Regulation of Phytochemicals by Molecular Techniques

August 6-10, 2000

United States Department of Agriculture
Beltsville, Maryland

2000 Plant Molecular Biology Conference

Mid-Atlantic Plant
Molecular Biology Society

Phytochemical Society
of North America

MAPMBS 2000

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Beltsville Agricultural Research Center
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PHYTOCHEMICAL SOCIETY OF NORTH AMERICA

Acknowledgments

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The Phytochemical Society of North America
and
The Mid Atlantic Plant Molecular Biology Society

Welcomes you to the 35th annual PSNA conference

and

the 16th annual MAPMBS meeting

Symposium on

Regulation of Phytochemicals by Molecular Techniques

held at
The United States Department of Agriculture
Beltsville Agricultural Research Center
Beltsville, MD 20705
U.S.A.
August 6-10, 2000
Program Organizers

James A. Saunders
Benjamin F. Matthews

Organizing Committee
Jonathan Arias
Rose Hammond
John Hammond
Kim Lewers
JJ Lin
Asha Manohar
B. Sue Mischke
Susan Nasr
Monica Pedroni
Janet Slovin
Emily Steiner
Frank Turano

PROGRAM SUMMARY

Sunday, Aug. 6, 2000

1:00-4:00 PM	PSNA Executive Meeting, USDA, Bldg. 50, Conference Rm.
4:00-8:00 PM	Registration, Holiday Inn, 10000 Baltimore Ave
6:00-9:00 PM	Welcome Reception - Holiday Inn, 10000 Baltimore Ave

Monday, Aug. 7, 2000

USDA, Bldg. 003, Auditorium
Symposium Session I
Plant/Environmental Interactions

Session Moderator Dr. Susan McCormick

8:00-8:30 AM	Registration	
8:30-9:00 AM	Welcoming remarks on Agriculture using Biotechnology	***Phyllis Johnson*** ***Susan McCormick*** ***Benjamin Matthews***
9:00-9:40 AM	Crop allelopathy enhancement through biotechnology.	*Stephen Duke* ***Brian Scheffler***
9:40-10:20 AM	Using chimeric hypoviruses to fine-tune the interaction between a pathogenic fungus (Chestnut blight). and its plant host.	***Donald Nuss***
10:20-10:40 AM	Coffee Break	
10:40-11:20 AM	A functional genomics approach to the enzymes of plant xenobiotic metabolism	***Daniel O'Keefe*** *S. J. Keeler,* *S. C. Lau,* *R. Perkins,*

		B. McGonigle
11:20-11:40 PM	Biosynthetic pathway of insect juvenile hormone III in the sedge, Cyperus iria L.	***J.C. Bede*** *P Teal, W.G. Goodman, S.S. Tobe*
11:40-1:00 PM	Lunch	

Monday, Aug. 7, 2000
USDA, Bldg. 003, Auditorium

Symposium Session II
Energy, Nutrition and Cyanogenic glucosides

Session Moderator Dr. Douglas Luster

1:00-1:40 PM	Production of Microbial Cellulases in Plants for Biomass Conversion.	***Kathleen Danna***
1:40-2:40 PM	Poster Session Oral Introductions.	*All persons presenting posters can present a one minute introduction of their poster topic (one slide maximum).*
2:40-4:00 PM	Poster Session I and Break	*Rm. 20, Bldg. 003*
4:00-4:40 PM	Metabolic engineering of cyanogenic glucoside synthesis.	***Birger L. Moller***
4:40-5:10 PM	The Mechanism of Substrate Specificity in ß-Glucosidases	***Asim Esen,*** *Mirjam Czjzeka, Muzaffer Cicek, Véronique Zamboni, Wim P. Burmeister, David R. Bevand, Bernard Henrissata*

Tuesday, Aug. 8, 2000
USDA, Bldg. 003, Auditorium
Symposium Session III
Arthur Neish Young Investigators' Minisymposium:
Molecular Manipulation of Alkaloids, Flavonoids, and Cyclases
Session Moderator Dr. Peter Facchini

9:00-9:35 AM	Isolation of regulators of genes in terpenoid indole alkaloid metabolism in *Catharanthus roseus via a* T-DNA activation tagging approach.	***Frederique Hilliou*** *Leslie van der Fits, Jan W Kijne, Johan Memelink*
9:35-10:10 AM	Regulation of flavonoid metabolism by MYB genes.	***Edward Braun*** *Anusha P.Dias*
10:10-10:35 AM	Coffee Break	
10:35-11:10 AM	Models for anthocyanin sequestration in plants.	***Lukas Mueller***
11:10-11:45 AM	The sesquiterpene cyclase gene family of *Nicotiana tabacum*: Hybrid origins and physiological roles.	***Mark Schoenbeck*** *Joseph Chappell.*

Tuesday, August 8, 2000
USDA, Bldg. 003, Auditorium
Symposium Session IV
Pharmaceuticals and Health Benefits from Bioengineering
Session Moderator Dr. J. J. Lin

1:00-1:40 PM	Resveratrol glucoside engineering: Plant and human health benefits.	***Nancy L. Paiva***
1:40-2:20 PM	Designing Transgenic Plant Expression Systems for the Production of Recombinant Proteins.	***Joseph G. Boothe***
2:20-2:40 PM	*In situ* hybridization and immunolocalization of dirigent protein and lignan reductases in *Forsythia intermedia* and *Pinus taeda.*	***M. Kwon*** *V. Burlat L.B. Davin N.G. Lewis*

2:40-3:10 PM	Coffee Break	
3:10-3:50 PM	Pharmaceutical production in plants.	***Vidadi Yusibov,*** *Hilary Koprowski*
3:50-4:10 PM	Malate Synthase C-terminal Peptide Interaction with its Peroxisomal Targeting Receptor in Castor Bean.	***Masoumeh Assadi*** *Robert P. Donaldson*
4:10-4:30 PM	Enzymology and flux control of Vanillin biosynthetic pathway in *Vanilla planifolia* tissue culture.	***Daphna Havkin-Frenkel,*** *Henrik Pedersen*
4:30-4:50 PM	Japanese use of beni-tengu-dake (*Amanita muscaria*) and the efficacy of traditional detoxification methods	***Allan G. Phipps*** *Bradley C. Bennett Kelsey R. Downum*

Wednesday, August 9, 2000
USDA, Bldg. 003, Auditorium

Symposium Session V
Current Advances in Molecular Tools

Session Moderator Dr. Kim Lewers

8:40-9:20 AM	EST and Microarray Technology	***John Quackenbush*** Erik Snesrud, Baoping Zhao, Brian Haas, Christopher D. Town
9:20-10:00 AM	Glutamate and GABA like receptors in Arabidopsis	***Frank Turano,*** *Mark Allard, Geraldine Glover, Michael McMahon, Michael Muhitch, Ganesh Panta, Peter Van Berkum*

10:00-10:40 AM	BREAK	
10:40-11:00 PM	An LC/MS-based metabolic profiling of the flavonoid pathway intermediates in the tissues and root exudates of Arabidopsis mutants and transgenics	***Kothandaraman Narasimhan*** *Thomas Payne Allan Lloyd Sanjay Swarup*
11:00-11:20 PM	Expression of soybean genes involved in resistance to the soybean cyst nematode	***Benjamin Matthews*** *Hunter S. Beard Margaret MacDonald Rana Khan Michael Yang, Kristina L. Pilitt Nadim Alkharouf*
11:20-11:45 PM	DNA fingerprinting in *Theobroma cacao*, the chocolate tree	***James A. Saunders*** *Sue Mischke Alaa Hemeida Monica J. Pedroni*
11:45-1:15 PM	LUNCH	

Wednesday, August 9, 2000
USDA, Bldg. 003, Auditorium
Symposium VI
Molecular modifications of the Phenylpropanoid pathway

Session Moderator Dr. Nichole R. O'Neill

1:15-1:55 PM	Mechanisms and applications of transcriptional control of phenylpropanoid metabolism.	***Cathie Martin*** Hailing Jin Kathy Schwinn
1:55-2:35 PM	Metabolic engineering of phenylpropanoid biosynthesis.	***Richard Dixon*** *F. Chen, D. Guo, X.-Z. He, C.J. Liu, C.L. Steele*

2:35-3:00 PM	BREAK	
3:00-3:35 PM	Ecdysone receptor-based chemical-inducible gene regulation for plants.	***Malla Padidam***
3:35-3:55 PM	Antisense expression of the flavonoid 3'-hydroxylase gene in transgenic "Mitchell' Petunia.	***D. H. Lewis*** *J.M. Bradley* *S. J. Bloor* *E. Swinny* *C. Winefield* *K. Davies* *S.C. Deroles*
3:55-5:00 PM	PSNA Annual General Meeting	***Susan McCormick***

Wednesday, August 9, 2000
Holiday Inn, 10000 Baltimore Blvd., College Park, MD
Session Moderator Dr. Rose Hammond

6:30-10:00 PM	Banquet and Dinner Speaker, Maize Genomics: Using ESTs and Transposon Tags to Discover Genes.	***Virginia Walbot***

Thursday, August 10, 2000

USDA, Bldg. 003, Auditorium

Symposium VII
Expression, Defense and Transcriptional Factors
Session Moderator Dr. Rose Hammond

8:50-9:30 AM	Pollen-pistil interactions.	***Sheila McCormick,*** *Ines Ezcurra,* *Sheila Johnson, Robyn Cotter,* *Wei-hua Tangk*

9:30-10:10 AM	Kill or cure: The enigma of plant virus satellite RNAs	***Anne Simon***
10:10-10:25 AM	Coffee Break.	
10:25-10:45 AM	Comparison of resistant and susceptible soybean genomes near a nematode resistance gene.	***Kim S. Lewers*** *Sasanda Nilmalgoda* *Halina Knap* *Benjamin F. Matthews*
10:45-11:05 AM	Antifungal and Molluscidal Activity of *Erigeron speciosus.*	**Kumudini M. Meepagala** George Sturtz David J. Wise David E. Wedge
11:05-11:25 AM	Nucleic-Acid Diagnostic Tools For The New Millennium	***Kenneth M. Haymes*** *David L. Scott*
11:25-12:05 PM	Members of the basic/leucine-zipper (bZIP) family of transcription factors play key regulatory roles in animals, yeast and plants.	***Jonathan Arias***

Sunday, August 6

1:00-4:00 PM – PSNA Executive Meeting
USDA, Bldg. 50, Conference Rm.

4:00-8:00 PM - Registration
Holiday Inn, 10000 Baltimore Ave

6:00-9:00 PM - Welcome Reception
Holiday Inn, 10000 Baltimore Ave

Monday, August 7

8:00-8:30 AM - Registration
USDA Bldg. 003, Auditorium

8:30-9:00 AM - Welcoming Remarks on Agriculture using Biotechnology
Phyllis Johnson, Director of Beltsville Agricultural Research Center,
Susan McCormick, President of Phytochemical Society of North America
Benjamin Matthews, President of Mid-Atlantic Plant Molecular Biology Society

9:00-10:20 AM - Symposium Session I, Plant/Environmental Interactions

10:20-10:40 AM - Coffee Break

10:40-11:40 AM - Symposium Session I, Plant/Environmental Interactions

11:40-1:00 PM - Lunch

1:00-1:40 PM - Symposium Session II, Energy, Nutrition and Cyanogenic glucosides

1:40-4:00 PM - Poster Session and Break

4:00-5:10 PM - Symposium Session II, Energy, Nutrition and Cyanogenic glucosides

Monday, August 7, 2000
Symposium Session I : Plant/Environmental Interactions
Session Moderator: Dr. Susan McCormick

9:00-9:40 AM
Symposium Speaker

Brian Scheffler, Stephen Duke
USDA, ARS, NPURU
PO Box 8048
University, MS 38677, USA

CROP ALLELOPATHY ENHANCEMENT THROUGH BIOTECHNOLOGY

Crop allelopathy has seldom been strong enough to be utilized by farmers in an effective weed management system. Traditional breeding methods have not been very successful or have not focused on incorporating/enhancing allelopathy traits. Crops have been made resistant to insects, pathogens, and herbicides with transgenes, but biotechnology has not resulted in crops that control weeds with allelochemicals. The strategies for producing allelopathic crops by biotechnology are relatively complex, usually involving multiple genes. One can choose to enhance production of allelochemicals already present in a crop or to impart the production of new compounds. The first strategy involves identification of the allelochemical(s), determination of enzymes and genes encoding key steps of the biochemical pathway, and then genetic engineering is needed to enhance production of the compound(s). The latter strategy employs altering existing biochemical pathways by insertions of transgenes to produce new allelochemicals. With either strategy there are potential problems with tissue-specific promoters, autotoxicity, metabolic imbalances, and proper movement of the compound to the rhizosphere. Allelopathic rice and sorghum will be used as examples.

9:40-10:20 AM
Symposium Speaker

Donald Nuss
Center for Agriculture Biotechnology
University of MD Biotechnology Inst.,
Plant Sciences Bldg., Rm 5115C,
College Park, MD 20742

USING CHIMERIC HYPOVIRUSES TO FINE-TUNE THE INTERACTION BETWEEN A PATHOGENIC FUNGUS AND ITS PLANT HOST.
Cytoplasmically-transmissible RNA viruses of the genus *Hypovirus* cause reduced virulence (hypovirulence) in the chestnut blight fungus *Cryphonectria parasitica*. The development of a full-length infectious cDNA clone of the prototypic hypovirus CHV1-EP713 has made it

possible to engineer hypovirulent *C. parasitica* strains with specific phenotypic traits and with improved biological control potential. The potential for practical and fundamental applications of this group of viruses was further enhanced by the recent construction of an infectious cDNA clone of a second hypovirus, CHV1-Euro7. By analogy with plant viruses, CV1-EP713 and CHV1-Euro7 can be viewed as severe and mild strains, respectively. By swapping domains of the two viruses, it has been possible to generate chimeric hypovirus-infected fungal isolates that exhibit a spectrum of defined colony and canker morphologies. These results demonstrated the feasibility of engineering hypoviruses to fine-tune the interaction between a pathogenic fungus and its plant host.

10:40-11:20 AM
Symposium Speaker

Daniel O'Keefe, S.J Keeler, S.C. Lau, R. Perkins, B.McGonigle
Dupont Central Research, Experimental Station, Wilmington, DE, 19880

A FUNCTIONAL GENOMICS APPROACH TO THE ENZYMES OF PLANT XENOBIOTIC METABOLISM.

Most pesticides are initially metabolized in higher plants by members of 2 gene families, the cytochrome P450s and glutathione S-transferases (GSTs). Large scale plant DNA sequencing programs reveal multiple members of these gene families, but background information is insufficient to ascertain any specific role in xenobiotic metabolism based solely on the sequence of an individual P450 or GST. To analyze the role of individual gene products we have taken a variety of approaches that are tailored to the different characteristics of the 2 gene families. Bacterial expression of the majority of maize and soybean GSTs allows us to analyze and compare the activity against a variety of substrates. With P450 genes, the numbers are much larger, and so microarray expression analysis is instructive to narrow the focus to a limited number of genes.

11:20-11:40 AM
Oral Contribution

Bede, J.C.1, Teal, P.2, Goodman, W.G.3 and S.S. Tobe1.
1. Department of Zoology, University of Toronto, Ontario
2. CMAVE, United States Department of Agriculture, ARS, Gainesville, Florida
3. Department of Entomology, University of Wisconsin, Madison

BIOSYNTHETIC PATHWAY OF INSECT JUVENILE HORMONE III IN THE SEDGE, *CYPERUS IRIA* L.

Juvenile hormones regulate metamorphosis and reproduction in most insect species. In the insect, these sesquiterpenoids are synthesized by small retrocerebral endocrine organs, the

corpora allata, via the classical MVA acid pathway. One of these compounds, juvenile hormone III (JH III), has also been identified in the sedge, Cyperus iria L (1). In higher plants, biosynthesis of the sesquiterpenoid backbone may proceed through two distinct pathways: the classical mevalonate (MVA) pathway or the 2-C-methyl erythritol 4-phosphate (MEP) pathway or through a combination of both (2). Cyperus iria cell suspension cultures were used as a model to elucidate the biosynthetic pathway of JH III in the plant. Labelling and enzyme inhibition studies demonstrate that the sesquiterpenoid backbone of JH III is, at least partially, synthesized via the MVA pathway. Precursor feeding studies also suggest that the later steps of the biosynthetic pathway are similar to the insect pathway.

1. Toong et al. (1988) Nature 333: 170-171.
2. Lichtenthaler (1999) Annu. Rev. Plant Physiology. Plant Mol. Biol. 50: 47-65.

Monday, August 7, 2000
Symposium Session II : Energy, Nutrition and Cyanogenic glucosides
Session Moderator: Dr. Douglas Luster

1:00 – 1:40 PM
Symposium Speaker

Kathleen J. Danna
Department of Molecular, Cellular, and Developmental Biology
University of Colorado, Boulder, CO 80309

PRODUCTION OF MICROBIAL CELLULASES IN PLANTS FOR BIOMASS CONVERSION

Plant biomass, the most abundant renewable resource on earth, is a potential source of fermentable sugars for production of alternative transportation fuels and other chemicals. Bioconversion of plant biomass to fermentable glucose involves enzymatic hydrolysis of cellulose, a major polysaccharide constituent of the plant cell wall. Commercially available microbial cellulases are prohibitively expensive for bioethanol processes. Manufacturing heterologous cellulases in crop plant bioreactors could significantly reduce costs associated with enzyme production and could offer a potentially high-volume alternative to traditional methods. Hydrolysis of cellulose by bacteria and fungi typically occurs through the synergistic action of three secreted enzyme classes. An endo-1,4-(-d-glucanase cleaves internal linkages in crystalline cellulose to create free ends. An exoglucanase releases oligosaccharides (e.g., cellobiose) from these ends. Finally, a (-D-glucosidase converts oligosaccharides to d-glucose. Enzymes from thermophilic organisms are particularly suited for industrial applications because they are typically thermostable, resistant to protease, and tolerant toward other stresses such as pH extremes. Moreover, enzyme specific activity typically increases as the temperature optimum increases. Genes for a variety of thermostable cellulase enzymes from fungi and bacteria have been cloned and sequenced. Much effort has been devoted to developing transgenic plants as bioreactors to produce heterologous proteins, including industrial enzymes such as cellulase. Maximum expression

levels of microbial cellulases have typically been well below 1% of total soluble protein (TSP) in plant extracts. Using an apoplast-targeting strategy, we have recently produced a thermostable bacterial endoglucanase -- the catalytic domain of E1 (E1-cat) from *Acidothermus cellulolyticus* -- in a model plant system. In leaves of transgenic *Arabidopsis thaliana*, E1-cat accumulates in the apoplast in amounts up to 25% of total soluble protein. The enzyme recovered from plants is active at the optimal temperature of 83°C, and plant extracts can be readily enriched for activity by heat treatment and centrifugation. Moreover, the transgenic plants are fertile and exhibit normal growth. This work provides proof of principle that industrially useful amounts of at least one cellulase component can be produced in transgenic plants. Further work is planned to determine if similar levels of expression can be obtained in a commercial crop and whether multiple cellulase components can be produced in a single plant using a variety of protein targeting strategies.

1:40-2:40 PM

Poster Session Oral Introductions

Persons presenting posters will present a one minute introduction of their poster topic.

2:40-4:00 PM

Poster Session and Break
Room 20, Bldg. 003

Poster Abstract 1

Filippo Imperato,
Dipartimento di Chimica
Università della Basilicata, 85100 Potenza, Italy

TWO NEW ACYLATED FLAVONOL GLYCOSIDES FROM THE FERN PTERIS VITTATA.

Kaempferol 3-O-(X", X"-di-protocatechuoylglucuronide) (1), quercetin 3-O-(X", X"-di-protocatechuoylglucuronide) (2), kaempferol 3-O- glucuronide (3) and astragalin (4) have been isolated from Pteris vittata L. Flavonoids (1) and (2) (new natural products) have been identified by UV-spectral analysis with shift reagents, electrospray mass spectra, acid hydrolysis and alkaline hydrolysis. Protocatechuic acid (3,4- dihydroxybenzoic acid) is a new acyl substituent in flavonoid glycosides. Electrospray mass spectrum of (3) showed the presence of a dimer, a trimer and a tetramer. Mass spectral data were provided by SESMA (CNR, Naples).

Poster Abstract 2

Betty C.R. Zhu[1], Feng Chen[2], Roger A. Laine[1] and Gregg Henderson[2]
Louisiana State University Agricultural Center, Louisiana State Experimental Station
Louisiana State University, Department of Biological Science[1]
Department of Entomology[2], Baton Rouge, LA 70803

COMPARISON OF TOXICITY AND REPELLENCY OF VETIVER OIL WITH SEVEN REPRESENTATIVE ESSENTIAL OILS AGAINST FORMOSAN SUBTERRANEAN TERMITES (*COPTOTERMES FORMOSANUS* SHIRAKI)

The repellency and toxicity of 8 essential oils, which were extracted from Vetiver, Cassia leaf, Clove bud, Cedarwood, *Eucalyptus globules, Eucalyptus citrodora*, Lemongrass and Geranium, were evaluated against Formosan subterranean termites, Coptotermes formosanus Shiraki. All 8 essential oils exhibited repellency at the lowest tested concentration of 10mg/cm2. However, vetiver oil was found to be the most effective repellent due to its bioactive compounds with long-lasting activity, while clove bud oil was the most toxic causing over 50% mortality of Formosan subterranean termites at a concentration of 50mg/cm2. The presence of vetiver oil substantially decreased the termite tunneling activity at the concentration as low as 5mg/g sand, and no tunneling was visible in the middle chamber at the concentration of vetiver oil higher than 25mg/g sand. The major component corresponding to each essential oil was identified by GC-MS.

Poster Abstract 3

* M. Farzad, ^R. Griesbach and * M. Weiss,
*Department of Biology,Georgetown University
^USDA, Floral and Nursery Plant Research

FLORAL COLOR CHANGE IN VIOLA CORNUTA: A MODEL SYSTEM TO STUDY REGULATION OF ANTHOCYANIN PRODUCTION.

Flowers of *V. cornuta* cv. Yesterday, Today and Tomorrow (YTT) change from white to lavender over 4 - 7 days. HPLC analysis was used to identify malvidin and myricetin as the major anthocyanidin and copigment in YTT. Possible ontogenetic changes in a) anthocyanin content,b) copigment content, or c) pH were investigated. The identities of the major pigment and copigment do not change over time. The amount of malvidin increases as color appears, while the myricetin concentration remains constant. Petal pH values do not vary over floral ontogeny. Comparison of emasculated and intact flowers shows that the presence of self-pollen on the stigma is necessary to produce the color change. YTT flowers exposed to light changed color while those maintained in darkness did not. Applying an ethylene inhibitor, silver thiosulphate, did not prevent color change. The increase in malvidin over time suggests that anthocyanin synthesis is upregulated by proximate triggers such as

pollination and light. YTT presents an excellent model system for the study of anthocyanin regulation because it is a floriferous annual, the appearance of color is easily triggered, and only one anthocyanin is involved.

Poster Abstract 4

Padolina, Isagani D. and Tom J. Mabry.
Molecular Cell and Developmental Biology Lab, University of Texas, Austin, TX 78712

DEOXYFLAVONOIDS IN ELICITED LIQUID CELL CULTURES OF *CEPHALOCEREUS SENILIS* (OLD MAN CACTUS): METABOLIC PROFILES AND MOLECULAR CELLULAR MECHANISMS

The existence and mode of operation of certain enzyme aggregates in the phenylpropanoid pathway is being established from the radio-concentration ratios of specific end products through the introduction of radiolabeled intermediates. Metabolic profiles of chitin-elicited cell cultures of *Cephalocereus senilis* were determined using radiolabeled phenylalanine and coumaric acid, and their incorporation into the phenypropanoid pathway was elucidated using HPLC with a radiodetector. The resulting deoxyflavonoids suggest a form of metabolite chanelling in which the usual hydroxylation step by C4H in unelicited cell cultures is bypassed. Immunoflourescence labeling is being developed to address the possible presence of enzyme complexes in this system.

Poster Abstract 5

Donald Keller, Craig Dubois, Mauricio Bustos
UMBC, Dept. of Biological Sciences
1000 Hilltop Circle
Baltimore, MD 21250

THE MOLECULAR CLONING AND CHARACTERIZATION OF ARABIDOPSIS ABI3 INTERACTING PROTEIN

Arabidopsis seed development includes desiccation of the seed and production of storage molecules to be used as fuel for the germinating plant. The transcription factor ABI3 activates genes in response to the plant hormone abscisic acid. Using a two-hybrid approach, we cloned the Arabidopsis ABI3 Interacting Protein 6 (AIP6) that interacts with ABI3 in yeast. A partial-length cDNA clone of AIP6 was isolated from an Arabidopsis library; the N-terminus of this clone contains a unique RING-H2 motif. Screening of an Arabidopsis BAC library revealed that the gene was located on chromosome III, near the embryo defective mutant emb51. Sequence data from this region shows that AIP6 contains 6 exons

and 5 introns, and is approximately 6 kb in length. Ongoing experiments in our laboratory suggest that a line carrying a T-DNA insertion in the AIP6 locus displays sucrose dependent seedling and root growth. Next, we plan to transform emb51 and AIP6[T-DNA] mutants with a copy of the full length gene. A comparison of phenotype and gene expression patterns between wild type and transformed plants versus the two mutants will help us to determine the nature of AIP6's function

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Poster Abstract 6

Monira Ahsan, Alexander I. Gray, SK. N. Islam and W.H. Stimson.
Dept of Pharm. Sci, Univ.of Strathclyde,
SIBS Bldg., 27 Taylor street, Glasgow G4 0NR, UK.

NOVEL DITERPENES FROM *SCOPARIA DULCIS* AND THEIR CYTOTOXIC ACTIVITIES ON HUMAN CANCER CELL LINES.

Scoparia dulcis (Fam.Scrophulariaceae), a perennial herb is reputed for its medicinal properties. Phytochemical investigation on this plant resulted in the isolation of three novel labdane-derived diterpenes. All these diterpenes contain a benzoyl group in position 6 and their structures were determined by spectroscopic methods, especially 2 D NMR techniques.The crude extracts and the pure compounds were tested for their cytotoxic activities on human cancer cell lines. Some of the products showed strong cytotoxicity (70-92% cell mortality), some moderate (30-70% cell mortality) and others indicated poor activity (10-30% cell mortality). The activities of the crude extracts as well as the pure compounds were found to be cell specific, i.e. the range of cytotoxities differed from one cell line to the other. The isolation, structure elucidation and cytotoxic properties of the compounds will be presented in the poster.

Poster Abstract 7

Md. Mukhlesur Rahman, Alexander I. Gray*, Allan J. Drummond and Proma Khondkar
Department of Pharmaceutical Sciences, University of Strathclyde,
SIBS Building, 27 Taylor Street, Glasgow G4 ONR, UK

ANTIMICROBIAL CONSTITUENTS FROM FERONIA LIMONIA

Feronia limonia Swingle (Fam. Rutaceae) is a common Bangladeshi tree which is reputed to have therapeutic properties. Phytochemical studies on the stem bark of the plant resulted in the isolation of a wide range of compounds- alkaloid, coumarin, lignan, flavanone, terpene and steroid types. Petroleum ether extraction afforded a novel pyranoflavanone along with bergapten and psoralen. The chloroform extract yielded a novel steroid, an acridone

alkaloid, a diprenylated flavanone, a lignan, demethylsuberosin, xanthotoxin and isopimpinellin. The structures of the compounds were established by spectroscopic methods. Most of the compounds were subjected to antimicrobial screening for MIC determination by a serial dilution technique. The MICs of compounds against the test organisms were found in the range of 25-100mg/ml.
*Author for correspondence

Poster Abstract 8

Jennifer L. Pelt, Cecilia A. McIntosh
Dept. of Biological Sciences, East Tennessee State University,
Johnson City, TN, 37614-0703.

A COMPARISON OF FLAVANONE-3-O-HYDROXYLASE (F3H) mRNA LEVELS IN PETUNIA HYBRIDA AND *CITRUS PARADISI*.

The goal of this research is to elucidate regulatory mechanisms controlling conversion of flavanones to dihydroflavonols by flavanone-3-0-hydroxylase (F3H). Southern and Northern blot analysis of *Citrus paradisi* indicates the F3H gene exists as a single copy with an approximate transcript size of 1.60 kb. Using semi-quantitative RT-PCR, potential points of regulation are studied by comparing F3H mRNA levels between the model plants petunia and grapefruit, which have heterogeneous flavonoid compositions. On the basis of conserved regions of F3H between several plant species, primers were designed and used to amplify an approximately 350bp region of citrus and petunia F3H. The linear range of PCR amplification of petunia and citrus F3H cDNA with their respective primers was established. Roots, leaves, and flowers at several developmental stages were ground to a fine powder (liq. N2) and samples removed from each separate pool for determination of F3H mRNA and enzyme activity levels to illuminate control mechanism(s) taking place for differential expression.

Poster Abstract 9

Sang-Un Park, Min Yu, Yinping Zhang, Peter J. Facchini
Department of Biological Sciences, University of Calgary,
Calgary, Alberta, T2N 1N4 Canada

METABOLIC ENGINEERING OF BENZYLISOQUINOLINE ALKALOID BIOSYNTHESIS IN OPIUM POPPY AND RELATED SPECIES

Plants remain the exclusive source for several benzylisoquinoline alkaloids of pharmaceutical importance including morphine, codeine, papáverine, berberine, and sanguinarine. The continued supply of these drugs, and the establishment of new plant production systems for novel pharmaceutical alkaloids, will benefit from the development of metabolic engineering strategies in transgenic medicinal plants. We have developed efficient

genetic transformation protocols for opium poppy and a variety of other benzylisoquinoline alkaloid-producing plants of medicinal value. Plants have been transformed with chimeric constructs composed of the constitutive cauliflower mosaic virus 35S promoter and the coding region from a variety of alkaloid biosynthetic genes [e.g., tyrosine/dopa decarboxylase, N-methylcoclaurine-3'-hydroxylase, and the berberine bridge enzyme (BBE)] in the sense or antisense orientation. For example, Eschscholzia californica (California poppy) expressing the sense BBE transgene produced higher alkaloid levels, including sanguinarine, whereas plants expressing the antisense BBE transgene produced virtually no alkaloids. Our results reveal new insights into the regulation of benzylisoquinoline alkaloid pathways, and demonstrate the feasibility of genetically manipulating product levels in transgenic medicinal plants.

Poster Abstract 10

Leon Adler, Srividya Ramesh, Mauricio Bustos
UMBC, Dept. of Biological Sciences, 1000 Hilltop Circle
Baltimore, MD 21250

AN ARABIDOPSIS MUTANT WITH SUCROSE DEPENDENT MERISTEM GROWTH

ABI3 interactive protein (AIP4) was isolated using a yeast 2-hybrid system. The protein is 708 aa long and contains a putative N-terminal kelch domain. AIP4 mutants have normal germination frequency and sensitivity to abscisic acid, but have retarded root and shoot meristem growth on minimal media. However, normal growth is observed on plates containing 25 mM sucrose, and AIP4 mutants are resistant to the effects of high concentrations of sucrose (6%), compared to WT seedlings. Preliminary results with 5-bromo deoxyuridine labeling of nucleic acids appear to indicate that the block to growth is at cell division, but have been difficult to quantify. Therefore, propidium iodine staining of whole seedlings to detect chromosomes at the metaphase plate is underway. Abnormalities in morphology and anatomy are detectable by light microscopy. Current efforts are to complement the AIP4 mutants with a genomic clone via Agrobacterium mediated transformation. AIP4-GFP fusions are being used to detect cellular localizations of the protein.

Poster Abstract 11

Walz, Alexander (A) (B) Park, Seijin (C), Ludwig-Mueller, Jutta (B) Slovin, Janet P. (A) Cohen, Jerry D. (C)
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(B): Institut fuer Botanik, TU Dresden, Zellescher Weg 22, 01062 Dresden, Germany
(C): University of Minnesota, Horticultural Science, 252 Alderman Hall,
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MOLECULAR CLONING AND CHARACTERIZATION OF THE IAA-PROTEIN GENE FROM *PHASEOLUS VULGARIS*.

IAA has been found in plants in several conjugated forms (e.g. IAA-sugars or IAA-amino acids). A class of IAA that is modified by an amide linkage to a protein was investigated. Antibodies raised against a bean 3.6 kDa IAA peptide detected several other polypeptides in bean seeds of varying molecular masses from 17-60 kDa. Using GC-MS analysis and immunoblotting, a major protein of 42 kDa with IAA covalently attached was found in bean seeds. Purified fractions of the 42 kDa protein analyzed by 2-D PAGE exhibited two polypeptides of different pIs upon silver staining. Amino acid compositions of the two proteins were essentially identical. The 2 spots were cut from several Ponceau S stained blots and subjected to microsequencing. Based on these results specific probes were made to screen a cDNA library made from 3-week old bean seeds. A partial cDNA clone was isolated and used to screen a genomic DNA library in order to obtain the 5' end of the gene and its promoter sequence. A full length 1143 bp sequence was obtained with an open reading frame of 380 amino acids. Southern hybridization indicated that this IAA modified protein is encoded by a single copy gene. Northern blot analysis indicated that the gene gets expressed only during the late stages of seed development. No RNA transcripts were detected in other plant tissues besides seeds. Supported by National Science Foundation grant IBN97-23999.

Poster Abstract 12

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INSECT GROWTH REGULATOR ACTIVITY OF EXTRACTS AND SOME METABOLITES OF MELIACEOUS PLANTS FROM MEXICO

The Mexican flora is very special and one of the highest in biodiversity in the world. The Meliaceae family is abundant in Mexico, we have studied some specimens of *Cedrela* and *Melia*. Extracts and some compounds as limonoids from Meliaceous plants have shown insecticidal activities under a artificial bioassay(1). The partitioned dichloromethane extract from aerial parts of *Cedrela dugessi*, bark of *Cedrela odorata* and seeds of *Melia azedarach*,

afforded some bioactive fractions and some limonoids with insect growth regulatory and insecticidal activities. In this opportunity the insect growth regulator activities of CH2Cl2 extract, some fractions and limonoids on the fall armyworm *Spodoptera frugiperda*, one of the principal insect pest on corn crops in Mexico, will be presented.
Céspedes, C. L., Calderón J, S., Lina L., and Aranda E. J.
Agric. Food Chem. 48, 1903-1908, (2000).
Acknowledgements: Funding CONACyT-Mexico Grant # 27975-N

Poster Abstract 13

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IDENTIFICATION OF *PSEUDOMONAS SYRINGAE* PV. TOMATO DC3000 HOST RANGE DETERMINANTS.

The *hrp* pathogenicity island (PAI) of *Pseudomonas syringae* pv. tomato DC3000 (Pto) encodes a type III protein secretion system that translocates effector proteins into plant cells. While some of the genes encoding effector proteins are part of the *hrp* PAI, others are dispersed throughout the Pto. genome but are coordinately regulated by *Hrp*L. In this study, a HrpL-linked promoter-trap assay was used to identify possible effector proteins. A genomic library of Pto fused to a lacZYA cassette was screened for promoter activity in the presence of an inducible hrpL construct. Of the approximately 8000 colonies screened, five colonies exhibited a HrpL-dependent phenotype. AvrPphE, previously not known to be present in Pto, and HrpW, previously identified in Pto, were identified by sequence analysis. One clone, identified thrice, exhibited HrpL-dependent promoter activity, carried a potential HrpL promoter and was a strong candidate to be a translocated effector. This 19.6 kD 181 amino acid protein had no homologs in the database, but was associated with peptide toxin biosynthesis genes, This gene may indicate the presence of pathogenicity satellites in the Pto genome.

Poster Abstract 14

Jonathan E. Poulton, Brianne K. Shepherd, Jiming Zhou

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Iowa City, IA 52242, USA

CYANOGENESIS IN ROSACEOUS STONE FRUITS: CLONING AND EXPRESSION OF *PRUNUS SEROTINA* AMYGDALIN AND PRUNASIN HYDROLASES.

Amygdalin hydrolase (AH) and prunasin hydrolase (PH), which play key roles in cyanogenesis in black cherry (*Prunus serotina* Ehrh.), occur as multiple forms in tissue homogenates. To understand better this multiplicity, the genes encoding these enzymes were cloned. The following cDNAs were isolated from seeds: AH1 (encoding isoform AH I), PH-S1 (encoding isoform PH I), and PH-S2. Four cDNAs, PH-L1 through PH-L4 (the latter three encoding isoforms PH A to C, respectively), were isolated from shoots. Genomic sequences corresponding to AH1, PH-S1, and PH-L1 through PH-L4 were obtained by PCR amplification of genomic DNA, allowing comparison of their intron-exon organizations. After expression of AH1, PH-L1, and PH-L3 in the yeast *Pichia pastoris*, the recombinant hydrolases, like native AH and PH, displayed strict aglycone specificities, being active only toward their respective substrates. However, the AH1 double mutant Y195I-G369D obtained by site-directed mutagenesis showed comparable AH and PH activities.

Poster Abstract 15

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ISOLATION, CLONING, AND REGULATION OF ß-GLUCOSIDASES INVOLVED IN COUMARIN BIOSYNTHESIS IN WHITE SWEETCLOVER (*MELILOTUS ALBA*).

Upon disruption of white sweetclover shoots, cis-o-hydroxycinnamic acid glucoside is rapidly hydrolyzed by a stereospecific b-glucosidase to coumarinic acid, which spontaneously lactonizes to coumarin. In this species, the levels of this hydrolase are controlled by the B gene, but it remains unclear whether this gene is structural or regulatory. To investigate this question, we have purified and cloned the sweetclover ß-glucosidase. In homogenates of both M. alba variety Spanish and mutant line N747, this enzyme occurs as multiple forms that are encoded by two different genes (GLU1 and GLU2). The corresponding cDNAs, which share ca. 90% nucleotide identity, predict the NEP and ITENG motifs characteristic of members of glycosyl hydrolase family I, a signal sequence, and several potential N-glycosylation sites. Western blotting showed that both genes are highly expressed in the homozygous dominant line N747 (BB), whereas their expression is severely reduced in the recessive line N745 (bb).

Poster Abstract 16

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PEACH ETR1 TRANSCRIPTS SHOW SOME TISSUE SPECIFICITY DURING WOUNDING

We are interested in the ethylene signal transduction pathway genes in peach (*Prunus persica*) due to the role of ethylene in biotic and abiotic stress responses. A PCR product from the peach genome encoding a portion of an ETR1 homologue (PpETR1) was used to screen a cDNA library from late ripening peach fruit and resulted in the identification of a cDNA clone representing PpETR1. Comparison with known cDNA and genomic sequences suggested that the peach cDNA clone was the result of incomplete splicing at the 3' terminus. A 3' RACE clone was obtained representing the full-length 3' end. Other RACE clones had polyA+ tails added at several sites within the sequence of a putative intron located just inside of the region of ETR1 encoding the response regulator domain. Translation of such a truncated transcript would lead to a product missing a large portion of the response regulator, similar to the ERS ethylene receptor. RT-PCR data indicate that both types of transcripts are present throughout fruit development and that PpETR1 expression during fruit development and maturation appears to be relatively constitutive across several peach cultivars and hybrids. This expression pattern also extends to wounded fruit; however, wounded leaves displayed a decline in expression 24 h after wounding.

Poster Abstract 17

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Delaware State University, Dept. of Agriculture and Natural Resources
Dover, DE, 19901-22779

VARIATION IN MOLECULAR CONSTITUENTS OF HYPERICUM PERFORATUM L. TISSUES DURING GERMINATION AND SEEDLING DEVELOPMENT IN RESPONSE TO SOURCES OF DIFFERENT LIGHT QUALITY.

Hypericum perforatum L. (St. John's wort) is widely marketed for use as nutritional supplements and medicines. Hypericin is one of the active ingredients in Hypericum, and it has been widely recognized that the bioactivity of hypericin is affected by light. The objectives of the study were to find out how light quality affects germination and to determine the variation in biomolecules among the treatments during their development. There were six (6) light treatments were provided to Hypericum seeds in Petri dishes. There were approximately 100 seeds per Petri and four (4) replications per treatment. Experiments were repeated several times, with several variations to the treatments and experimental set-up. Percent germination was significantly different among treatments. The best germination responses were to Sylvania white soft light, and sources with red and yellow light. The worst response was to black, blue, and Sylvania Grow Lux light. Analysis of carbohydrates and

proteins in the tissues showed interesting variation among the treatments. There is clear evidence of metabolic differences caused by light quality.

Poster Abstract 18

Alister D. Muir[1], Ron Wilen[2], and Neil D. Westcott[1]
[1]Crop Utilization, Saskatoon Research Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, Canada, S7N 0X2
[2]Dept of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8

ACCUMULATION OF THE LIGNAN IN DEVELOPING FLAX SEED.

Accumulation of the lignan secoisolariciresinol diglucoside (SDG) was studied in developing flax seed in greenhouse and field grown material. Samples were collected at intervals beginning 5 days post anthesis and continuing until seed maturity. Accumulation of SDG which began about 9 days post anthesis was preceded by rapid accumulation of coniferin and coniferyl alcohol. Coniferin and coniferyl alcohol levels declined to undetectable levels as SDG accumulation occurred. Similar patterns of accumulation were observed in both greenhouse and field grown material.

Poster Abstract 19

Daniel K. Owens[1], Lori, J. Wilson[2], Cecilia A. McIntosh[1].
East Tenn. St. Univ., Dept. Biol. Sci. [1] & Chem. [2],
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DEVELOPMENT OF A SENSITIVE QUANTITATIVE ASSAY FOR FLAVANONE-3-HYDROXYLASE USING CAPILLARY ELECTROPHORESIS.

One goal of our lab is to elucidate control of an early branch point in flavonoid biosynthesis. At this branch point, flavanones such as naringenin (NAE) can either undergo functional ring group substitution (e.g. glycosylation), or serve as a substrate for further core derivation. In the latter case isoflavones, flavanones, or dihydroflavanols can be produced. Flavanone-3-Hydroxylase (F3H) catalyzes production of the dihydroflavanol dihydrokaempherol (DHK) from NAE. Detailed information about F3H activity in plant tissues is somewhat lacking due to problems with current assays. These problems include expense (custom synthesis of radiolabeled substrates) or relatively low resolution coupled with semi-quantitative analysis (TLC). A quantitative assay employing capillary electrophoresis (CE) to detect DHK production has been developed. The relationship between peak area and DHK concentration is linear from 0-1 nmol/ml with a detection limit of 0.005 nmol/ml. Assay precision is high with 0.07% CV. This assay is being used to

quantify F3H levels in petunia and grapefruit leaf tissue at different developmental stages to help elucidate potential mechanisms regulating the metabolic fate of NAE.

Poster Abstract 20

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AURONE BIOSYNTHESIS IN *ANTIRRHINUM*.

We are using the model plant antirrhinum (*Antirrhinum majus*) as the basis of a genetical and molecular approach to identify the biosynthetic gene(s) involved in the production of aurones, which are yellow flavonoid pigments. Through EMS mutagenesis of a sulfurea line (which produces aurones throughout the petals), a new mutant was identified that is specifically inhibited in aurone production. Additionally, differential and subtractive cDNA approaches have been used to identify cDNA clones with enhanced expression in regions of the flower synthesizing aurones. One of the clones, pPAM1, has a deduced amino acid sequence similar to polyphenol oxidase (PPO). Isolation of the pPAM1 allele from the aurone-lacking mutant line is in progress to determine whether pPAM1 corresponds to an aurone biosynthetic gene.

Poster Abstract 21

Teresita Flores, Nancy L. Paiva
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PHYTOCHEMICAL ANALYSIS OF TOBACCO PLANTS EXPRESSING RESVERATROL SYNTHASE.

Previous studies involving expression of resveratrol synthase (RS) in tobacco reported accumulation of resveratrol (Hain et al., 1993 Nature 361: 153-156; Fischer et al. 1997 The Plant J. 11: 489-498). Quantitation of the product primarily relied upon ELISA assays with an antibody raised against resveratrol conjugated to a protein. Recent studies using a similar construct in alfalfa revealed the accumulation of trans-resveratrol-3-O-beta-D-glucopyranoside, and no resveratrol aglycone (Hipskind and Paiva, 2000 MPMI 13, 551-562). The alfalfa studies utilized HPLC and other chemical analyses. To determine the source of the discrepancies in the reported product profiles, the construct from the alfalfa studies was introduced into tobacco. Preliminary HPLC analysis indicates that while resveratrol aglycone was present in acetone extracts of fresh tobacco leaves, variable levels

of a resveratrol glucoside was also detected. Factors affecting the ratio of resveratrol aglycone to glucoside are being investigated, since glycosylation may affect the phytotoxicity, bioavailabilty, and stability of resveratrol.

Poster Abstract 22

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STIMULATION OF SPORE GERMINATION IN A PLANT-PATHOGENIC FUNGUS IN RESPONSE TO HOST FLAVONOIDS.

Legumes produce a variety of flavonoids to signal and influence microbes in the rhizosphere, however pathogens may use these compounds as host-specific cues to recognize their hosts. Studies of the ascomycete *Nectria haematococca* MPVI (*Fusarium solani*) indicate that specific flavonoids produced by its host garden pea (*Pisum sativum*) can trigger spore germination. Stimulatory flavonoids are pisatin, the pterocarpan isoflavonoid phytoalexin of pea, and rhizobium nod-inducing flavones and flavanones. This study is directed at identifying these flavonoids in pea root exudates and assessing their relative roles in stimulating the germination. Evidence for flavonoid stimulation of germination suggests a possible mechanism by which fungi achieve host-specific recognition, responding to a characteristic host flavonoid fingerprint. We have established this fingerprint for pea root exudate through analytical HPLC, quantified the germination-stimulating activity of crude exudate, and, subsequently, tracked this activity in HPLC fractions of the exudate. Results show that pisatin plays a major role as the active stimulatory component of root exudates.

Poster Abstract 23

Jennifer S. Smith, Lee M. Pike
East Tenn. St. Univ., Johnson City, TN 37614.

BRYOPHYTE PHYLOGENY: EVIDENCE BASED ON THE CHLOROPLAST psbA GENE SEQUENCE.

Bryophytes are a primitive group of small, nonvascular land plants. They are the most likely transitional group between green algae and tracheophytes. Their relationships to each other and to other land plants remain a subject of interest. We used the highly conserved chloroplast psbA gene sequence to collect data pertaining to bryophyte phylogeny. Through PCR amplification and cloning, the sequence of 971 nucleotides of the 1,062 bp psbA gene was obtained for nine bryophytes. Maximum parsimony analysis of 19 taxa (10 sequences were obtained through GenBank) suggests that bryophytes are paraphyletic, and that hornworts form a clade sister to the vascular plants. Mosses form a clade sister to the hornwort + tracheophyte lineage; liverworts are monophyletic and form the basal lineage.

Bootstrap analysis shows very strong support for the basal placement of liverworts, but weak support for the placement of hornworts and mosses. Distance measurement analyses also result in the basal placement of liverworts. We believe studies of relationships among early land plants may benefit from the inclusion of the psbA gene sequence in combined data sets (molecular + morphological information).

Poster Abstract 24

Daniel C. Tanner, Cecilia A. McIntosh
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STRUCTURAL ELUCIDATION OF FLAVANONE-7-O-GLUCOSYLTRANSFERASE FROM *CITRUS PARADISI*.

The goal of this study is to elucidate factors influencing the transformation of one flavonoid to another by structural analysis of a grapefruit enzyme that "captures" flavanones before they can be used to make other flavonoids. This enzyme, flavanone 7-O-glucosyltransferase (7GT), catalyzes glucosylation of naringenin to form prunin which is subsequently converted to the bitter flavanone diglycoside, naringin. This enzyme has been characterized and purified to near homogeneity (McIntosh et al., 1990), however yields were too low for amino acid sequencing. The challenge was to adapt the purification protocol so that 7GT was separated from other contaminating flavonoid GTs in levels that were amenable for amino acid sequencing. The adapted procedure involves salt fractionation, gel filtration chromatography, and an extended Mono Q anion exchange protocol and resulted in 600-fold purification of 7GT. Samples were run on 10% SDS-PAGE gels and either internal fragments sequenced, or gels blotted onto PVDF for N-terminal sequencing (U. Va.). Amino acid sequences will be used to design degenerate oligonucleotide primers for RT-PCR and products used to screen a grapefruit leaf cDNA library (G. Moore) to obtain a full-length clone.

Poster Abstract 25

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** Department of Chemistry, Regional Engineering College,
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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF A FLAVONOID GLYCOSIDE FROM *SPILANTHES CALVA*

Spilanthes calva belonging to Compositae is an herb occurring throughout the greater parts of India, with the stems erect or decumbent at base and appears more or less hairy. All parts of the plant are acrid but the flower heads are by far the most pungent. They are chewed to relieve toothache, affections of throat and gums, and paralysis of the tongue. The herb may be used against scurvy. Ethanolic extract of the herb was found to affect the blood pressure in dogs and cats. Spilanthol has been reported earlier from ether extract. There is no report on the chemical investigation of flavonoids. Hence, in our present study we have isolated a flavonoid glycoside, which was characterized as tetrahydroxdihydrochalcone 3'-0-glucoside by color reactions, chromatography and hydrolysis. UV, PMR and C13 spectral studies supported the identification. The compound possessed hypoglycemic activity in mice where diabetes was induced by streptozotocin.

Poster Abstract 26

Harhash M. M.[1]; Khafid, A. M.[1]., Hemeida, A. Alaa.[2]
[1] Faculty of Agriculture Saba Bacha, Alexandria University, Egypt.
[2] Genetic Engineering and Biotechnology Research Institute. (GEBRI)
Sadat City, Minufiya University, Egypt.

GENETICAL AND ENVIRONMENTAL EFFECTS ON DATE PALM PEROXIDASE ISOZYME PATTERNS.

Peroxidase isozyme patterns were detected on seven date palm varieties grown in three different localities, i.e., El-Meadia (M), Edko (E) and Rashid (R). These three localities are located in northwest delta of Egypt. Five bands of isoperoxidases were found for all varieties (genotypes) in all localities. Two bands migrated towards the anode (Px-A1 and Px-A2) while the other three bands migrated towards the cathode (Px-C1, Px-C2 and Px-C3). All five bands seemed to be controlled by inducible loci expressing themselves in response to environmental stresses. The activities of Px-A1 and Px- C1 bands were predominant in all genotypes of the three localities and seem to be genotype-specific as well as locality-specific. Genetic distances and similarity values among different varieties in relation to different localities were detected and discussed.

Poster Abstract 27

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[2]Shanghai Inst. of Plant Physiol., Chinese Acad. of Sci., P.R.C.,
[3]School of Chem. and Chem. Eng., Zhongshan Univ., P.R.C.

P450-CATALYZED HYDROXYLATION OF (+)-δ-CADINENE.

Cadinene synthase catalyses conversion of (E,E)-farnesyl diphosphate to (+)-δ-cadinene, the parent compound in the biosynthesis of gossypol in cotton (Gossypium). A cytochrome P450, whose cDNA was cloned from G. arboreum, was found by RNA analysis to be expressed in developing seeds of glanded G. hirsutum cultivars, but not in seeds of a glandless cultivar (Luo et al., manuscript in preparation). Microsomes from a yeast clone expressing this P450 catalyzed hydroxylation of [3H](+)-δ-cadinene to a more polar product detected by radio-HPLC. GC-MS analysis revealed a product with a molecular mass of 220, consistent with that of mono-hydroxy-cadinene, which has not previously been reported from cotton. Structure of the product is being investigated by NMR spectroscopy. This reaction is probably the second committed step of gossypol biosynthesis. Supported by Cotton Incorporated, National Science Foundation of China, and the Okla. Agric. Expt. Sta.

Poster Abstract 28

Lan Liu-Gitz[1], Dennis C. Gitz[2], Joseph H. Sullivan[2] and Steven J. Britz[1];
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University of Maryland, College Park, MD 20742

TEMPORAL SEPARATION OF BLUE LIGHT CONTROLLED STOMATAL FORMATION AND FLAVONOID ACCUMULATION IN A SOYBEAN ISOLINE EXPRESSING AN UNUSUAL KAEMPFEROL TRIGLUCOSIDE (K9)

Stomata, plant epidermal pores, have a fundamental role controlling photosynthesis and transpiration, and, hence, water use efficiency, drought sensitivity and yield. In spite of major advances in understanding plant organogenesis and development via modern techniques, little is known of the specific cellular and molecular processes that underlie formation of stomata. We have used a soybean line expressing a unique branched kampferol triglycoside (K9) to study stomatal development. One of the characters tightly linked with K9 expression is extreme reduction of stomatal density on upper leaf surfaces. We reported earlier that the presence of blue light inhibits stomatal formation and induces K9 accumulation. To understand the relation between blue light, stomatal formation and K9 expression, we asked whether an increase in K9 was obligatorily coupled to altered stomatal formation under the influence of blue light. Plants were raised under high irradiance with or without blue and transferred to the alternate spectral quality at different stages of first trifoliolate leaf development. We clearly showed that blue light affected stomatal development at the guard mother cell stage, about 9 days after emergence. In contrast, flavonoid induction occurred 1-2 days later. Both processes were sensitive to light within narrow developmental windows, but were temporally and causally separate. These results should be important for understanding the molecular basis of blue light regulated gene expression and stomatal formation.

Poster Abstract 29

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Palmerston North, New Zealand.

OVER EXPRESSION OF THE PHYTOENE SYNTHASE CAROTENOID GENE IN 'MITCHELL' PETUNIA.

'Mitchell' Petunia ((*Petunia axillaris x (P.axillaris x P.hybrida*) plants transformed with the phytoene synthase gene from tomato, had increased carotenoid levels in both leaf and flower tissue. A concentration greater than double that of the control plants was detected in some transgenic lines. A series of crosses between the transgenic lines and 'Summer Sun', a yellow petunia variety with carotenoids already present in the petal tissue, further enhanced carotenoid production. The crosses demonstrated the stability of the transgene and produced flowers with a deeper pigmentation than the control cross progeny The chemistry and biochemistry of carotenoid biosynthesis are well defined and a number of cDNA clones encoding the biosynthetic enzymes have been isolated. We utilized this technology to increase carotenoid production in an ornamental plant. The ultimate aim is to control the accumulation of specific carotenoids and to produce novel flower colours.

4:00-4:40 PM
Symposium Speaker

Birger Lindberg Moller
Plant Biochemistry Laboratory,
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40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark.

METABOLIC ENGINEERING OF CYANOGENIC GLUCOSIDE SYNTHESIS.

Cyanogenic glucosides are amino acid derived natural products found in more than 2.500 different higher plants including important crop plants like cassava, sorghum, flax and almonds. Upon disruption of the plant tissue, the glucosides are degraded thereby causing the release of hydrogen cyanide. Foods derived from crop plants like cassava that contains large amounts of cyanogenic glucosides thus require careful processing.
The biosynthetic pathway is highly channeled and is catalyzed by two multifunctional microsomal cytochrome P450s (CYP79 and CYP71E) and a soluble UDPG-glucosyl transferase. The complete pathway for cyanogenic glucoside biosynthesis has been reconstituted in vitro using the heterologously expressed proteins. When the two P450s and the glucosyltransferase were transformed into Arabidopsis and tobacco, cyanogenic plants were obtained indicating the ability of these previously acyanogenic plants to properly

transport and store "foreign natural products". The production of "unnatural natural products" in plants as a result of the introduction of new points of cross-talk between the pathways for synthesis of cyanogenic glucosides and glucosinolates and based on the broad substrate specificity of CYP71E1 and the glucosyltransferase will be documented. Acyanogenic cassava plants are being generated using anti-sense constructs towards the two different CYP79 genes present in this allotetraploid species.

4:40-5:10 PM
Oral Contribution

Mirjam Czjzeka Muzaffer Cicek [b], Véronique Zamboni [a], Wim P. Burmeister [c], David R. Bevand, Bernard Henrissata, Asim Esen [b].
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THE MECHANISM OF SUBSTRATE SPECIFICITY IN ß-GLUCOSIDASES

The mechanism and the site of substrate (i.e., aglycone) recognition and specificity were directly investigated in maize ß-glucosidase by x-ray crystallography using cocrystals of a catalytically inactive mutant (ZMGlu1E191D) in complex with the natural substrate 2-O-b-D-glucopyranosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOAGlc), the free aglycone DIMBOA, and unhydrolyzed competitive inhibitor para-hydoxy-S-mandelonitril ß-glucoside (dhurrin). The structures of these complexes and uncomplexed mutant were solved at 2.0 Å resolution. The structural data from the complexes allowed us for the first time to visualize an intact substrate, free aglycone, or an unhydrolyzed competitive inhibitor in the slot-like active site of a ß-glucosidase. These data show that the aglycone moiety of the substrate is sandwiched between W378 on one side and F198, F205, and F466 on the other. Thus, specific conformations of these four hydrophobic amino acids and the shape of the aglycone binding site they form determine aglycone recognition and substrate specificity in maize ZMGlu1. In addition to these four residues, D261 indirectly (via a water molecule) interacts with the 3-one group and A467 directly with the 7-methoxy group of DIMBOA. All but W378 of these sites are variable among ß-glycosidases that differ in substrate specificity, supporting the conclusion that these sites are the basis of aglycone recognition and binding (i.e., substrate specificity) in ß-glycosidases. The data also provide a plausible explanation for the competitive binding of dhurrin to maize ß-glucosidases with high affinity without being hydrolyzed.

Tuesday, August 8

9:00-10:10 AM - Symposium Session III, Molecular Manipulation of Alkaloids, Flavonoids, and Cyclases
USDA Bldg. 003, Auditorium

10:10-10:35 AM - Coffee Break

10:35-11:45 AM - Symposium Session III, Molecular Manipulation of Alkaloids, Flavonoids, and Cyclases

1:00-2:40 PM - Symposium Session IV, Pharmaceuticals and Health Benefits from Bioengineering

2:40-3:10 PM - Coffee Break

3:10-4:50 PM - Symposium Session IV, Pharmaceuticals and Health Benefits from Bioengineering

Tuesday, August 8, 2000
Symposium Session III
Arthur Neish Young Investigator's Minisymposium:
Molecular Manipulation of Alkaloids, Flavonoids, and Cyclases
Session Moderator: Dr. Peter Facchini

9:00 – 9:35 AM
Symposium Speaker

Frédérique Hilliou, Leslie van der Fits, Jan W Kijne, Johan Memelink
Institute of Molecular Plant Sciences, Leiden University,
Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

ISOLATION OF REGULATORS OF GENES IN TERPENOID INDOLE ALKALOID METABOLISM IN *CATHARANTHUS ROSEUS* VIA A T-DNA ACTIVATION TAGGING APPROACH

In *Catharanthus roseus*, the formation of a class of secondary metabolites called terpenoid indole alkaloids (TIAs) is induced by the plant stress hormone jasmonic acid (JA), which is synthesized via the octadecanoid pathway. TIAs are derived from the central intermediate

strictosidine, consisting of a terpenoid (secologanin) and a tryptophan-derived indole moiety. JA coordinately induces genes in the TIA pathway, as well as in primary precursor pathways, suggesting the existence of a shared signal transduction pathway. In order to isolate components of this JA signaling pathway, C. roseus cells were transformed with a T-DNA activating construct carrying a constitutive promoter reading towards the border. Cells were selected for increased TDC activity on the toxic 4-methyl-tryptophan and further selected for high Tdc and Str expression levels. The T-DNA flanking plant DNA rescued from one of the positive cell lines encoded a JA-responsive AP2-domain transcription factor, called ORCA3 (Octadecanoid-Responsive Catharanthus AP2-domain). Overexpression of ORCA3 in stably transformed C. roseus cell lines increased the expression of genes involved in the tryptophan and terpenoid precursor pathways, as well as in the TIA pathway, resulting in increased primary or secondary metabolite levels depending on the growth conditions. Analysis of other positive T-DNA tagged lines is in progress and can lead to the isolation of additional regulators.

9:35 – 10:10 AM
Symposium Speaker

Edward L. Braun, Anusha P. Dias, Todd J. Matulnik, J. Marcela Hernandez and Erich Grotewold
Department of Plant Biology and Plant Biotechnology Center,
Ohio State University, Columbus OH 43210.

REGULATION OF FLAVONOID METABOLISM BY MYB GENES

Plant transcription factors exhibiting homology to the DNA-binding domain of the vertebrate c-Myb proto-oncoprotein are encoded by a diverse gene family with more than one hundred distinct members in the higher plants. Several plant myb genes have been implicated in the regulation of phenolic compounds including flavonoids and phenylpropanoids. We are interested in the use of evolutionary analyses to make functional inferences regarding members of the myb gene family. Various molecular techniques are then used to explore the implications of these functional inferences in terms of genome dynamics and metabolic diversity. Our analyses suggest that the myb gene family underwent a remarkable expansion near the origin of the land plants, coincident with the early evolution of some phenolic secondary compounds, and indicate more recent expansions of specific subgroups within the grasses. Striking differences in the evolutionary rate of distinct groups of myb genes were documented, which allowed us to target specific genes for additional analyses. The independent control of two branches of maize flavonoid biosynthesis by the myb genes P and C1 provided an unique opportunity to investigate how sequence differences within Myb domains contribute in distinct ways to the regulatory specificity of related Myb transcription factors, and the insights gained from analyses of this system will be discussed.

10:10-11:35 AM Coffee Break

10:35-11:10 AM
Symposium Speaker

Lukas Mueller
Carnegie Institute of Washington
260 Panama Street, Stanford, CA 94305

MODELS FOR ANTHOCYANIN SEQUESTRATION IN PLANTS

The anthocyanin pathway is arguably the best understood plant secondary metabolite biosynthetic pathway. Whereas all anthocyanin biosynthetic genes have been mutated to define function in vivo and cloned to analyze gene structure, little is known about how anthocyanins are exported to their final site of storage, the vacuole. In maize and petunia, glutathione S-transferases (GSTs) are required for the vacuolar sequestration of anthocyanins. We investigated how AN9, the GST from *Petunia hybrida* required for pigment sequestration, mediates anthocyanin export to the vacuole. For many xenobiotics, a covalent glutathione "tag" is required for recognition of molecules destined for vacuolar sequestration by a tonoplast-localized ATP binding cassette (ABC) pump.
We investigated if AN9 could similarly catalyze the formation of glutathione conjugates with flavonoid substrates. Using HPLC analysis of reaction mixtures containing enzyme, glutathione and flavonoids, including anthocyanins, we could detect neither conjugates nor a decrease in the free thiol concentration. These results suggest that no conjugate is formed in vitro. We further showed that AN9 binds flavonoids using three assays: inhibition of the GST activity of AN9 towards the common GST substrate CDNB, equilibrium dialysis, and tryptophan quenching. We conclude that AN9 is a flavonoid binding protein, and propose that in vivo it serves as a cytoplasmic flavonoid carrier protein.

11:10 – 11:45 AM
Symposium Speaker

Mark A. Schoenbeck, Joseph Chappell
Department of Agronomy, University of Kentucky
N221-W Agricultural Sciences North, Lexington, KY 40506

THE SESQUITERPENE CYCLASE GENE FAMILY OF *NICOTIANA TABACUM*: HYBRID ORIGINS AND PHYSIOLOGICAL ROLES.

5-epi-aristolochene synthase (EAS) is a sesquiterpene cyclase of *Nicotiana tabacum* which catalyzes the first step in the synthesis of the antimicrobial phytoalexin capsidiol. EAS is a member of a gene family with 12-15 members, some of which are induced within hours by

incompatible pathogens and elicitor molecules. Members of the EAS gene family have a conserved gene structure within the coding region, but diverge significantly in their flanking genomic sequences, including the promoter regions. Some EAS genes are interrupted by transposable elements or have suffered deletions, and appear to be no longer functional. N. tabacum is the product of hybridization between an ancestors of *N. sylvestris* and *N. tomentosiformis,* followed by chromosome doubling that allowed meiotic pairing. DNA blot analysis *N. sylvestris* and *N. tomentosiformis* genomes shows that each ancestor likely contributed unique members of the EAS gene family. We are comparing the induction of EAS gene expression in N. sylvestris, N. tomentosiformis, and a modern synthesis of *the N. sylvestris x N. tomentosiformis* hybrid to assess the contribution of the ancestral genomes to modern defense response, which has undergone continued modification as a result of breeding efforts.

Tuesday, August 8, 2000
Symposium Session IV : Pharmaceuticals and Health Benefits from Bioengineering
Session Moderator: Dr. J. J. Lin

1:00 – 1:40 PM
Symposium Speaker

Nancy L. Paiva,
Plant Biology Division
Samuel Roberts Noble Foundation
Ardmore, OK, 73401

RESVERATROL GLUCOSIDE ENGINEERING: PLANT AND HUMAN HEALTH BENEFITS.

Resveratrol (3,5,4'-trihydroxystilbene) accumulates in a wide range of plant species. Several studies have indicated that resveratrol exhibits activities beneficial to human health, suggesting its consumption could potentially decrease cancer and heart disease. However, little or no resveratrol accumulates in the edible portions of most crop plants. We have introduced a resveratrol synthase coding region driven by a constitutive promoter into various plant species which do not naturally accumulate resveratrol. In alfalfa (*Medicago sativa*), a perennial forage legume, plants accumulated trans-resveratrol-3-O-beta-D-glucopyranoside in leaves and stems, and no resveratrol aglycone. Resveratrol glucoside accumulation produced no visible negative phenotype in alfalfa, and conferred increased resistance to one fungal pathogen. Because alfalfa is a highly nutritious animal feed, we are using this material in animal studies to assess the possible nutritional benefits of resveratrol glucoside-containing diets. An identical product is accumulated in transgenic soybean cell cultures, while in preliminary studies tobacco leaves appear to accumulate a mixture of free resveratrol and a resveratrol glucoside. Glycosylation may affect the bioavailabilty, stability, and phytotoxicity of resveratrol.

1:40 – 2:20 PM
Symposium Speaker

Joseph G. Boothe
SemBioSys Genetics Inc., 500, 3605 - 29th Street N.E., Calgary, Alberta, T1Y 5W4.

DESIGNING TRANSGENIC PLANT EXPRESSION SYSTEMS FOR THE PRODUCTION OF RECOMBINANT PROTEINS.

High capacity coupled with low upstream production costs make plants attractive vehicles for the manufacture of recombinant proteins. Plant-based systems also lend themselves to a variety of different product applications. These include products that can delivered within the plant tissue such as feed additives and edible vaccines, to those for which either moderate or high levels of purity are required such industrial enzymes and biopharmaceuticals. The use of transgenic plants as vehicles for commercial production requires that the recombinant proteins be expressed at high levels, are stable within the host tissue, and can be recovered in a cost-effective manner. The design of plant expression systems to meet these requirements will be discussed with specific reference to the technology we have developed at SemBioSys for the manufacture of recombinant proteins in oilseeds.

2:20 – 2:40 PM
Oral Contribution

Kwon, M., Burlat, V., Davin, L. B., Lewis, N. G.
Institute of Biological Chemistry, Washington State University,
Pullman, WA 99164-6340.

***IN SITU* HYBRIDIZATION AND IMMUNOLOCALIZATION OF DIRIGENT PROTEIN AND LIGNAN REDUCTASES IN *FORSYTHIA INTERMEDIA* AND *PINUS TAEDA*.**

Monolignol partitioning into the lignan pathway has been demonstrated in vascular (woody) forming tissues using both *F. intermedia* and *P. taeda*. The dirigent involved in pinoresinol formation was demonstrated to be expressed exclusively in the vascular cambium and differentiating xylem regions of *F. intermedia* by in situ hybridization and immunolocalization. Additionally, the downstream reductases, pinoresinol-lariciresinol and phenylcoumaran benzylic ether reductases, in F. intermedia and P. taeda, respectively, were expressed/localized in the vascular cambium as well as along the axial/radial parenchyma cells in the developing secondary xylem. These results thus establish how woody plants, in addition to monolignol partitioning, have developed mechanisms for protection of their vascular apparatus e.g., for heartwood formation and preservation.

2:40 – 3:10 PM Coffee Break

3:10 – 3:50 PM
Symposium Speaker

Vidadi Yusibov, Hilary Koprowski
Biotechnology Foundation Laboratories, Thomas Jefferson University, Philadelphia.

PHARMACEUTICAL PRODUCTION IN PLANTS

Vaccines are proven to be one of the most efficient approaches to prevent infectious diseases of human and animals. There are number of vaccines, based on full pathogens, used successfully to prevent infectious diseases of human and animals. Nevertheless, the growing need of world's population in effective and easily accessible vaccine products require new approaches to vaccine development. During last 2 decades application of molecular biology resulted in characterization of number of genes of humans, animals and their pathogens critical for certain disease conditions. These achievements in molecular biology created opportunity for new approaches to the development and production of vaccines and other biomedicals. As a result of studies several systems for the production and delivery of biomedicals were developed. More recently plants became one of the new approaches resulting in a significant progress during short period of time. This review will focus on transgenic plants and engineered plant viruses used for the development and production of vaccines and other important biomedicals.

3:50 – 4:10 PM
Oral Contribution

Masoumeh Assadi, Robert P. Donaldson,
Biological Sciences Department, George Washington University

MALATE SYNTHASE C-TERMINAL PEPTIDE INTERACTION WITH ITS PEROXISOMAL TARGETING RECEPTOR IN CASTOR BEAN.

Peroxisomal matrix proteins are synthesized in cytosol and translocated post-translationally into the peroxisomal matrix. Peroxisomal targeting sequence 1 (PTS1) is a C-terminal tripeptide (SKL or conservative variants) that is sufficient to direct a peroxisomal matrix protein to peroxisomes in plants, animals, and yeast. Results from our lab and other groups have shown that upstream charged residues at the C-terminal of the PTS1-containing proteins play a role in interacting with the peroxisomal receptor(s). The peroxisomal targeting process has been somewhat characterized in yeast and human. Several peroxisomal

membrane associated and cytosolic factors, including PTS1 receptor (PEX5), have been identified in these systems. But, much less information is available for plants. We have attempted to determine localization of the peroxisomal targeting receptor in the endosperm of germinating castor bean. Also we were interested in the role of the charged residues near the C-terminal of the PTS1 proteins in interacting with the receptor in castor bean and pumpkin. Peptides labeled with I-125 were synthesized

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4:10 – 4:30 PM
Oral Contribution

Daphna Havkin-Frenkel, Henrik Pedersen,
Rutgers University, New Brunswick, NJ

ENZYMOLOGY AND FLUX CONTROL OF VANILLIN BIOSYNTHETIC PATHWAY IN *VANILLA PLANIFOLIA* TISSUE CULTURE

Vanilla extract is widely used in the food and the confectionery industry. Vanillin is the most abundant component of vanilla extract. An understanding of the biosynthetic pathway of vanillin may be important for regulating the production of the compound in plants. It is agreed that vanillin (C6-C1) is a product of phenylpropanoid compounds. It was shown that in *Vanilla planifolia* tissue cultures the benzoate derivative pathway operates using phenylpropanoid substrates, leading from trans-cinnamic acid to flavor compounds, arising from phenylalanine and to less extent tyrosine as precursor. The branch point between the C6-C3 and C6-C1 pathways most likely occurs at the level of p-coumaric acid. Starting with chain shortening of p-coumaric acid to p-hydroxy benzaldehyde and following hydroxylation and methylation the pathway yields vanillin as final product. We examine the control of flux of metabolites in the vanillin biosynthetic pathway in vanilla embryo culture. Metabolite feeding studies suggest that the rate-controlling step in the vanillin pathway is hydroxylation of 4-hydroxybenzyl alcohol.

4:30-4:50 PM
Oral Contribution

Allan G. Phipps; Bennett, Bradley C.; Downum, Kelsey R.
Florida International University, Miami, Florida

JAPANESE USE OF BENI-TENGU-DAKE (*AMANITA MUSCARIA*) AND THE EFFICACY OF TRADITIONAL DETOXIFICATION METHODS

The poisonous fruiting bodies of beni-tengu-take (*Amanita muscaria* (L. ex Fr.) Pers. ex Hook.) are harvested by rural inhabitants of Sanada Town, Japan. These mountain villagers consume beni-tengu-take as a local delicacy, despite its potential hallucinogenic effects. The Japanese use several methods to detoxify beni-tengu-take, but believe pickling the

mushrooms to be the safest. Other methods of preparation include grilling and drying the mushrooms. The preparation and consumption of each detoxification method were documented through local interviews with Japanese informants. Ion-interaction rp-HPLC was used to quantify the hallucinogenic compounds, ibotenic acid and muscimol, and determined the efficacy of each traditional detoxification method. Fresh mushrooms contained 6.17mmol/kg of ibotenic acid (LD50 in mice is 0.9 mmol/kg when administered orally) and 0.93mmol/kg of muscimol (LD50 in mice is 0.4 mmol/kg when administered orally). Grilling and drying increased the toxicity of the mushrooms. The pickling process removed all detectable amounts of both hallucinogenic compounds.

Wednesday, August 9

8:40-10:00 AM - Symposium Session V, Current Advances in Molecular Tools
USDA Bldg. 003, Auditorium

10:00-10:40 AM - Break

10:40-11:40 AM - Symposium Session V, Current Advances in Molecular Tools

11:45-1:15 PM - Lunch

1:15-2:35 PM - Symposium Session VI, Molecular modifications of the Phenylpropanoid pathway

2:35-3:00 PM - Break

3:00-3:55 PM - Symposium Session VI, Molecular modifications of the Phenylpropanoid pathway

3:55-5:00 PM - PSNA Annual General Meeting

6:30-10:00 PM - Banquet Dinner Speaker,
Maize Genomics: Using ESTs and
Transposon Tags to Discover Genes
Holiday Inn, 10000 Baltimore Ave

Wednesday, August 9, 2000
Symposium Session V
Current Advances in Molecular Techniques
Session Moderator: Dr Kim Lewers

8:40 – 9:20 AM
Symposium Speaker

John Quackenbush, Erik Snesrud, Baoping Zhao, Brian Haas, Christopher D. Town,
The Institute for Genomic Research, Rockville MD

WHOLE-CHROMOSOME GENE EXPRESSION ANALYSIS IN *ARABIDOPSIS THALIANA* USING DNA MICROARRAYS.

Genomics has produced a revolution in plant biology and genome sequencing projects in plants are progressing rapidly. While the sequencing of the *Arabidopsis thaliana* genome will be completed within months, the identification and annotation of the genes and the role they play in development, disease, and response to environmental stress remains a significant challenge. In Arabidopsis, there are an estimated 30,000 genes, but fewer than 1,500 have annotated, full-length coding sequences in GenBank and the nearly 40,000 EST sequences represent only a fraction of total gene content; nearly three quarters of the annotated genes have no direct experimental support. Using the gene predictions for the completed sequence of Arabidopsis chromosome II as a model, we have begun a program to validate the gene predictions using cDNA microarrays. We have designed primers and amplified the 3' ends of the 4,442 predicted genes on the chromosome and constructed microarrays containing those genes. These arrays are being used to assess patterns of gene expression in a variety of plant tissues and developmental stages in order to provide experimental validation for the gene predictions and to develop an expression map of the chromosome. In addition, the arrays contain genomic sequencing clones from a minimal tiling path spanning a 1.5 Mb region that will allow us to assess the frequency with which genes were missed in the initial annotation and may provide additional information on the accuracy of the gene models.

9:20 – 10:00 AM
Symposium Speaker

Frank J. Turano[1], Mark Allard[1], Geraldine Glover[2], Michael McMahon[3], Michael Muhitch[4], Ganesh Panta[1], Peter Van Berkum[5]
The George Washington University, Department of Biological Sciences,Washington, DC[1],
Maryland University, Center for Agricultural Biotechnology, College Park, MD[2]
Foreign Disease-Weed Science, USDA, ARS, Fort Detrick, MD[3]
National Center for Agricultural Utilization Research, Mycotoxin Research Unit, USDA, ARS, Peoria, IL[4]
Soybean and Alfalfa Research Laboratory, USDA, ARS, Beltsville, MD[5]

GLUTAMATE AND GABA-LIKE RECEPTORS IN ARABIDOPSIS.

In animals there are different types of excitatory or inhibitory amino acid neurotransmitter receptors. These large groups of receptors, referred to as superfamilies, are genetically, pharmacologically and structurally distinct from each other. Although sequence similarities among members of the superfamilies have been reported in the literature, a viable evolutionary history of the animal receptors remains elusive. We demonstrate that a large gene family of receptors in plants, which has been designated the putative glutamate receptor (GLRs), are related to two distinct superfamilies of neurotransmitter receptors in animals via unique evolutionary mechanisms. Analysis of the spatial and temporal expression of the plant GLRs indicates that the transcripts for several receptors are differentially processed. The gene encoding for GLR2 is processed into two distinct transcripts, GLR2a and GLR2b, by a combination of events, the initiation of transcription at unique sites and differential splicing of the resulting transcripts. However, transcription of the gene encoding for GLR4 appears to be initiated at the same site but the transcript is differentially processed to produce a full-length peptide, GLR4a, and a truncate peptide, GLR4b. The accumulation of the distinct transcripts corresponding to GLR2 and GLR4 were determined to be temporally and spatially regulated. The physiological significance of alternative slicing of the plant transcripts remains unknown.
We have constructed transgenic plants carrying antisense GLR constructs to determine the functional role of the GLRs. The antisense plants accumulate less GLR peptide than wild-type plants and exhibit altered phenotypes. Preliminary results from plants expressing antisense constructs suggest that the GLRs may play roles in plant development and responses to different environmental stresses.
The cellular localization of the GLR peptides suggests that they may function in intracellular or intercellular signaling, however, the possibility that they may function in amino acid transport can not be refuted at this time. Collectively, our preliminary data suggest that the plant GLRs may be fundamentally and functionally different than the highly specialized neurotransmitter receptors found in animal neurons.

10:40-10:40 AM Coffee Break

10:40-11:00 AM
Oral Contribution

[1]Kothandaraman Narasimhan, [2]Thomas Payne,[2]Allan Lloyd and [1]Sanjay Swarup.
[1]Molecular Plant-Microbe Interaction Laboratory, Department of Biological Sciences, National University of Singapore, Singapore 117543
[2]The Department of Botany, The Institute of Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712

AN LC/MS-BASED METABOLIC PROFILING OF THE FLAVONOID PATHWAY INTERMEDIATES IN THE TISSUES AND ROOT EXUDATES OF ARABIDOPSIS MUTANTS AND TRANSGENICS.

Detection, identification and quantitation of intermediate metabolic compounds in the biosynthetic pathway are crucial for engineering metabolic pathways for biotechnological applications. Flavonoids are important ubiquitous secondary metabolites in plants with diverse biological activities. Arabidopsis provides a model for the analysis of changes induced in flavonoid biosynthetic pathway due to the availability of a series of metabolic mutants. We have used Liquid Chromatography/Electron Spray Ionization Mass Spectrometry (LC/ESI-MS) to characterize the flavonoid profiles of a number of metabolic mutants and transgenics in Arabidopsis tissues and root exudates. No detailed LC/MS based studies are reported in the literature. Therefore, based on RP-HPLC studies, Arabidopsis mutants that are known to overaccumulate (ttg/ler), have altered profiles (tt8/ler) and low or no accumulation of flavonoids (tt4/ler) (Shirley et al., 1999) were included in this study. Additionally three uncharacterized mutants for the intermediates (tt9/ler, tt5/ler, tt6/ler) were also profiled. All the lines showed differences in tissue-specific profiles. Only the flavonol quercetin and its glycosides were detected in the root exudates, whereas kaempferol and its glycosides could be additionally detected in various tissues. Only non-conjugated quercetin was detected in the root exudates of the mutant tt8/ler. However the transgenics carrying both the full length and the truncated Lc (myb-type) gene accumulated kaempferol and quercetin with their glycosides in both root exudates and plant tissues. In situ localization with epifluorescence microscopy was carried out by using the flavonol-specific fluorescent stain, diphenyl boric acid amino ethyl ester (DPBA). Root flavonoids were conspicuously localized to the root hairs.

11:00-11:20 PM
Oral Contribution

Benjamin F. Matthews, Hunter S. Beard, Margaret H. MacDonald, Rana Khan, Michael Yang, Kristina L. Pilitt, Nadim Alkharouf
USDA-ARS, Soybean & Alfalfa Research Laboratory, Beltsville, MD. USA 20705

EXPRESSION OF SOYBEAN GENES INVOLVED IN RESISTANCE TO THE SOYBEAN CYST NEMATODE.

The soybean cyst nematode (SCN), *Heterodera glycines*, is the major pest of soybean worldwide and is responsible for damage estimated at $1.5 million each year in the U.S. We are identifying genes involved in the resistance response of soybean to SCN using array techniques. Over 1000 ESTs were sequenced from a cDNA library constructed from cv. Peking mRNA two days after invasion of roots by SCN race 3. Numerous genes were identified by BLAST searches, including genes involved in secondary metabolism, signaling, and the defense response. Several genes involved in phenylpropanoid synthesis were found including phenylalanine ammonia lyase, chalcone synthase, cinnamyl alcohol dehydrogenase, 4-coumarate coA ligase, and others. Numerous peroxidases, protein kinases and transcription factors also were identified. Insert sizes range from ca. 400 bp to over 3.2 kb. Some clones were added to the database from differential display experiments that identified SCN-induced soybean genes. A set of 96 clones also was added from a cDNA library made from cotyledons to serve as non-induced genes in the array. Inserts of several clones are completely sequenced and contain complete reading frames: these are from genes that were available in this or other USDA laboratories. The database containing clone information is searchable by clone address, identity and size. The database can be viewed by collaborators at http://bldg6.arsusda.gov/benlab. A subset of the array is being hybridized to cDNA made from mRNA representing genes expressed in resistant and susceptible soybean inoculated and not inoculated with SCN to identify genes greatly induced in response to SCN.

11:20-11:45 PM
Oral Contribution

James A. Saunders[1], Sue Mischke[1], Alaa A. Hemeida[2], and Monica J. Pedroni[1]
[1]USDA, ARS, Climate Stress Lab, Bldg. 50, Rm. 100, Beltsville, MD 20705
[2]Genetic Engineering & Biotechnology Research Institute (GEBRI), Sadat City, Minufiya University, Egypt.

DNA FINGERPRINTING IN *THEOBROMA CACAO*, THE CHOCOLATE TREE.

AFLP patterns from various *Theobroma cacao* genotypes were determined to establish relationships among populations of trees in germplasm collections. DNA fragments were selectively amplified, labeled with fluorescent dyes, and separated by capillary electrophoresis after selective PCR amplification. The peaks corresponding to DNA fragment patterns were reproducible and consistent within a common genotype. A similarity dendrogram was produced based on the combined analysis of multiple AFLP primer sets of polymorphic peaks between 50 and 400 base pairs detected from electropherograms using a PE/ABI 310 DNA analyzer. AFLP analyzes by this procedure defines individual accessions, identifies duplications of genotypes within germplasm collections, and establishes genetic similarity of breeding lines for potential crosses. This information allows for selection of appropriate germplasm stocks to be used in *Theobroma cacao* breeding programs to produce cultivars with improved tolerance/resistance to fungal diseases.

Wednesday, August 9, 2000
Symposium Session VI
Molecular Modification of Phenylpropanoid Pathway
Session Moderator: Dr. Nichole R. O'Neill

1:15 – 1:55 PM
Symposium Speaker

Cathie Martin, Hailing Jin, Kathy Schwinn
Department of Genetics, John Innes Centre, Colney, Norwich, NR4 7UH, UK

MECHANISMS AND APPLICATION OF TRANSCRIPTIONAL CONTROL OF PHENYLPROPANOID METABOLISM

In the field of valuable plant products most biotechnological strategies involve making more or less of a product or making a novel product and many actually involve two of these requirements; making more of a novel product. A central task in pathway engineering is therefore to engineer flux. Because flux control is usually vested in multiple enzyme steps the best means for its manipulation is to use regulatory proteins that co-ordinately modulate the activity of several biosynthetic steps. Our basic understanding of the transcriptional control of secondary metabolism comes from the regulatory genes that control anthocyanin accumulation. These are known to encode conserved proteins in different species, although the structural genes they regulate depend on the requirements for metabolic flexibility of the particular plant species. Study of members of the MYB gene family show how plants regulate the patterning and intensity of floral pigmentation and provides tools for further pathway manipulation. Closely related proteins, of the same regulatory gene family, control other branches of phenylpropanoid metabolism. In Arabidopsis the gene AtMYB4 negatively controls synthesis of sinapoyl esters. This repressor action is unusual in the control of secondary metabolism, but derepression can effectively enhance the synthesis of UV-protecting sunscreens. The output from manipulating this pathway includes increased UV-tolerance in plants and reduced lignin for paper and forage products. Our understanding of the regulatory genes of phenylpropanoid metabolism allow for imaginative and flexible manipulation. The genes controlling synthesis of other plant metabolites remain to be discovered. The most direct means to identify novel regulators is through the functional genomics of plant transcription factors a research goal we are pursuing in a Pan-European project.

1:55 – 2:35 PM
Symposium Speaker

Richard A. Dixon*, F. Chen, D. Guo, X.-Z. He, C.J. Liu, C.L. Steele,
Samuel Roberts Noble Foundation, Ardmore, OK.
METABOLIC ENGINEERING OF PHENYLPROPANOID BIOSYNTHESIS

The phenylpropanoid pathway leads to the production of the abundant biopolymer lignin and to a range of bioactive natural products including flavonoids, isoflavonoids and lignans. Genetic engineering of lignin content and composition can result in improvements to the digestibility of forage crops. Such studies are outlined utilizing down-regulation of caffeic acid 3-O-methyltransferase and caffeoyl CoA 3-O-methyltransferase in the forage legume alfalfa. The isoflavonoids of the leguminosae function as antimicrobial phytoalexins, and we have demonstrated improved disease resistance of transgenic alfalfa with an increased phytoalexin response due to over-expression of isoflavone O-methyltransferase. The simple isoflavones daidzein and genistein have been shown to have important potential as dietary cancer chemopreventants in humans and other mammals. We have characterized the gene encoding the first committed step of isoflavonoid biosynthesis, the cytochrome P450-mediated aryl migration of a flavanone to yield an isoflavone. We describe the use of this gene to introduce the isoflavonoid pathway into Arabidopsis as a model for the metabolic engineering of health-promoting nutraceuticals in food crops.

2:35 – 3:00 PM Coffee Break

3:00 – 3:35 PM
Symposium Speaker

Malla Padidam
Biotechnology Research,Rohm and Haas Company
727 Norristown Road, Spring House, PA 19477-0904.

ECDYSONE RECEPTOR-BASED, CHEMICAL-INDUCIBLE GENE REGULATION FOR PLANTS

An inducible system to activate or inactivate plant gene expression has many potential applications in basic understanding of gene function, in manipulating complex developmental pathways, and in plant biotechnology. We are developing an inducible system with potential for field application using spruce budworm ecdysone receptor (EcR) and non-steroidal ecdysone agonists. Chimeric transcription factors were made using different DNA binding and activation domains and EcR ligand binding domain. Reporter gene or gene of interest was cloned behind a DNA element where chimeric EcR transcription factor can bind. Such transcription factor does not activate or inhibit transcription in the absence of a ligand. Addition of bisacylhydrazine ligand, which has an exceptional health and environmental safety profiles, activates or inhibits the transcription of the gene(s) of interest. We used our system in transient assays with tobacco protoplasts and in tobacco and Arabidopsis plants. Results show that this system based on EcR is very effective and can be used to express high levels of protein. Data from transient and transgenic experiments will be presented.

3:35 – 3:55 PM
Oral Contribution

D. H. Lewis[1] , J.M. Bradley[1], S. J. Bloor[2], E. Swinny[2], C. Winefield[1], K. Davies[1], S.C. Deroles[1].
[1]Crop & Food Research, Private Bag 11 600, Palmerston North. NZ.
[2]Industrial Research Ltd. PO Box 31-310, Lower Hutt,NZ

ANTISENSE EXPRESSION OF THE FLAVONOID 3'-HYDROXYLASE GENE IN TRANSGENIC "MITCHELL' PETUNIA.

'Mitchell' Petunia was transformed with an antisense flavonoid 3'-hydroxylase (F3'H) constuct under the control of the 35S promoter, as part of a project examining the genetic manipulation of flavonoid biosynthesis. A series of independent transformants with different levels of F3'H activity were produced. 'Mitchell' Petunia is a white flowered line so flower colour was not altered, however there was a change in the pattern of flavonol accumulation in both leaf and flower tissue. The total level of flavonols in flower limb tissue did not change but the quercetin:kaempherol ratio, for the line with the greatest change in enzyme activity, was reduced from 12: 1 to 1:1. No changes in plant morphology were observed but there was a significant reduction in pollen germination in some transgenic lines.

3:55 – 5:00 PM PSNA General Business Meeting
Susan McCormick Presiding

Wednesday, August 9, 2000
Banquet and Dinner Speaker, Genomics
Holiday Inn, 10000 Baltimore Blvd., College Park, MD
Speaker Introductions Dr. Rose Hammond

6:30 – 10:00 PM

Virginia Walbot
Department of Biological Sciences, Stanford University, Stanford, CA 94305

MAIZE GENOMICS: USING ESTS AND TRANSPOSON TAGS TO DISCOVER GENES

The goals of the NSF-funded Maize Gene Discovery Project are to produce a database of maize ESTs, DNA microarrays built from the ESTs, genomic DNA sequences flanking Rescue Mu transposon insertions, and phenotypes of plants generated in the transposon tagging. This comprehensive project combines gene discovery with functional genomics by using transposons to both mutate genes and to clone the genes. Progress in gene discovery (~62,000 ESTs to date define ~20,000 maize genes) and characterization will be presented as well as a description of the resources available to public sector scientists. You will be encouraged to use these new resources and to provide "expert information" for the annotation of genes and phenotypes.

Thursday, August 10

8:50-10:10 AM - Symposium Session VII, Expression, Defense and Transcriptional Factors
USDA Bldg. 003, Auditorium

10:10-10:25 AM - Coffee Break

10:25-12:05 AM - Symposium Session VII, Expression, Defense and Transcriptional Factors

Thursday, August 10, 2000
Symposium Session VII
Expression, Defense and Transcriptional Factors
Session Moderator: Dr. Rose Hammond

8:50 – 9:30 AM
Symposium Speaker

Sheila McCormick, Ines Ezcurra, Sheila Johnson, Robyn Cotter, Wei-hua Tang
Plant Gene Expression Center, USDA/ARS-UC-Berkeley, Albany, CA

POLLEN-PISTIL INTERACTIONS

Mature pollen grains are released from the anther in a partially dehydrated state and re-hydrate after they contact the female tissue; the pollen tube emerges from one of the apertures and grows by tip growth through the extracellular matrix of the pistil, in order to deliver the sperm cells to the embryo sac. Signaling between the two partners is crucial for completion of this intricate process.
As one entrée into understanding pollen-pistil communication, we have characterized three tomato receptor kinases (LePRK1, 2 and 3) that are specifically expressed in pollen. These kinases have extracellular domains composed of 5-6 leucine-rich repeats, a transmembrane domain, and other characteristic features that distinguish them from other plant receptor kinases. Because antibodies raised against the extracellular domains of the tomato kinases label the pollen tubes, it is likely that these kinases play a role during pollen-pistil signaling. We are using the yeast two-hybrid system to isolate potential ligands for these kinases: in yeast, the extracellular domains of these kinases interact with several secreted proteins that are expressed either in the stigma/style or in the pollen grain itself. We are expressing the candidate ligands and receptor kinases in heterologous systems in order to determine if the interactions are biologically relevant. Receptor kinases frequently heterodimerize but we have only three tomato kinases in hand - there may be others. We have therefore taken

advantage of the nearly complete genome sequence of Arabidopsis to identify (thus far) 10 such receptor kinases and confirmed pollen expression of several using RT-PCR. This complete data set and reverse genetics tools will allow us to systematically generate mutations in the genes encoding each kinase and potential ligand.
In a separate study, we are characterizing a mutant in Arabidopsis (raring-to-go) whose pollen can apparently bypass the requirement for contact with the female - the initial steps of pollen tube hydration and germination occur inside the anther.

9:30 – 10:10 AM
Symposium Speaker

Anne Simon
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KILL OR CURE: THE ENIGMA OF PLANT VIRUS SATELLITE RNAS

Many plant RNA viruses are associated with nonessential subviral RNAs including satRNAs that can modulate the symptoms of the helper virus. Accumulation of satRNAs can have either no effect on symptoms or can attenuate or exacerbate symptoms. SatC, which normally intensifies the symptoms of its helper virus TCV, can attenuate viral symptoms if TCV expresses the coat protein of a related virus or produces a reduced level of wild-type TCV coat protein. The ability to form virions is not required for symptom attenuation since an N-terminal mutation in the TCV coat protein that eliminates virion formation does not preclude either virus movement or symptoms attenuation. Symptom attenuation is associated with an inability of the virus to translocate from the initially inoculated leaf, suggesting that the presence of the satRNA in the context of reduced coat protein levels inhibits virus long-distance movement. The satC determinants involved in symptom attenuation were mapped to the 3' terminal hairpin, which is also the promoter for minus-strand synthesis. Symptom attenuation was directly correlated with the binding affinity of the coat protein for the hairpin structure.

10:10-10:25 AM
Coffee Break

10:25-10:45 AM
Oral Contribution

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COMPARISON OF RESISTANT AND SUSCEPTIBLE SOYBEAN GENOMES NEAR A SOYBEAN CYST NEMATODE RESISTANCE GENE.

Soybean cyst nematode (SCN) is the most serious pest of soybeans in the US. The most desirable control method is the use of soybean cultivars having SCN resistance genes. The SCN resistance gene, Rh94, is located near the I locus controlling seed coat color. From published sequence of the I locus, we developed a PCR marker. In addition, the molecular marker, pBLT65, is close to Rhg4. Both markers were used to screen a 'Williams 82' (susceptible) Bacterial Artificial Chromosome (BAC) library and identified one 150kb BAC. SSR markers and a subclone library were developed from this BAC. Subclones from the BAC were sequenced: 1) to identify genes in this region, and 2) to develop additional markers to use in finding the same region in resistant genotypes. These markers identified 87 BACs from a PI 437.654 (resistant) BAC library. Restriction fragment analysis using FPC (fingerprinted contigs) software at several stringency levels assigned BACs to contigs. RFLP markers pBLT65 and the I locus were assigned by FPC at all stringency levels to separate contigs. However, connection of contigs containing these markers was confirmed by PCR primers developed from BAC end sequencing. Because these primers did not amplify a product from the Williams 82 BAC, and because the physical distance between pBLT65 and the I locus marker is greater in PI 437.654 than in Williams 82, we are investigating the possibility that there may be an insertion in PI 437.654 relative to Williams 82. This is of interest, because at least three resistance genes have been found in insertions in plant genomes. We are focusing our efforts on two BACs that connect the contigs containing pBLT65 and the I locus marker. We are taking two approaches: 1) complementation tests through transformation, and 2) sequencing and assembly of subclones of the two BACs.

10:45 – 11:05 AM
Oral Contribution

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ANTIFUNGAL AND MOLLUSCIDAL ACTIVITY OF *ERIGERON SPECIOSUS*.

The steam-distilled oil of the aerial parts of *Erigeron speciosus* was screened for antifungal activity against strawberry fungi that cause anthracnose (*Colletotrichum spp.*) and against the intermediate host-snails (*Planobdella trivolvis*) for trematodes that infest catfish in commercial ponds in the Mississippi Delta region. Preliminary bioassay of Erigeron oil indicated the presence of phytochemicals with antifungal activity against *Colletotrichum fragariae, C. gloeosporioides,* and *C. acutatum* and lethal toxicity to *P. trivolvis* sp. Bioactive compounds were isolated by bioassay-guided fractionation and chromatographic techniques and identified by 1H and 13C NMR spectroscopic techniques.

11:05 – 11:25 AM
Oral Contribution

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NUCLEIC-ACID DIAGNOSTIC TOOLS FOR THE NEW MILLENNIUM.

Diagnostic testing is one of the fastest growing applications of biotechnology. The key to successful nucleic acid based diagnostics is reliable procedures for the recovery of nucleic acids amendable to the numerous diagnostic formats. The goal of D-squared BioTechnologies is to develop reliable nucleic extraction systems for use with our diagnostic kits for the detection of pathogen nucleic acid sequences. We have combined DNA/RNA sequencing, recombinant nucleic acid technology, and bioinformatic tools to develop a proprietary database of genus- and species-specific probes for agricultural and food-borne pathogens. These probes can be used for a number of nucleic acid-based diagnostic methods including PCR and DNA/RNA hybridization. Armed with these tools, diagnosticians are better equipped to meet the demands of diagnosis of agricultural diseases in the new millennium.

11:25 – 12:05 PM
Symposium Speaker

Jonathan Arias
University of Maryland, College Park, MD

MEMBERS OF THE BASIC/LEUCINE-ZIPPER (BZIP) FAMILY OF TRANSCRIPTION FACTORS PLAY KEY REGULATORY ROLES IN ANIMALS, YEAST AND PLANTS.

Biotic and industrial activities result in the environmental accumulation of organic toxins. Cellular protection against such xenobiotic stress agents is afforded by increasing the rate of transcription of nuclear detoxification genes through changes in the activity of basic/leucine-zipper (bZIP) transcription factors. Research in my laboratory is aimed at understanding how trans-activation by a tobacco bZIP transcription factor, termed TGA1a, is augmented by xenobiotic stress. We have found that xenobiotic stress potentiates the activation potential and DNA-binding activity of this factor. Our studies implicate a TGA1a-binding nuclear protein, with characteristics of a xenobiotic stress-reversible co-repressor, in the regulation of these activities. Results of these studies have led to the design of modified TGA1a factors with unique transcriptional activities. Because TGA1a is implicated in the regulation of plant detoxification and defense genes, studies with these modified transcription factors will be important for advancing basic knowledge of cellular defense processes, and for developing effective genetic strategies to manipulate natural protective genes of plants. Because xenobiotic toxins accumulate in crop plants and retain significant latent activity, these studies may also have important implications for human health.

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