed for the 40% expanded leaves except that the higher concentrations of N^6-benzyladenine did not increase shoot production. Previous reports of the formation of adventitious shoots from *in vitro* grown sugarbeet seedlings, shoots, leaf pieces, or thin layer cells of these tissues were presumed to have been due to pre-formed meristematic initials induced during the *in vitro* cultures and were therefore deemed not amenable for direct selection or transformation. Highly regenerative hormone automonous callus from leaf pieces had previously been used for transformation. Now, fresh insight into simpler and more efficient genetic transformation of sugarbeet enables the use of leaves, or pieces of them for transformation.

PROTEOMIC ANALYSIS OF THE SYMBIOSOME MEMBRANE FROM NITROGEN-FIXING ROOT NODULES IN MEDICAGO TRUNCATULA

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Nitrogen fixing root nodules form from the symbiotic relationship between legume plants and rhizobia bacteria. In this symbiosis, the rhizobia differentiate and are enclosed by a membrane derived from the plant plasma membrane. This membrane is called the symbiosome membrane and is the focus of our research. The symbiosome membrane is essential because it separates the rhizobia from the plant cell cytoplasm and controls the exchange of metabolites and nutrients between the symbiotic partners. One research focus has been to fully document the infection process between the two partners using light microscopy. Documentation has been made to determine at what point in the infection process certain events occur. A second research focus has been to biochemically characterize the symbiosome membrane to understand cell surface interactions and symbiosome formation. Using subcellular fractionation and two-dimensional SDS-polyacrylamide electrophoresis along with mass spectroscopic sequence analysis, we are biochemically characterizing the symbiosome membrane and have found an abundant amount of proteins in the membrane. Using two-dimensional SDS-polyacrylamide electrophoresis, we have generated a standard map for the symbiosome membrane in order to gain a better understanding of its contribution to nodule formation and function.

In Vitro Cultivation of Date Palm; 1- Micropropagation of adult and juvenile date palm

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In vitro propagation of adult and juvenile date palm (*Phoenix dactilifera* L. cv. Zagloul) through somatic embryogenisis and organogenisis have been established. Regenerated plants from shoot tip and leaf primordial have been reported from adult and juvenile date palm trees. Direct organogenisis and embryogenisis developed on explants when cultured on a medium containing auxins (NOA and NAA at 5mg/L) and cytokinin (kinetin and 2iP at 3mg/L). There are no visible differences between shoot tip and leaf primordial for response on the previous medium (MI) while this is not the case on MS medium supplemented with 100mg/L 2, 4-D and 3mg/L 2iP (MII). Adventitious roots were induced in medium containing low levels of auxin (0.1 mg/L). The resultant plantlets from activated charcoal have thin and long roots while plantlets produced from MS medium without activated charcoal have short roots. The highest rooting percentage was recorded for the plantlets produced from leaf primordial for adult and juvenile date palm. Survival percentage in the greenhouse ranged from 63 to 69 percent for plantlets derived from adult and juvenile date palm trees respectively when plantlets were transferred to plastic pots containing soil (peat moss, sand, and vermiculite at equal ratios). This method offers the opportunity for mass production of true-to-type plants from adult and juvenile date palm since the callus stage is avoided.

In Vitro Cultivation of date palm; 2- Factors affecting growth and development of in vitro date palm through embryogenisis

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Shoot tip explants of young date palm off shoots cv. Zaghloul were used to produce date palm plantlets via somatic embryogenisis. Plantlets were used to investigate factors affecting the rooting stage and subsequent survival in acclimatization stage as determined by salt strength and sucrose concentrations. The plantlets were utilized to study the influence of activated charcoal and light intensity and the root and shoot formation through rooting stage and their viability during acclimatization stage. Root and shoot formation was affected by MS salt strength, sucrose concentrations and light intensity. The best medium for rooting was ¾ MS salt strength supplemented with 50g/L sucrose, 0.1mg/L NAA, 2mg/L glutamine, and 6g/L Agar to enhance root-shoot formation. Individual young plants ere cultured on this medium for two passages and then transferred to root in medium with activated charcoal under 9000 lux light intensity for shoot growth enhancement. In this technique, we have addressed first three steps to produce a vigorous and healthy root-shoot system of date palm plantlets, where the fourth step to in vitro preacclimatization stage. In the fourth step, the process of preacclimatization began while plantlets were still in vitro, since date palm cultured on sugar-free medium in order to change from heterophic to autotrophic. Finally, the highest survival percentage of date palm plants after 3 months were 93 percent when we used plantlets that were 10-20 cm in length with a well developed adventitious roots system and 2-3 erect leaves, and we transplanted to special pots (8 by 15 cm) containing peat moss, vermiculite, and sand at equal volume.

In vitro Cultivation of date palm; 3- Date palm abnormalities during micro propagation via embryogenisis

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In cultures developed for mass propagation of date palm (*Phoenix dactylifera* L. cv. Zaghloul), through somatic embryogenisis are considered in the present day. Embryogenetic callus was obtained from shoot tips and leaf primordial cultured on a modified murashige and skoog (MS) medium containing 10.0mg/L NOA, 5.0mg/L 2-4-D and 1.0mg/L 2iP or 100.0mg/L 2-4-D, 3mg/L kinetic and 3mg/L 2iP. Somatic embryos developed leaves and roots when recultured on nutrient medium devoid of activated charcoal and containing 0.1mg/L NAN. Changes in plantlets in both developmental and morphological characters were obtained as malformed growth during in vitro stages (embryo, elongation, and rooting), while some malformed leaves were observed in greenhouse-grown plants (ex. Vitro stages). Date palm embryo abnormalities included swelling embryos, a thread growth embryo, vitrified embryos, abortion embryos, embryo inhibited cotyledonary sheath open, high curling embryo and very thin newly leaf emergence. During rooting stage seven types of abnormalities were recorded (shoot without primary root development, shoot with aerial root growth, shoot with green colored roots, an open shoot bare swelling roots with absent fiber root, shoot with inactive brown roots and flowering plantlets). Addition of auxins were found to be necessary for enhancing callus, embryogenetic callus formation and their subsequent development as well. Abnormalities were apparently affected and increased by high auxin concentration used in this study. Shoot tips which were cultured on medium devoid of height content of auxins, were found to have lower percentage of morphological changes either in vitro or ex vitro. These abnormalities were described in details. This study clearly suggests that the percentage of morphological changes is, to a large extent, media-dependent event. Molecular biology techniques can be used to determine the mechanisms that determine their development.

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