IN VITRO

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CELLULAR & DEVELOPMENTAL BIOLOGY

2006 In Vitro Biology Meeting Minneapolis

Minnesota, June 3 - 6, 2006

Journal of the Society for In Vitro Biology

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Program Schedule

Sunday, June 4

Monday, June 5

Tuesday, June 6

Wednesday, June 7

Abstracts

SIVB Program Committee

Program Chair:
Mark C. Jordan, Agriculture and Agri-Food Canada

Program Co-Chairs:
Guido Caputo, Canadian Forest Service
John W. Harbell, Mary Kay Inc.

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Ron Phillips, University of Minnesota
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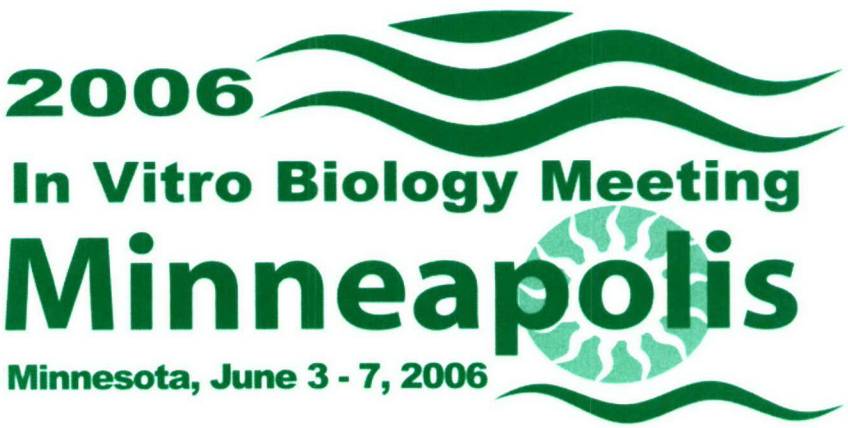
Meeting Secretariat
Marietta Wheaton Ellis, Society for In Vitro Biology



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In Vitro Cellular & Developmental Biology — Plant

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Aims and scope

In Vitro Cellular & Developmental Biology– Plant publishes peer-reviewed original research and reviews concerned with the latest developments and state-of-the-art research in plant cell and tissue culture and biotechnology from around the globe. Four issues cover cellular, molecular and developmental biology research using *in vitro* grown or maintained organs, tissues or cells derived from plants. Two special IAPTC&B issues deal with plant tissue culture, and molecular and cellular aspects of plant biotechnology. The IAPTC&B and SIVB maintain completely separate and independent International Editorial Review boards for their issues.

Topics covered by the Journal include:

- biotechnology/genetic transformation
- developmental biology/morphogenesis
- micropropagation
- functional genomics
- molecular farming
- metabolic engineering
- plant physiology
- cell biology
- somatic cell genetics
- secondary metabolism

2006 In Vitro Biology Meeting
Program-at-a-Glance

Time	Saturday, June 3	Location	Time	Sunday, June 4	Location	Time
Morning **7:00 am - 8:00 am**			**Morning** **7:00 am - 8:00 am**	**7:00 am - 8:00 am** Plant Biotechnology Program Planning Breakfast	Salon A	**Morning** **7:00 am - 8:00 am**
				Publications Committee Meeting	Directors Row 2	
8:00 am - 12:00 pm	**8:00 am - 3:00 pm** SIVB Board of Directors Meeting	Board Room 3	**8:00 am - 10:00 am**	**8:00 am - 10:00 am** Molecular Nutrition	Salon C	**8:00 am - 10:00 am**
			10:00 am - 10:30 am	**Coffee Break**	Salon D,E,F,G	**10:00 am - 10:30 am**
Afternoon **12:00 pm - 1:30 pm**		Salon D,E,F,G	**10:30 am - 12:30 pm**	**10:30 am - 12:30 pm** Cell-based Bioassays as Essential Tools in Drug Development	Duluth	**10:30 am - 12:30 pm**
				Functional Genomics	Salon C	
				Genomic Approaches to Host-pathogen Interactions	Salon A	
			12:30 pm - 1:30 pm	**12:30 pm - 1:30 pm** Exhibitors/SIVB Refreshment Break	Salon D, E, F, G	**12:30 pm - 1:30 pm**
				Lunch on your own		
				Membership Committee Meeting	Directors Row 1	
				In Vitro - Plant Editorial Board Meeting	Directors Row 2	
Afternoon **1:30 pm - 5:00 pm**	**3:00 pm - 6:00 pm** Poster Set-up **4:00 pm - 5:00 pm** 2006 Program Planning Committee Meeting	Board Room 3	**Afternoon** **1:30 pm - 6:00 pm**	**1:30 pm - 3:00 pm** Plant Biotechnology After the Farmers' Fields - Testing, Tracking and Economics	Salon B	**Afternoon** **1:30 pm - 6:00 pm**
				Plant Transformation Contributed Paper Session	Salon A	
				Recent Advances in Invertebrate Immunity Using In Vitro Techniques	Duluth	
				3:00 pm -5:30 pm Opening Ceremony *Announcement of Fellow Award Winners* *Distinguished Service Award Presentations* *Lifetime Achievement Award Presentations*	Salon C	
				Keynote Symposium *"Ever-expanding Horizons"*		
Evening **5:00 pm - 10:00 pm**	**5:00 pm - 7:00 pm** Invertebrate Section Business Meeting and Social	Off Property	**Evening** **6:00 pm - 10:00 pm**	**5:30 pm - 7:30 pm** Opening Ceremony Reception	Salon D,E,F,G	**Evening** **6:00 pm - 11:00 pm**
	7:00 pm - 9:00 pm Opening Reception	Salon D,E,F,G		**6:30 pm - 7:30 pm** Poster Session Odd Poster Authors in Attendance	Salon D,E,F,G	
	City of Lakes Silent Auction Begins	Salon D,E,F,G				
	7:30 pm - 8:30 pm Poster Session Even Poster Authors in Attendance	Salon D,E,F,G				

Registration 7:00 am - 7:00 pm Red Wing Room

Registration 7:00 am - 6:00 pm Red Wing Room
Exhibits and Posters 10:00 am - 2:00 pm Salon D,E,F,G

Registration 7:00 am - 6:30 pm Red Wing Room
Exhibits and Posters 10:00 am - 3:30 pm Salon D,E,F,G

Monday, June 5	Location	Time
7:00 am - 8:00 am Education Committee Meeting	Board Room 3	**Morning** **7:00 am - 8:00 am**
SIVB/IAPTC&B/Springer Meeting	Board Room 1	
8:00 am - 10:00 am Bioreactors: Application of Cultured Eukaryotic Cells for Biopharmaceutical Production	Salon C	**8:00 am - 10:00 am**
Coffee Break Nominating Committee Meeting	Salon D,E,F,G Board Room 3	**10:00 am - 10:30 am**
10:30 am - 12:30 pm 3D Cell Constructs for Tissue Engineering	Duluth	**10:30 am - 12:30 pm**
Global Commercial Micropropagation: Challenges and Opportunities	Salon B	
Plant-made Pharmaceuticals	Salon A	
12:30 pm - 1:30 pm Lunch on your own		**12:30 pm - 1:30 pm**
Long Range Planning Committee Meeting Luncheon	Directors Row 1	
1:30 pm - 2:30 pm Poster Session Even Poster Authors in Attendance	Salon D,E,F,G	**Afternoon** **1:30 pm - 6:00 pm**
2:30 pm - 3:30 pm Animal Cell Sciences Contributed Paper Session	Duluth	
Interactive Poster Sessions: Embryogenesis/Regeneration/ Micropropagation Invertebrate Secondary Products and Biotechnology	Salon D,E,F,G	
3:30 pm - 5:00 pm Bioinformatics Tools for Biotechnologists	Salon C	
Conserving Endangered Orchids: An In Vitro Perspective	Salon A	
Stem Cells	Duluth	
5:00 pm - 6:00 pm Nutritional Requirements of Mammalian Cells in Culture: Design and Optimization of the Cell Culture Medium and Processes	Duluth	
Student Professional Development	Salon A	
6:00 pm - 7:30 pm Plant Biotechnology Business Meeting	Salon A	**Evening** **6:00 pm - 10:00 pm**
6:00 pm - 8:00 pm Animal Cell Sciences Business Meeting	Duluth	
7:00 pm - 10:00 pm Plant Biotechnology Social	Salon B	
8:00 pm - 10:00 pm Animal Cell Sciences Social	Duluth	

Registration 7:00 am - 5:00 pm Red Wing Room
Exhibits and Posters 10:00 am - 3:30 pm Salon D,E,F,G

Tuesday, June 6	Location	Time
7:00 am - 8:00 am Student Affairs Breakfast	Directors Row 1	**Morning** **7:00 am - 8:00 am**
Development Committee Meeting	Board Room 3	
8:00 am - 10:00 am Controlling Transgene Delivery and Integration	Salon C	**8:00 am - 10:00 am**
Coffee Break Constitution and Bylaws Committee Meeting	Salon D,E,F,G Directors Row 1	**10:00 am - 10:30 am**
10:30 am Closing of the Silent Auction	Salon D,E,F,G	
10:30 am - 12:30 pm Alternative Crops/Uses	Salon C	
Commercialization of Transgenic Crops	Salon A	
Cryopreservation	Duluth	
12:30 pm - 1:30 pm Announcement of Silent Auction Winners	Red Wing Room	**12:30 pm - 1:30 pm**
Lunch on your own		
2007 Program Planning Committee Meeting	Directors Row 1	
1:30 pm - 2:30 pm Poster Presentations Odd Poster Authors in Attendance	Salon D,E,F,G	**Afternoon** **1:30 pm - 6:00 pm**
2:30 pm - 3:30 pm **Interactive Poster Sessions:** Animal Cell Sciences In Vitro Tools, Techniques and Optimization Plant Transformation	Salon D,E,F,G	
3:15 pm - 5:00 pm Plant Biotechnology Contributed Paper Session	Salon C	
3:30 pm - 5:00 pm Linking Species for Genome Analysis	Salon A	
Three-dimensional Tissue Models of Cancer Cell Invasion and Metastasis	Duluth	
5:00 pm - 5:45 pm SIVB Business Meeting *Student Award Presentations*	Rochester	
6:00 pm - 10:00 pm **An Evening at the Mill City Museum**	Mill City Museum	

Registration 7:00 am - 12:00 pm Red Wing Room

Wednesday, June 7	Location
8:00 am - 10:05 am Advances in Plant Transformation	Salon C
Embryogenesis/Regeneration/ Micropropagation Contributed Paper Session	Duluth
Coffee Break	Ballroom Foyer
10:30 am - 12:30 pm **Distinguished Plant Symposium:** *"Agrobacterium Transformation: Past, Present, and Future"*	Salon C
12:30 pm - 1:30 pm Lunch on your own	
2:00 pm - 5:00 pm Tour of the Center for Microbial and Plant Genomics	Off Property

2006 In Vitro Biology Meeting

Tentative Schedule of Functions

TIME	TYPE OF FUNCTION	ROOM
FRIDAY, JUNE 2		
6:00 pm – 8:00 pm	SIVB Current and Incoming Board of Directors Reception	President's Suite
SATURDAY, JUNE 3		
7:00 am – 7:00 pm	**Registration**	Red Wing Room
8:00 am – 3:00 pm	SIVB Board of Directors Meeting	Board Room 3
3:00 pm – 6:00 pm	Poster Set-up	Salon D, E, F, G
4:00 pm – 5:00 pm	2006 Program Planning Committee Meeting	Board Room 3
5:00 pm – 7:00 pm	Invertebrate Business Meeting and Social	Off Property
7:00 pm – 9:00 pm	Opening Reception	Salon D, E, F, G
7:00 pm – 9:00 pm	City of Lakes Silent Auction Kickoff	Salon D, E, F, G
SUNDAY, JUNE 4		
7:00 am – 6:00 pm	Registration	Red Wing Room
7:00 am – 8:00 am	Publications Committee Meeting	Directors Row 2
7:00 am – 8:00 am	Plant Biotechnology Program Committee Meeting	Salon A
10:00 am – 10:30 am	Coffee Break	Salon D, E, F, G
10:00 am – 2:00 pm	**Exhibits and Posters**	Salon D, E, F, G
12:30 pm – 1:30 pm	Exhibitors/SIVB Refreshments Break	Salon D, E, F, G
12:30 pm – 1:30 pm	Membership Committee Meeting	Directors Row 1
12:30 pm – 1:30 pm	In Vitro – Plant Editorial Board Meeting	Directors Row 2
5:30 pm – 7:30 pm	Opening Ceremony Reception	Salon D, E, F, G
MONDAY, JUNE 5		
7:00 am – 6:30 pm	Registration	Red Wing Room
7:00 am – 8:00 am	Education Committee Meeting	Board Room 3
7:00 am – 8:00 am	SIVB/IAPTC&B/Springer Business Meeting	Board Room 1
10:00 am – 3:30 pm	**Exhibits and Posters**	Salon D, E, F, G
10:00 am – 10:30 am	Coffee Break	Salon D, E, F, G
10:00 am – 10:30 am	Nominating Committee Meeting	Board Room 3
12:30 pm – 1:30 pm	Long Range Planning Committee Meeting	Director's Row 1
12:30 pm – 1:30 pm	Plant Cell Reports Editors Meeting	Board Room 3
6:00 pm – 7:30 pm	Plant Biotechnology Section Business Meeting	Salon A
6:00 pm – 8:00 pm	Animal Cell Sciences Section Business Meeting	Duluth
7:30 pm – 10:00 pm	Plant Biotechnology Section Social	Salon B
8:00 pm – 10:00 pm	Anima! Cell Sciences Section Social	Duluth
TUESDAY, JUNE 6		
7:00 am – 5:00 pm	Registration	Red Wing Room
7:00 am – 8:00 am	Student Affairs Breakfast	Directors Row 1
7:00 am – 8:00 am	Development Committee Meeting	Board Room 3
10:00 am – 3:30 pm	**Exhibits and Posters**	Salon D, E, F, G
10:00 am – 10:30 am	Coffee Break	Salon D, E, F, G
10:00 am – 10:30 am	Constitution and Bylaws Committee Meeting	Directors Row 1
10:30 am	Closing of the City of Lakes Silent Auction	Salon D, E, F, G
12:30 pm	Posting of the City of Lakes Silent Auction Winners	Red Wing Room
12:30 pm – 1:30 pm	2007 Program Planning Committee Meeting	Directors Row 1
3:30 pm – 5:00 pm	Poster Breakdown and Removal	Salon D, E, F, G
5:00 pm – 5:45 pm	SIVB Business Meeting	Rochester
6:00 pm – 10:00 pm	Evening at the Mill City Museum	Mill City Museum
WEDNESDAY, JUNE 7		
7:00 am – 12:00 pm	Registration	Red Wing Room
10:00 am – 10:30 am	Coffee Break	Ballroom Foyer
2:00 pm – 5:00 pm	Afternoon Tour of the Center for Microbial and Plant Genomics	Cargill Building Center

Note: Additions and changes to functions will be posted on a bulletin board located in the registration area. Please check the bulletin board daily.

SATURDAY, JUNE 3

7:00 am – 7:00 pm	Registration	Red Wing Room
8:00 am – 3:00 pm	SIVB BOARD OF DIRECTORS MEETING	Board Room 3
4:00 am – 5:00 pm	2006 PROGRAM PLANNING COMMITTEE MEETING	Board Room 3
7:00 pm – 9:00 pm	**2006 IN VITRO BIOLOGY MEETING OPENING RECEPTION**	Salon D, E, G, F
7:00 pm – 9:00 pm	**CITY OF LAKES SILENT AUCTION KICKOFF**	Salon D, E, G, F

Saturday, June 3
Even Poster Authors will be present
7:30 pm – 8:30 pm
(See list of posters on pages 26-A to 49-A)

SUNDAY, JUNE 4

7:00 am – 6:00 pm | Registration | Red Wing Room

MOLECULAR NUTRITION

Conveners: Jeffrey W. Adelberg, Clemson University, and Linda B. Jacobsen, Roche Applied Science

8:00 am – 10:00 am | Plenary Symposium | Salon C

(See abstract page 2-A)

All life started in the sea and we still have saline solutions bathing all vital tissues. The concentration and organic complexation of nutrients confers specificity for their physiological roles. Higher plants are non-motile and meet environmental challenges with altered enzyme responses. Nutritional and defense compounds from plants can have similar functions in regulating heterologous systems found in plants and animals. In vitro systems are a powerful tool to ask vital questions on how chemicals become nutrients, and how nutrients affect the target organism. It is known that animal cells directly or indirectly concentrate their nutrients from plants and require more concentrated nutrients than plant cells. Thus key to understanding the total picture is the identification of chemicals/nutrients in culture media that are required by animal cells to reproduce and function.

8:00 Introduction (J. Adelberg and L. Jacobsen)

8:05 PS-1 Advances in Molecular Nutrition: Phytochemicals and Antioxidant Response Pathways as Critical Control Points for Chemoprevention of Disease
Kalidas Shetty, University of Massachusetts

9:00 PS-2 Genomic and Proteomic Approaches to Understanding and Manipulating Nutritional Requirements in Mammalian Cell Culture Processes
Laurel Marie Donahue, *SAFC Biosciences*

10:00 am – 10:30 am | Coffee Break | Salon D, E, G, F

CELL-BASED BIOASSAYS AS ESSENTIAL TOOLS IN DRUG DEVELOPMENT

Convener: John W. Harbell, Mary Kay Inc.

10:30 am – 12:00 pm | Animal Symposium | Duluth

(See abstract page 5-A)

Cell-based bioassays have become a mainstay in drug discovery and development. These assays range from simple screens for basic toxicity or efficacy to systems involving multiple cell types and a complex array of end points. Even the most "simple" of these assays involves a series of independent variables that must be defined and controlled in the study design. Factors such as the selection of the target cells, cell number, duration of exposure, and endpoints will impact the resulting data and predictive capacity of the test. An understanding of the design and validation processes that have gone into successful bioassays is key to the design and validation of future assays. This symposium will present examples of three successful and large scale bioassay programs.

10:30 Introduction (J. Harbell)
10:35 A-1 The NCI 60 Tumor Cell Line Screen: An Information-rich Screen Informing on Mechanisms of Toxicity
Robert H. Shoemaker, *National Cancer Institute*
11:00 A-2 High-fidelity In Vitro Modeling of Clinically-defined, Chemically-induced Organ Toxicity in Man Using Adverse Effects of Oncology Drugs as Ethical Learning Sets
Ralph E. Parchment, *NCI-Frederick Cancer Research Facility*
11:25 A-3 Assessing Hepatotoxicity Through Multiple Endpoints
James M. McKim, *CeeTox, Inc.*

FUNCTIONAL GENOMICS

Conveners: Baochun Li, University of Kentucky, and Allan R. Wenck, BASF Plant Sciences

10:30 am – 12:30 pm Plant Symposium Salon C
(See abstract page 10-A)

Studying the function of plant genes and developing new tools for functional genomics studies are of fundamental interest and advances in this field are being made at a very dramatic pace. The speakers from this session will update their research on the following areas: molecular mechanisms that govern plant development and floral induction; molecular mechanisms that underlie plant responses to harsh environment such as soil salinity, drought and cold temperatures; small RNA pathways in plants; and a highly efficient *Agrobacterium-tumefaciens*-mediated transient gene expression system using TMV-based binary vectors.

10:30 Introduction (B. Li and A. Wenck)
10:35 P-1 Remembering Winter: Vernalization as an Environmentally Induced Epigenetic Switch
Richard M. Amasino, *University of Wisconsin*
11:00 P-2 Role of miRNAs and siRNAs in Abiotic Stress Repsonses
Jian-Kang Zhu, *University of California*
11:25 P-3 Diverse Small RNA-directed Pathways in Plants
Zhixin Xie, *Texas Tech University*
11:50 P-4 High Throughput Gene Assembly and Expression Using Viral RNA Replicons Delivered by *Agrobacterium*
Yuri Gleba*, Icon Genetics*
12:15 Discussion

GENOMIC APPROACHES TO HOST-PATHOGEN INTERACTIONS

Convener: Gary Muehlbauer, University of Minnesota

10:30 am – 12:30 pm Plant Symposium Salon A
(See abstract page 11-A)

Plant pathogens cause significant economic damage to the agricultural production worldwide by direct reduction of yields and losses of market and export. How do plant pathogens infect host plants and establish disease interactions with host plants? What are the host responses and gene expression networks induced during infection? The science of genomics is leading to an advanced understanding of how genes function in a coordinate fashion that results in various organismal characteristics or phenotypes. Functional, structural, and comparative genome analysis of plant pathogens and their hosts will provide insights to understand the questions. These understandings are essential to the development of effective and environmentally sound strategies for disease control. This symposium will provide an update on host-pathogen interactions.

10:30 Introduction (G. Muehlbauer)
10:35 P-5 Signaling Networks Controlling Disease Resistance Responses in *Arabidopsis*
Jane Glazebrook, *University of Minnesota*
11:00 P-6 Profiles in Scourge: Gene Expression Analysis of a Crop Killer
H. Corby Kistler, *USDA-ARS*
11:25 P-7 *In Planta* Transcriptional and Functional Patterns of an Agriculturally Relevant R Gene
James M. Bradeen, *University of Minnesota*
11:50 P-8 Use of a High-performance, Custom Microarray for Elucidation of Signaling Networks Controlling Plant Defense Responses
Fumiaki Katagiri, *University of Minnesota*
12:15 Discussion

PLANT BIOTECHNOLOGY AFTER THE FARMERS' FIELDS – TESTING, TRACKING, AND ECONOMICS

Convener: Raymond D. Shillito, Bayer CropScience

1:30 pm – 3:00 pm Plant Symposium Salon B
(See abstract page 12-A)

Once a transgenic has been commercialized, many economic issues affect its acceptability and adoption. Plant varieties must meet performance requirements - so tests are needed for ensuring purity. Once the crop leaves the farm gate it may be segregated for use in certain markets. Thus testing is needed in order to satisfy the needs of those involved in trade of grain and foodstuffs to comply with regulatory and labeling requirements. The segregation and testing of grain and food and feed for the presence or absence of transgenic crop products can have major economic impacts. This session will describe the types of tests available for detecting and quantifying transgenic seeds and grain, describe how these are applied in the grain industry, and look at the economic issues raised by these testing regimes.

1:30 Introduction (R. Shillito)
1:35 P-9 Testing Methods for DNA and Proteins in Transgenic Crops
Raymond D. Shillito, *Bayer CropScience*
2:00 P-10 Applications of Testing Methods in the Grain Industry
Randal Giroux, *Cargill. Inc.*
2:25 P-11 A Global Perspective on the Economic Impact of Transgenic Crop Varieties
Greg Traxler, *Auburn University*

PLANT TRANSFORMATION

Moderator: Dennis J. Gray, University of Florida

1:30 pm – 3:00 pm Plant Contributed Paper Session Salon A
(See abstract page 20-A to 21-A and 34-A)

1:30 P-1000 RNAi-mediated Silencing of Maize Chromatin Genes Confer Increased Transformation Efficiency in Maize
Mary Ann McGill, *University of Wisconsin-Madison, S. M. Kaeppler, and H. F. Kaeppler*

1:45 P-1001 Stable Transformation of *Taxus*
Helena Mathews, *Exelixis Plant Sciences, Nikolaus Matheis, Fira Negru, Karin Connors, Vaka Reddy, Al Lammers, Debra Schuster, Mylavarapu Venkatramesh, and D. Ry Wagner*

2:00 P-1002 A Novel Shoot Organogenesis and Transformation System for *Nicotiana obtusifolia* Accession PI 555573
Baochun Li*, University of Kentucky, Qingwei Huang, and Hui Qiu*

2:15 P-1003 Selecting Disease Resistant Transgenic Grapevine for Field Tests
Dennis J. Gray, *University of Florida, Z. T. Li, S. A. Dhekney, M. Dutt, M. Van Aman, J. Tattersall, and K. T. Kelley*

2:30 P-1004 The Immunodominant Allergan Ara h2 is Silenced in Transgenic Peanut Via the RNA Interference (RNAi) Strategy
Koffi N. Konan, *Alabama A&M University, O. M. Viquez, and H. W. Dodo*

2:45 P-1005 Antibiotic Marker Free Approach for Obtaining Salt Stress Tolerant *Vigna mungo* (Blackgram)
Neera Bhalla Sarin, *Jawaharlal Nehru University, P. Bhomkar, C. P. Upadhyay, S. DebRoy, R. Rajwanshi, A. Muthusamy, M. Saxena, N. Shiva Prakash, M. Pooggin, and T. Hohn*

RECENT ADVANCES IN INVERTEBRATE IMMUNITY USING IN VITRO TECHNIQUES

Conveners: Cynthia L. Goodman, USDA/ARS/BCIRL, and Guy Smagghe, Ghent University

1:30 pm – 3:00 pm Animal Symposium Duluth
(See abstract page 5-A to 6-A)

Understanding and manipulating insect immunity has important implications for the control of agriculturally and medically important insects, including lepidopterans that destroy crops as well as dipterans that vector human and animal diseases. The innate immune system in insects is quite complex, with both humoral and cellular components. By using cell and tissue culture techniques, these components can be isolated and their mechanisms and specific effects more directly studied (Fallon and Sun 2001, Insect Biochem. Molec. Biol. 31:263). One aspect of immunity that can be readily investigated using in vitro methods is the identification of the signaling pathways involved in controlling the various immune responses. Eicosanoids (Stanley 2006, Annu. Rev. Entomol. 51: 25-44) and insect hormones (Franssens et al., Dev. Comp. Immunol., submitted) play important roles in this arena. To summarize, this symposium will discuss a number of important topics related to insect immunity and the in vitro methodologies used to dissect them, with potential field applications of these studies also being noted.

1:30 Introduction (C. Goodman and G. Smagghe)

1:35 A-4 Eicosanoids in Invertebrate Immunity: An In Vitro Approach
David Stanley, *USDA/ARS/BCIRL*

2:00 A-5 Experimental Approaches to the Evaluation of Immune Functions in Mosquito Cell Lines
Ann Fallon, *University of Minnesota*

2:25 A-6 Determination of the Effects of Ecdysteroids and JH on Nodulation Responses
Vanessa Franssens, *University of Leuven*

Sunday, June 4

2006 IN VITRO BIOLOGY OPENING CEREMONY

Program Chair: Mark C. Jordan, Agriculture and Agri-Food Canada
Program Co-Chairs: Guido F. Caputo, Canadian Forest Service
John W. Harbell, Mary Kay Inc.

3:00 pm – 5:30 pm — Opening Ceremony — Salon C

Conveners: David W. Altman, IPR Consulting, Inc
Mark C. Jordan, Agriculture and Agri-Food Canada
Paul J. Price, Invitrogen

3:00 Welcome and Opening Remarks:
David W. Altman, SIVB Past President, Society for In Vitro Biology
Paul J. Price, President, Society for In Vitro Biology

3:10 2006 Fellow Award Recipients *(Awards to be presented at Section Meetings)*:
Guido F. Caputo, *Canadian Forest Service*
Dennis A. Laska, *Eli Lilly & Company*
Shirley A. Pomponi, *Harbor Branch Oceanographic Institution*
Nancy A. Reichert, *Mississippi State University*
Amy A. Wang, *GlaxoSmithKline*

3:20 2006 Distinguished Service Award Presentations:
Delia R. Bethell, *Ventria Bioscience*
Nancy A. Reichert, *Mississippi State University*
David D. Songstad, *Monsanto Company*

3:30 2006 Lifetime Achievement Award Recipients:
Bob V. Conger, *University of Tennessee (Introduction presented by David D. Songstad)*
Wei-Shou Hu, *University of Minnesota (Introduction presented by David W. Jayme)*

4:00 Keynote Symposium

Introduction (D. W. Altman and M. C. Jordan)
KS-1 Ever-expanding Horizons
Ronald L. Phillips, *University of Minnesota*
(See abstract page 1-A)

5:30 pm – 7:30 pm — **2006 IN VITRO BIOLOGY MEETING OPENING CEREMONY RECEPTION** — Salon D, E, G, F

Sunday, June 4

5:30 pm – 7:30 pm	Exhibits and Posters	Salon D, E, G, F

Sunday, June 4
Odd Poster Authors will be present
6:30 pm – 7:30 pm
(See list of posters on pages 26-A to 49-A)

MONDAY, JUNE 5

7:00 am – 6:30 pm Registration Red Wing Room

BIOREACTORS: APPLICATION OF CULTURED EUKARYOTIC CELLS FOR BIOPHARMACEUTICAL PRODUCTION

Conveners: Michael E. Horn, Phyton Biotech, Inc., and David Jayme, Brigham Young University – Hawaii

8:00 am – 10:00 am Plenary Symposium Salon C

(See abstract page 2-A to 3-A)

There often exist multiple options for the production of biopharmaceuticals, many of them involving eukaryotic cell culture processes. Process development requires comparative analysis of these options and appreciation of their relative strengths and limitations to project the superior route for bioproduction. This plenary session invites speakers experienced with the production of biological compounds in vertebrate, invertebrate and plant cell-based cultures. Using case studies from their personal experiences, they will explore the utility of their respective culture systems to achieve bioproduction objectives.

8:00 Introduction (M. E. Horn and D. Jayme)
8:05 PS-3 Animal Cell Culture: The Future Beyond Bioreactors and Metabolic Engineering
Wei-Shou Hu, *University of Minnesota*
8:40 PS-4 Baculovirus Recombinant Protein Production in Insect and Mammalian Cells
Thomas A. Kost, *GlaxoSmithKline*
9:15 PS-5 Commercial Production of Paclitaxel by Plant Cell Culture
Venkatesh Srinivasan, *Phyton Biotech, Inc.*
9:50 Discussion

10:00 am – 10:30 am Coffee Break Salon D, E, G, F

3D CELL CONSTRUCTS FOR TISSUE ENGINEERING

Convener: Robert Tranquillo, University of Minnesota

10:30 am – 12:00 pm Animal Symposium Duluth

(See abstract page 6-A to 7-A)

The success of tissue engineering hinges on an appropriate combination of cells, polymer scaffolds, and culture conditions, often involving controlled mechanical and chemical signals. This session will provide state-of-art studies among a range of key applications that explore these critical factors.

10:30 Introduction (R. Tranquillo)
10:35 A-7 Multipotent Adult Progenitor Cells for Vascular Repair
Catherine M. Verfaillie, *University of Minnesota*
11:00 A-8 Cell Sourcing for Fibrin-based Heart Valve -equivalents
Chrysanthi Williams, *Bose Corporation*

11:25 A-9 Engineering Large, Mineralized Bone Tissue Constructs Using Human Mesenchymal Stem Cells
Gordana V. Vunjak-Novakovic, *Columbia University*

GLOBAL COMMERCIAL MICROPROPAGATION: CHALLENGES AND OPPORTUNITIES

Conveners: Michael E. Kane, University of Florida, Michael Becwar, ArborGen LLC, and Jeffrey W. Adelberg, Clemson University

10:30 am – 12:30 pm Plant Symposium Salon B
(See abstract page 12-A to 13-A)

Micropropagation, the application of tissue culture for efficient clonal plant production, has been used commercially since the 1960's and is possibly the oldest example of commercial biotechnology. In the United States, the micropropagation industry has developed primarily to service the temperate and tropical ornamental plant industry. Production of elite stock plants for small fruit and vegetable crops is a secondary area of activity. Clonal propagation of coniferous forest trees via somatic embryogenesis is being pursued for large-scale production in the US. Manufactured seed and automation technologies in combination offer a cost effective method of producing, selecting and deploying somatic embryos into conifer nurseries. Tropical plantation crops, outside this country have been commercially micropropagated for many years. Recently, bioreactor technology has been implemented for efficient large-scale propagation of several fruit and timber crops. Our speakers will provide differing perspectives on the critical challenges and technology developments that impact this industry.

10:30 Introduction (M. E. Kane, M. Becwar, and J. W. Adelberg)
10:35 P-12 Current Status and Impact of Commercial Plant Tissue Culture
Steve McCulloch, *Mountain Shadow Nursery*
11:00 P-13 Status of Commercial Tropical Foliage Plant Micropropagation
Gary Hennen, *Oglesby Plants International, Inc.*
11:25 P-14 Temporary Immersion Bioreactor: An Efficient Technology for Scaling-up Plant Production
Maritza Escalona Morgado, *University of Ciego de Avila*
11:50 P-15 Manufactured Seed - An Efficient Method for Delivery of Somatic Embryos to Nurseries
William C. Carlson, *Weyerhaeuser*
12:15 Discussion

PLANT-MADE PHARMACEUTICALS

Convener: Delia R. Bethell, Ventria Bioscience

10:30 am – 12:30 pm Plant Symposium Salon A
(See abstract page 13-A to 14-A)

The production of protein therapies using plants as the expression system is gaining steady acceptance. The technology is moving beyond the theoretical and the lab bench and into the clinic. This session will explore the progress being made in the clinical application of plant made proteins and pharmaceuticals.

10:30 Introduction (D. Bethell)
10:35 P-16 Therapeutic Protein Expression in the Plant-based LEX System™
Vincent Wingate, *Biolex*

11:10 P-17 Production of Biodefense-related Proteins in Tobacco
Keith Wycoff, *Planet Biotechnology, Inc.*
11:45 P-18 Transgenic Expression and Recovery of Biologically Active Recombinant Human Insulin from *Arabidopsis thaliana* Oilseeds
Elizabeth W. Murray, *SemBioSys Genetics, Inc.*
12:20 Discussion

Monday, June 5
Even Poster Authors will be present
1:30 pm – 2:30 pm
(See list of posters on pages 26-A to 49-A)

ANIMAL CELL SCIENCES

Moderator: Eugene Elmore, University of California – Irvine

2:30 pm – 3:30 pm Animal Contributed Paper Session Duluth
(See abstract page 19-A)

2:30 A-1000 Response of Rosiglitazone, UAB 30, and Atorvastatin in the Human Melanoma Prevention Assay
Eugene Elmore, *University of California – Irvine, A. Jain, L. Kopelovich, F. L. Meyskens, V. E. Steele, and J. L. Redpath*
2:45 A-1001 Lineage Specificity and Interspecies Variation in Hematopoietic Toxicity Testing
Cindy Miller, *StemCell Technologies, Inc., Carla Pereira, Jackie Damen, and Emer Clarke*
3:00 A-1002 Computer-aided Tissue Engineering: Predicting Self-assembly of Prostate Cancer Spheroids
Kim C. O'Connor, Tulane University, H. Song, and S. Clejan
3:15 A-1003 Characterization of Neuroblastoma Cells Cultured in Three-dimensional, Microgravity Rotary Bioreactor: Organoid Formation and Free Cell Dynamics
Robert Asbury Redden, *The Children's Hospital of Philadelphia, and E. J. Doolin*

EMBRYOGENESIS / REGENERATION / MICROPROPAGATION

Moderator: Vicki Magnusson, North Dakota State University

2:30 pm – 3:30 pm Plant Interactive Poster Session Salon D, E, G, F
(See abstract page 37-A to 38-A)

P-2012 In Vitro Propagation of Silver Maple
Vicki Magnusson, *North Dakota State University, and Wenhao Dai*

P-2013 In Vitro Specificity Exhibited by Fungal Mycobionts of *Spiranthes floridana* (Orchidaceae) within the Congener *Spiranthes brevilabris*
Scott L. Stewart, *University of Florida, and M. E. Kane*

P-2014 Optimization of Regeneration in *Pongamia pinnata* (L.) Pierre - a Potential Biodiesel Plant Using Taguchi Approach
B. Srinivas, *Sreenidhi Institute of Science and Technology (SNIST), and S. Ramgopal Rao*

P-2015 Callus Establishment and Shoot Proliferation in *Jatropha curcas*: A Biodiesel Plant Through Nodal Explant Culture
Shailendra Kumar Tiwari, *Plant Propagation & Biotechnology Division State Forest Research Instituite, P. K. Shukla, Amit Pandey, and M. P. Goswami*

P-2016 Recovery from Gross Apical Damage in Dicotyledonous Seedlings
Victor Gaba, *ARO Volcani Center, L. Jashi, S. Amutha, K. Kathiravan, S. Singer, I. Shomer, and B. Steinitz*

P-2017 In Vitro Propagation of Rare and Disappear Plants
Magfrat P. Muminova, *Institute of Genetics & PEBAS of Uzbekistan, O. I. Isaeva, I. N. Grigina, and S. S. Khamrakulov*

INVERTEBRATE

Moderator: Guido Caputo, Canadian Forest Service

2:30 pm – 3:30 pm Invertebrate Interactive Poster Session Salon D, E, G, F
(See abstract page 32-A to 33-A)

I-2000 Eicosanoids Influence Insect Cell-viral Interactions
Cynthia L. Goodman, *USDA/ARS/ BCIRL, A. McIntosh, and D. Stanley*

I-2001 Stable Transformation of a Tick (*Ixodes scapularis*) Cell Line with the Sleeping Beauty Transposon System
Timothy J. Kurtti, *University of Minnesota, R. F. Felsheim, J. T. Mattila, G. D. Baldridge, N. Y. Burkhardt, and U. G. Munderloh*

I-2002 RNAi-mediated Silencing of a DsRed2-expressing *Ixodes Scapularis* (Acari: Ixodidae) Cell Line
Joshua T. Mattila, *University of Minnesota, G. D. Baldridge, R. F. Felsheim, N. Y. Burkhardt, U. G. Munderloh, and T. J. Kurtti*

I-2003 Electroporation of *Crithidia ricardoi* with pNUS-GFPH
Moses A. McDaniel, *Elizabeth City State University, Gieira Jones, Margaret M.Young, Gary L. Harmon, and Ronald H. Blackmon*

I-2004 Functional Analysis of Nictaba in Insect Midgut Cells
Guy Smagghe, *Lab Agrozoology, Ghent University, G. Vandenborre, T. Soin, L. Jacobsen, G. Caputo, and E. J. M. Van Damme*

I-2005 Validation Analysis of an Ecdysteroid Receptor Agonist Assay Using Intact Cultured Lepidoptera Cells
Guy Smagghe, *Lab Agrozoology, Ghent University, H. Mosallanejad, L. Decombel, C. Goodman, and T. Soin*

I-2006 Biologically Potent Broad Spectrum Antibiotics Obtained from the Tetrodotoxin Rich Organs of Puffer Fishes
***Joey D. Mangadlao**, University of the Philippines*

SECONDARY PRODUCTS AND BIOTECHNOLOGY

Moderator: Mitchell L. Wise, USDA/ARS

2:30 pm – 3:30 pm Plant Interactive Poster Session Salon D, E, G, F
(See abstract page 34-A to 35-A)

P-2000 Elicitation of *Pueraria lobata* (Kudzu) Cell and Root Cultures for Radiolabeling of Isoflavones
***Nelson Adam Reppert**, University of Illinois Urbana-Champaign, G. Yousef, R. B. Rogers, and M. A. Lila*

P-2001 Copper Chloride Elicitation of In Vitro Red Clover Isoflavones
***Nancy Engelmann**, University of Illinois, Urbana, Randy Rogers, Padmapriya Vattem, Jeevan Prasain, and Mary Ann Lila*

P-2002 Biosynthesis of Avenanthramides in Chitin Elicited Oat (*Avena sativa*) Suspension Cultures
***Mitchell L. Wise**, USDA/ARS*

P-2003 Cell-free Expression of the Tobacco Lectin
***Guy Smagghe**, Ghent University, D. Breite, E. Daniel, G. Vandenborre, N. Lannoo, L. Jacobsen, and E. J. M. Van Damme*

P-2004 Rapid Screening of Silent Mutations for Cell-free Production of the Plant *Lectin nictaba*
***Erica Daniel**, Indiana University, D. Breite, L. Jacobsen, E. J. M. Van Damme, and G. Smagghe*

P-2005 Tissue Culture of *Nerium oleander* Possesses Cytotoxic Activity for Human Cell Lines In Vitro
***Nelli A. Hovhannisyan**, Yerevan State University*

BIOINFORMATICS TOOLS FOR BIOTECHNOLOGISTS

Conveners: Peggy J. Ozias-Akins, University of Georgia, and Sylvia Adjoa Mitchell, University of the West Indies

3:30 pm – 5:00 pm Plant Symposium Salon C
(See abstract page 11-A to 12-A)

Bioinformatics combines aspects of the biological sciences with information technology and thereby provides a means to store and mine complex biological data sets. Databases are essential components of bioinformatics and should be carefully designed in order to optimize subsequent mining through queries. This workshop is intended for the novice as an introduction to the design concepts of relational databases, the computational environments and programming skills that are required, and the potential applications to biotechnology.

3:30 Introduction (P. J. Ozias-Akins and S. A. Mitchell)
3:35 TBD
Zheng Jin Tu, *University of Minnesota*

CONSERVING ENDANGERED ORCHIDS: AN IN VITRO PERSPECTIVE

Conveners: Valerie C. Pence, Cincinnati Zoo and Botanical Gardens, and Barbara M. Reed, USDA/ARS

3:30 pm – 5:00 pm Plant Symposium Salon A
(See abstract page 14-A to 15-A)

Conservation efforts to preserve rare and endangered plants involve both in situ preserves and ex situ collections. In vitro culture of rare orchids can provide a secure storage form for these popular flowers as well as providing specimens for population augmentation, reintroduction, and establishment. This symposium will include several perspectives on the use of in vitro culture to preserve and protect rare and endangered orchid species.

3:30 Introduction (V. C. Pence and B. M. Reed)
3:35 P-19 An Overview of the Orchid-fungal Sybiosis in Nature, and Its Application In Vitro to Promote Conservation
Lawrence W. Zettler, *Illinois College*
4:00 P-20 Symbiotic and Asymbiotic Orchid Seed Germination as Tools in Conservation
Scott L. Stewart, *University of Florida*
4:25 P-21 In Vitro Strategies for Conservation of Madagascar's Endemic Orchids
Marge Mary From, *Omaha's Henry Doorly Zoo*
4:50 Discussion

STEM CELLS

Convener: Gordana V. Vunjak-Novakovic, Columbia University

3:30 pm – 5:00 pm Animal Symposium Duluth
(See abstract page 7-A)

Stem cells have the potential for self-renewal and the potential for differentiation into any specific cell lineage. Our ability to utilize this unique potential depends on in vitro methods that can recapitulate some of the conditions present during normal development and thereby regulate stem cell differentiation. Focus of this session is on stem cells, in the context of their application in tissue engineering and regenerative medicine.

3:30 Introduction (G. V. Vunjak-Novakovic)
3:35 A-10 Stem Cell Based Artificial Heart
Doris Taylor, *University of Minnesota*
4:00 A-11 Blood and Endothelial Cell Development from Human Embryonic Stem Cells
Dan S. Kaufman, *University of Minnesota*
4:25 A-12 Muscle Stem Cell: Satellite Cell and Sca-1-positive Cell
Atsushi Asakura, *University of Minnesota Medical School*
4:50 Discussion

NUTRITIONAL REQUIREMENTS OF MAMMALIAN CELLS IN CULTURE: DESIGN AND OPTIMIZATION OF THE CELL CULTURE MEDIUM AND PROCESSES

Convener: Paul J. Price, GIBCO Invitrogen

5:00 pm – 6:00 pm | Animal Symposium | Duluth

(See abstract page 7-A)

A classical cell culture medium is made up of a buffered isotonic salt solution supplemented with vitamins, amino acids, a source of energy such as glucose or glutamine and a protein supplement such as serum. As the science of media formulation progressed from serum supplemented to serum-free and then to chemically-defined, substitutes for the serum component and then for serum derived proteins had to be found. Many segments of Industry are presently moving away from media and reagents containing any component of animal or human origin. The outbreak of BSE and CJV in England heightened the awareness of the risk of contamination of therapeutics by prions or viruses and prompted regulatory pressure for companies to address this potential risk. This teaching seminar will cover the basics of a cell culture medium and the advantages and disadvantages of the serum supplement. It will then progress into the construction of serum-free and chemically defined formulations and then to the elimination of all components of animal origin. Ways to optimize both cell growth and productivity will be presented as well as the design of media for specific applications. Emphasis will be placed on reducing apoptosis by controlling osmolality, ammonia, and free radical production and through optimization of the media formulation.

5:00 A-13 Nutritional Requirements of Mammalian Cells in Culture: Design and Optimization of the Cell Culture Medium and Processes
Paul J. Price, *GIBCO Invitrogen*

STUDENT PROFESSIONAL DEVELOPMENT

Moderator: Mary Ann McGill, University of Wisconsin

5:00 pm – 6:00 pm | Joint Symposium | Salon A

One of the most beneficial aspects of the SIVB meetings is the chance to interact with researchers from academic, industry, and government institutions. As students get closer to graduating, they start to ask themselves questions like "academia or industry?" or "If I go to industry without doing a postdoc, can I come back to an academic institution?" and other questions of that nature. This workshop will feature a discussion panel with representatives from academia, industry, and government research institutions. The session will consist of a student-run question and answer session followed by an interactive Q&A segment with the audience. So, come and learn all the answers to life's big questions (or at least get an idea of what you want to do in your career.)

Panelists:
Michael E. Kane, *University of Florida*
Mary Ann McGill, *University of Wisconsin*
Sylvia Adjoa Mitchell, *University of the West Indies*
Nancy A. Reichert, *Mississippi State University*
Elizabeth J. Roemer, *State University of New York – Stony Brook*
Carol M. Stiff, *Kitchen Culture Kits*

TUESDAY, JUNE 6

7:00 am – 5:00 pm | Registration | Red Wing Room

CONTROLLING TRANSGENE DELIVERY AND INTEGRATION

Conveners: Michael Bosela, Indiana University - Purdue University at Fort Wayne, and Theodore M. Klein, Pioneer Crop Genetics

8:00 am – 10:00 am | Plenary Symposium | Salon C

Processes for delivering transgenes into cells and tissues have advanced dramatically in recent years. However, for most eukaryotes, with the notable exception of yeasts and some specialized animal cell lines (e.g., murine embryonic stem cells, etc.), DNA integration occurs primarily by illegitimate recombination at random locations in the genome. In contrast with homologous recombination, where gene integration occurs at regions of DNA sequence homology, illegitimate recombination is characterized by decreases in transgene fildelity (with truncation and transgene arrangements being relatively common), more complex integration patterns (and associated increases in the frequency of homology dependent gene silencing), and the possibility of collateral damage resulting from integration into gene coding sequences or control elements (promoters, enhancers, etc). In addition, forward genetics and gene therapy (gene repair or conversion) are only possible via homologous recombination. This session focuses on means to more precisely manipulate transgene delivery; with the goal of increasing the degree of experimenter control over the location and type of gene integration. Two approaches are emphasized. The first involves the use of heterologous or synthetic DNA metabolism enzymes (e.g., recombinases, targeted endonucleases, etc) to stimulate site-specific integration and/or homologous recombination. The second approach involves the development of artificial chromosomes, both as a means to circumvent difficulties associated with random integration and as a platform for the delivery of large sets of genes. The speakers will emphasize the applications of the new technologies and their utility across both plant and animals, and model and non-model organisms.

8:00 Introduction (M. Bosela and T. M. Klein)
8:05 PS-6 Cre/lox Mediated Site-specific Gene Integration in Plants
Vibha Srivastava, *University of Arkansas*
8:30 PS-7 Homologous DNA Integration in Plants
Avraham A. Levy, *The Weizmann Institute of Science, Israel*
8:55 PS-8 Using Zinc-fingered Nuclease Mediated Homologous Recombination to Manipulate the Mammalian Genome
Matthew H. Porteus, *University of Texas*
9:20 PS-9 Chromosome-based Gene Expression Platforms for Cell Engineering
Edward Perkins, *University of Minnesota Medical School - Duluth*
9:45 Discussion

10:00 am – 10:30 am | Coffee Break | Salon D, E, G, F

ALTERNATIVE CROPS/USES

Conveners: Nancy A. Reichert, Mississippi State University, and Margaret M. Young, Elizabeth City State University

10:30 am – 12:30 pm | Plant Symposium | Salon C
(See abstract page 15-A)

There are thousands of plant species grown throughout the world, yet we rely on a select few to provide us with products for input into primarily food/feed/fiber markets. Thinking outside the box, those "traditional" crops are being looked at for input into alternative uses and markets. Also, plants not thought of as traditional "crops" are also being improved to contribute value added benefits and products. This session will focus on research that could expand our definition of crops and their uses.

10:30 Introduction
10:35 P-22 Expanding the Utility of Alfalfa
Richard A. Dixon, *Samuel Roberts Noble Foundation*
11:10 P-23 From Crops to Biorefineries
Olga V. Selifonova, *Cargill, Inc.*
11:45 P-24 TBA
Maurice M. Moloney, *SemBioSys Genetics, Inc.*
12:20 Discussion

COMMERCIALIZATION OF TRANSGENIC CROPS

Conveners: David D. Songstad, Monsanto Company

10:30 am – 12:30 pm Plant Symposium Salon A
(See abstract page 15-A to 16-A)

The commercialization of transgenic crops has now moved into its second decade and provides an opportunity to reflect upon the progress and look forward to the future. Over the first decade, progress has been made toward the global acceptance of transgenic crops with a cumulative planting of biotech crops now reaching one billion acres spanning 17 countries. In 2004 alone, 200 million acres of biotech crops were planted and it is projected that this will increase by 20% for each of the 2005 and 2006 plantings. The future for transgenic crops looks very promising as the commercial climate grows and expands into new crops and traits. In this session, presentations will cover both the progress made over the first ten years of plant biotech crops and also the future as new crops and traits come to the marketplace.

10:30 Introduction
10:35 P-25 Development of Roundup ReadyÒ Alfalfa Varieties
Mark McCaslin, *Forage Genetics International*
11:10 P-26 Development and Characterization of Alfalfa Populations Tolerant to Glyphosate
Glenn Rogan, *Monsanto Company*
11:45 P-27 Impact of Yieldgard Rootworm on Corn Rootworm Control
Scott C. Johnson, *Monsanto Company*

CRYOPRESERVATION

Convener: Lia H. Campbell, Cell and Tissue Systems, Inc.

10:30 am – 12:30 pm Animal Symposium Duluth
(See abstract page 8-A)

Cryobiology is the study of cells at temperatures below the physiological temperature of 37°C. It encompasses many aspects of preservation including hypothermic storage, cryopreservation, and freeze-drying among others. While most researchers freeze cells, their experience with other aspects of cryobiology is limited. This symposium seeks to introduce participants to other applications involving sub-physiological temperatures.

10:30 Introduction (L. H. Campbell)
10:35 A-14 Cryopreservation of Hepatocytes: Role of Culture Configuration on Survival
Allison Hubel, *University of Minnesota*
11:00 A-15 Biophysical and Molecular Changes Associated with Cryopreservation of Sperm
Ken Roberts, *University of Minnesota Medical School*
11:25 A-16 Thermal Injury Characterization for Biomedical Applications: In Vitro Model Systems
John C. Bischof, *University of Minnesota*
11:50 Discussion

Tuesday, June 6
Odd Poster Authors will be present
1:30 pm – 2:30 pm
(See list of posters on pages 26-A to 49-A)

ANIMAL CELL SCIENCES

Moderator: Lia H. Campbell, Cell & Tissue Systems, Inc.

2:30 pm – 3:30 pm Animal Interactive Poster Session Salon D, E, G, F
(See abstract page 26-A to 27-A)

A-2000 Preservation of Cells by Freeze Drying
Lia H. Campbell*, Cell & Tissue Systems, Inc., Kristy Sarver, Sarah Miller, Brian Leman, and Kelvin G. M. Brockbank*
A-2001 Use of a Cancer Cell Line Profiling Array to Evaluate the Effect of Chemotherapeutic Agents on Cullin 5 (Cul5) mRNA Expression
K. Zaffarkhan, *Midwestern University, M. J. Fay, and C. Koch*
A-2002 Calcium Oxalate Crystals in Commercial Fetal Bovine Serum: Implications for Cell Culture, Phagocytosis and Biomineralization Studies In Vitro
Claudio E. Pedraza*, McGill University, and M. D. McKee*
A-2003 Antioxidant and Antiproliferative Effects of Turkish *Rheum Ribes* Ethyl Acetate Extract
Pembegul Uyar*, Middle East Technical University*
A-2004 In Vitro Tolerance of Filamentous Fungi to Environmental Pollutants: A Potential for Mycoremediation
Naiza Mae Natividad De Los Santos*, University of the Philippines in the Visayas, Krysta Laureen Palma, and Nelson D. Aclan*

Tuesday, June 6

IN VITRO TOOLS, TECHNIQUES, AND OPTIMIZATION

Moderator: Lindsey K. Tuominen, Orbital Technologies Corporation

2:30 pm – 3:30 pm — Plant Interactive Poster Session — Salon D, E, G, F
(See abstract page 38-A to 39-A)

P-2019 Effects of Auxin Inclusion During Indirect Shoot Regeneration in Model Plant Species
***Michael J. Bosela**, Indiana University-Purdue University at Fort Wayne*

P-2020 Time Course Study of Turmeric (*Curcuma longa* L.) Microrhizome Development in Large and Small Vessels of Liquid Media with Varied PGR Concentrations.
***Jeffery Adelberg,** Clemson University, and Matthew Cousins*

P-2021 Miniature Sensors and LED Lighting for Advanced In Vitro Experimentation
***Lindsey K. Tuominen**, Orbital Technologies Corporation, D. J. Smith, A. G. Vermaak, J. C. Vignali, M. J. Mischnick, and R. C. Morrow*

P-2022 Germination and Plantlet Regeneration of Encapsulated Somatic Embryos from Transgenic Grape (*Vitis vinifera* L.)
***Niraj Kumar Nirala**, Hamdard University, D. K. Das, M. K. Reddy, P.S. Srivastava, S. K. Sopory, and K. C. Upadhyaya*

P-2023 Identification and Cloning of RAPD Markers Linked to WA CMS in Rice (*Oryza sativa* L.)
***Asadollah Ahmadikhah,** Russian State Agricultural University, and G. I. Karlov*

PLANT TRANSFORMATION

Moderator: Maureen M. M. Fitch, USDA/ARS

2:30 pm – 3:30 pm — Plant Interactive Poster Session — Salon D, E, G, F
(See abstract page 20-A and 35-A to 36-A)

P-2006 Marker Gene Removal During Gene Transfer *In Vitis* spp.: A Technological Approach Toward an Improved Science-society Communication
***Lucia Martinelli**, Istituto Agrario San Michele All'Adige, L. Dalla Costa, I. Vaccari, V. Poletti, and F. Guzzo*

P-2007 Transformation of Anthurium with Transgenes for Bacterial Blight and Nematode Resistance
***Maureen M. M. Fitch,** USDA/ARS, T. Leong, H. Albert, S. Schenck, P. Moore, and D. Gonsalves*

P-2008 Genetic Transformation of *Cyamopsis tetragonoloba* (GUAR)
***Sanchita Vaghchhipawala**, The Samuel Roberts Noble Foundation, and Richard A. Dixon*

P-2009 *Agrobacterium*-mediated Cotton Transformation and Regeneration- Using Sucrose as Carbohydrate Source and Selecting of Transgenics with Kanamycin Selection
***Shubha Subbarao**, Monsanto Company, J. Layton, N. Sidorova, L. Tan, J. Washam, E. Jakse, and D. Duncan*

P-2010 Development of an Efficient *Agrobacterium*-mediated Gene Transfer System for Multiple Sweetpotato Cultivars
***Jessica A. Scoffield**, Tuskegee University, M. Egnin, B. Bey, M. Quain, C. S. Prakash, and D. Mortley*

P-2011 Assessment of *Agrobacterium*-mediated Transformation Methods: Simplifying the Induction Process and Factors to Consider in Plant/Strain Competence Selection
***Anwar A. Alsanabani**, Alabama A & M University, Caula A. Beyl, and Anthony Ananga*

PLANT BIOTECHNOLOGY

Moderator: Charles Neal Stewart, Jr., University of Tennessee

3:15 pm – 5:00 pm Plant Contributed Paper Session Salon C
(See abstract page 21-A to 22-A and 25-A)

3:15 P-1020 Mineral Nutrient Requirements for Regulating the Growth of Plant Tissue
***Randall P. Niedz**, USDA-ARS, and T. J. Evens*

3:30 P-1006 In Vitro Production of Turmeric (*Curcuma longa* L.) Microrhizomes as a Potential Source for Secondary Metabolites
***Matthew Cousins,** Clemson University, Jeffrey Adelberg, Feng Chen, and James Reick*

3:45 P-1007 Castor Seed Development and Storage Lipid Biosynthesis
***Grace Q. Chen,** USDA/ARS, Yeh-Jin Ahn, and Louisa Vang*

4:00 P-1008 Production of Biologically-active Acidothermus Cellulolyticus Endo-1, 4-β-gluconase (E1) Enzyme in Transgenic Rice Plants for Alcohol Fuels and Cleaner Environment
***Hesham Farouk Oraby,** Michigan State University, Balan Venkatesh, Bruce Dale, Rashid Ahmad, Callista Ransom, James Oehmke, and Mariam Sticklen*

4:15 P-1009 High-yields and Extended Serum Half-life of Therapeutic Proteins Expressed as Fusion Glycoproteins in Tobacco Cells
***Jianfeng Xu,** Ohio University, S. Okada, K. J. Goodrum, J. J. Kopchick, and M. J. Kieliszewski*

4:30 P-1010 Plant Transformation and Horizontal Gene Flow of a Plant ABC Transporter Gene
***Charles Neal Stewart, Jr.,** University of Tennessee, R. J. Millwood, J. S. Davis, K. P. Burris, A. Mentewab, and J. N. Burris*

4:45 P-1011 Quantitative Multiplex Real-time PCR as a Screening Tool for Estimating Transgene Copy Number in Transgenic Citrus
***Ahmad Al-Sayed Omar,** University of Florida, M. G. H. Dekkers, H. J. Graham, and J. W. Grosser*

LINKING SPECIES FOR GENOME ANALYSIS

Conveners: Peggy J. Ozias-Akins, University of Georgia, and Heidi F. Kaeppler, University of Wisconsin

3:30 pm – 5:00 pm | Plant Symposium | Salon A

(See abstract page 16-A to 17-A)

Genomics research benefits greatly from cross-species comparison for sequence characterization, genetic dissection and functional analysis of genomes. Although species often are distinguished by their inability to mate, sexual compatibility is no longer a barrier to linking genomes. In this symposium, various interpretations of "linking" species will be addressed. These include the development and application of radiation hybrids containing chromosomes mainly of one species but being distinguished by the presence of a chromosome from a different species whose similarities promote analysis but whose differences allow characterization that could not be accomplished in its own genetic background. Another application is derived from the discovery that genomic similarities among species are extensive. These similarities have been useful for genetic mapping and gene isolation as well as expanding our understanding of species evolution.

3:30 Introduction
3:35 P-28 Wide-cross Whole-genome Radiation Hybrid Mapping in Cotton
David M. Stelly, *Texas A&M University*
4:00 P-29 Evolution of Chromatin Structure and Function
Shawn M. Kaeppler, *University of Wisconsin*
4:25 P-30 Allium Genomics: Exploiting Model Plants for Analyses of Enormous Nuclear Genomes
Michael J. Havey, *University of Wisconsin – Madison*

THREE-DIMENSIONAL TISSUE MODELS OF CANCER CELL INVASION AND METASTASIS

Convener: Jonathan Garlick, Tufts University

3:30 pm – 5:00 pm | Animal Symposium | Duluth

(See abstract page 8-A to 9-A)

It is now clear that the tumor microenvironment plays an essential role in all stages of cancer progression. This is based on observations showing that cancer cells demonstrate complex cross-talk with the surrounding stroma and with its constituent cells during invasion and metastasis. In this light, cancer development is dependant on reciprocal interactions regulated by the manner in which cancer cells communicate with neighboring cells, with adjacent stroma and with soluble growth factors. As a result, it is very important that experimental paradigms designed to study these processes manifest these complex cellular interactions as they occur *in vivo*. In recent years, three-dimensional (3D) human tissue models that mimic various stages of cancer progression have been developed to significantly advance our ability to study cancer in a biologically-meaningful tissue context. The goal of this symposium is to provide an overview and update on studies using these human, 3D experimental models that have been used to study how the tumor microenvironment regulates invasion and metastasis during cancer development. This will be accomplished through three presentations that will describe 3D, human tissue models that have revealed mechanisms through which defined stages of breast cancer, squamous cell carcinoma and melanoma are controlled by signals from the tumor microenvironment.

3:30 Introduction
3:35 A-17 Three-dimensional Microenvironment and Breast Cancer Progression
Penney Gilbert, *University of Pennsylvania*
4:00 A-18 3D In Vitro Models Reveal the Invasive, Drug Resistant Phenotype of Metastatic Melanoma
Keiran Smalley, *The Wistar Institute*

Tuesday, June 6

4:25 A-19 Reconstructing and Deconstructing the Progression of Human Squamous Cell Carcinoma in 3D Tissue Models
***Addy Alt-Holland**, Tufts University*
4:50 Discussion

5:00 pm – 5:45 pm **SIVB Business Meeting** Rochester
(All Members are Urged to Attend)

6:00 pm – 10:00 pm **An Evening at the Mill City Museum Event** Mill City Museum
Admittance by Advance Ticket Holders Only

WEDNESDAY, JUNE 7

7:00 am – 12:00 pm Registration Red Wing Room

ADVANCES IN PLANT TRANSFORMATION

Conveners: Kan Wang, Iowa State University, and John J. Finer, Ohio State University

8:00 am – 10:05 am Plant Symposium Salon C

(See abstract page 17-A to 18-A)

Plant genetic transformation is the essence of plant biotechnology and is critical for functional genomics studies. Although much progress has been made in recent years using *Agrobacterium*-mediated transformation and physical delivery methods such as particle bombardment, transformation efficiencies still need to be increased to meet the increasing demands in gene functional analysis. In addition to simply generating more transgenics, high efficiency transformation will naturally lead to high throughput functional analysis and recovery of higher quality transformants. This session will highlight some recent development in the area of tissue culture and transformation methods, strategies for generating quality transformants, transgene expression tracking, and evaluation of different nanotechnology tools for DNA delivery to plant cells. These advances in plant transformation could influence the future direction of plant biology research.

8:00 Introduction (K. Wang and J. J. Finer)

8:05 P-31 High Efficiency and High Throughput Transformation of Cereals Mediated by *Agrobacterium* for Functional Genomics
Toshihiko Komari, *Japan Tobacco, Inc.*

8:25 P-32 Plant Tissue Transformation Using Periodic Arrays of Vertically Aligned Carbon Nanofibers
Timothy Eric McKnight. *Oak Ridge National Laboratory*

8:45 P-33 DNA-coated Nanoparticles Mediated Transgene Expression in Plant Cells
François Torney, *Iowa State University*

9:05 P-34 Transfection: A Reliable and Efficient Method for Maize Transformation
Michael E. Horn, *Phyton Biotech, Inc.*

9:25 P-35 A Novel Plant Transformation Technology – Lipoic Acid
Yinghui Dan, *Virginia Polytechnic Institute and State University-Blacksburg*

9:45 P-36 Evaluation of an Automated Image Analysis System for Factors Which Stabilize Gene Expression
Joseph Chiera/John J. Finer, *OARDC/The Ohio State University*

EMBRYOGENESIS / REGENERATION / MICROPROPAGATION

Moderator: Barbara M. Reed, USDA/ARS

8:00 am – 10:00 am Plant Contributed Paper Session Duluth

(See abstract page 23-A to 24-A)

8:00 P-1012 Abstract has been withdrawn

8:15 P-1013 Medium-term In Vitro Storage of Pear (*Pyrus* L.) Germplasm
Barbara M. Reed, *USDA/ARS*

Wednesday, June 7

8:30 P-1014 Indirect Shoot Organogenesis from Leaves of Dieffenbachia: A Method to Produce Somacolonal Variants
Xiu Li Shen, University of Florida, M. E. Kane, and J. Chen

8:45 P-1015 Micropropagation of *Calopogon tuberosus*, a Native Terrestrial Orchid, by Shoot Production from Corm Explants
Philip Joseph Kauth, *University of Florida, W. A. Vendrame, and M. E. Kane*

9:00 P-1016 Comparative In Vitro Shoot Multiplication, Rooting, and Ex Vitro Acclimatization of Sea Oats (*Uniola paniculata* L.) Genotypes
Michael E. Kane, *University of Florida, N. L. Philman, P. Kauth, X. Shen, P. Sleszynski, S. Stewart, and C. Valero-Aracama*

9:15 P-1017 In Vitro Regeneration of Periwinkle
Andrea Swanberg, *North Dakota State University, and Wenhao Dai*

9:30 P-1018 Comparison of Different Callus and Plant Regeneration from Different Explants in Triploid and Tetraploid Turf-type Bermudagrasses
Song Zhang, *University of Georgia, Wayne Hanna, and Peggy Ozias-Akins*

9:45 P-1019 The HBK3 Homeobox of Knox Gene is Responsible for Developmental Control and Shoot Apical Meristem Determination in Maturing Somatic Embryos of *Picea abies*
Mark F. Belmonte, *University of Manitoba, M. Tahir, and C. Stasolla*

10:00 am – 10:30 am Coffee Break Ballroom Foyer

DISTINGUISHED PLANT SYMPOSIUM
AGROBACTERIUM TRANSFORMATION: PAST, PRESENT, AND FUTURE

Convener: Kan Wang, Iowa State University

10:30 am – 12:00 pm Distinguished Plant Symposium Salon C
(See abstract page 18-A)

The discovery of *Agrobacterium tumefaciens* and the use of its ability to deliver DNA into plant cells have practically redefined the plant biology and agriculture today. In this plant keynote session, Professor Marc Van Montagu, the world renowned scientist and the founding father (together with late Prof. Jeff Schell) of *Agrobacterium*-mediated transformation technology, will address the audience with his experiences in the history of the discovery, his perspective on the present development of the technology and his insight regarding the future of plant biology.

10:30 Introduction (K. Wang)
10:40 P-37 Agrobacterium Mediated Gene Transfer in Plants
Marc Van Montagu, *Ghent University*

Animal Cell Science Posters

SATURDAY, JUNE 3	SUNDAY, JUNE 4	MONDAY, JUNE 5	TUESDAY, JUNE 6
7:00 pm – 9:00 pm	8:00 am – 10:00 pm	8:00 am – 10:00 pm	8:00 am – 3:30 pm

Posters mounted Saturday, June 3, 3:00 pm – 6:00 pm.
Posters removed from Exhibit Hall from 3:30 pm – 5:00 pm, June 6
Poster authors will be present at their posters the following days and times:

SATURDAY, JUNE 3	SUNDAY, JUNE 4	MONDAY, JUNE 5	TUESDAY, JUNE 6
Even Authors Present	Odd Authors Present	Even Authors Present	Odd Authors Present
7:30 pm – 8:30 pm	6:30 pm – 7:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:30 pm

CELLULAR & MOLECULAR BIOLOGY

A-2005 Establishment and Characterization of 13 Human Colorectal-carcinoma Cell Lines
***Ja-Lok Ku**, Laboratory of Cell Biology, Cancer Research Institute, Kyung-Hee Kim, Jin-Sung Choi, Sung-Hye Hong, Young-Kyoung Shin, Hong-Sun Kim, Jae-Hyun Park, Il-Jin Kim, and Jae-Gahb Park*

CELLULAR IMMUNOLOGY

A-2006 Animal Component Free T-Cell Culture
***John H. Manwaring**, HyClone, B. B. Barnett, and W. G. Whitford*

A-2007 Signal Transduction Targets of Modeled Microgravity
***Alamelu Sundaresan**, Texas Southern University, Kamleshwar Singh, Neal R. Pellis, and James DuMond, Jr.*

CELLULAR MODELS

A-2008 Formation of Intact Membrane Structures with High Transepithelial Electrical Resistance in Culture Inserts with Mouse Embryonic Stem Cells
***Frank A. Barile**, St. John`s University, and R. Konsoula*

A-2009 BEAS-2B Cells as a Model for the Reductive Activation of Hexavalent Chromium in Human Lung Epithelial Cells
***Charles R. Myers**, Medical College of Wisconsin, Griselda R. Borthiry, and William E. Antholine*

A-2010 Comparative Extraction Profiles of Medical Devices Using a Cell Growth Inhibition Assay
***David Tan,** CIBA Vision Corporation, Ann M. Wright, Alana Renaud, Alicja Sills, and Mary Mowrey McKee*

SILENT ABSTRACTS

A-2011 Up-regulation of Inducible Nitric Oxide Synthase by Rosiglitazone in Sinusoidal Endothelial Rat Liver Cells In Vitro
***Miguel Reyes**, Faculty of Medicine UJED, Claudia Reyes-Estrada, and Brissia Lazalde*

A-2012 Integrin Regulation of Mouse Embryonic Stem Cell Self-renewal

J. Denry Sato, *Mount Desert Island Biological Laboratory, Yohei Hayashi, Miho Furue, Kiyoshi Ohnuma, Yasufumi Myoishi, Takanori Abe, Ryu-ichiro Hata, Tetsuji Okamoto, and Makoto Asashima*

Education Poster & High School Award Winning Silent Abstracts

SATURDAY, JUNE 3	SUNDAY, JUNE 4	MONDAY, JUNE 5	TUESDAY, JUNE 6
7:00 pm – 9:00 pm	8:00 am – 10:00 pm	8:00 am – 10:00 pm	8:00 am – 3:30 pm

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Even Authors Present	Odd Authors Present	Even Authors Present	Odd Authors Present
7:30 pm – 8:30 pm	6:30 pm – 7:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:30 pm

EDUCATION POSTERS

E-2000 Use of Lettuce Tissue Culture for Developing Transformation Techniques in High School
Carol A. Harrison, *Tuskegee University, M. Egnin, J. Scoffield and B. Bey*

HIGH SCHOOL STUDENT SCIENCE AWARD WINNING SILENT ABSTRACTS

E-2001 Resistance of *Serratia marcescens*
Mary E. Crowther Kent

E-2002 The Effects of Road De-icer ($MgCl_2$) on Plant Germination
Caren Michelle Collins, *North Garland High School, Jay Ingram, and David Muirhead*

E-2003 Herbal Alternatives to Antibiotics and Their Effects on *E. coli* k12
Simran S. Grewal, *Huron High School*

E-2004 Microbiology Evaluation of Toothbrushes
Vitor Hugo Fernandes, *Fundação Educacional Montes Claros, and D. L. César*

E-2005 Proteomics Analysis of Osteosarcoma Cells
Michael McCord Polmear, *University of Colorado at Denver, L. K. Polepeddi*

E-2006 Determining the Effects of Vitamin A on Short-term Memory Ability
Amanda Marie Thomas, *School of the Osage*

E-2007 The Development of a Novel Gene Knockout Technique Utilizing RNA-interference
Jason Chao Zhang, *Yorktown High School*

Plant Biotechnology Posters

SATURDAY, JUNE 3	SUNDAY, JUNE 4	MONDAY, JUNE 5	TUESDAY, JUNE 6
7:00 pm – 9:00 pm	8:00 am – 10:00 pm	8:00 am – 10:00 pm	8:00 am – 3:30 pm

Posters mounted Saturday, June 3, 3:00 pm – 6:00 pm.
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SATURDAY, JUNE 3	SUNDAY, JUNE 4	MONDAY, JUNE 5	TUESDAY, JUNE 6
Even Authors Present	Odd Authors Present	Even Authors Present	Odd Authors Present
7:30 pm – 8:30 pm	6:30 pm – 7:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:30 pm

BIOTECHNOLOGY

P-2024 Marker-assisted Progeny Test for the Use in Mapping Experiments
Asadollah Ahmadikhah, *Russian State Agricultural University of Timiriazev, G. I. Karlov, and V. S. Sheveloukha,*

P-2025 Characterization of *Populus tremuloides* COMT, 4CL1 and 4CL2 Gene Promoters to Identify Regulatory Elements
Edward Odhiambo Anino*, Michigan Technological University, S. Blumer, P. Pechter, S. Harding, and C.-J. Tsai*

P-2026 Suppression of Phospholipase D in Soybean Seed
Jung-Hoon Lee*, Kansas State University, William T. Schapaugh, Xuemin Wang, and Harold N. Trick*

P-2028 Development of Transgenic Rice Using Electroporation Technique After the Vacuum Treatment
Sea-Kwan Oh*, National Institute of Crop Science, T. Hagio, A. Sunaga, K. Konagaya, S. Kamachi, S. Ando, M. Tsuda, J. Mochizuki, Y. Tabei, and H.-Y. Kim*

P-2029 Plant Transcriptional Responses to RDX (Royal Demolition Explosive)
Murali Raghavendra Rao*. University of Tennessee*

P-2030 Modified Leaf Phenolics and European Corn Borer Herbivory in Oxalate Oxidase Transformed Corn
John Alfred Simmonds*, Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, J. Mao, K. Hubbard, I. Altosaar, and J. Arnason*

DICOT TRANSFORMATION

P-2031 WHISKERST-mediated Transformation of Cotton
Jeffrey R. Beringer*, Dow AgroSciences, LLC, L. W. Baker, C. Clifford, A. Miller, A. M. Palta, D. Pareddy, T. Strange Moynahan, L. Schulenberg, and J. F. Petolino*

P-2032 A Novel Plant Transformation Enhancer
Yinghui Dan*, Virginia Polytechnic Institute and State University, C. L. Armstrong, J. Dong, X. Feng, J. E. Fry, G. E. Keithly, B. J. Martinell, K. A. Rayford, G. A. Roberts, C. Rommens, L. A. Smith, L. Tan, and D. R. Duncan*

P-2033 In Vitro Regeneration and Transformation in Chilli Pepper (*Capsicum annuum* L.)
Karampuri Subhash*, Kakatiya University, Peddaboina Venkataiah, and Thamidala Christopher*

Plant Biotechnology Posters

DISEASE RESISTANCE

P-2034 Microarray Analysis of Gene Expression in Barley During *Fusarium graminearum* Infection
***Hatice Bilgic**, University of Minnesota, Seungho Cho, Lexingtons Nduulu, Kevin Smith, and Gary J. Muehlbauer*

EDIBLE VACCINES

P-2035 Expression and Stability of the Respiratory Syncytial Virus-F Gene in Advanced Generations of Tomato
***Joann Lau,** University of Illinois at Urbana-Champaign, and Schuyler S. Korban*

P-2036 Transgenic Apple Lines Expressing an Antigenic Protein Against the Human Respiratory Syncytial Virus
***Joann Lau,** University of Illinois at Urbana-Champaign, and Schuyler S. Korban*

EMBRYOGENESIS/REGENERATION/MICROPROPAGATION

P-2037 Molecular Characterization of PgAGO, a Novel Conifer Gene of the ARGONAUTE Family Expressed in the Apical Cells and Required for Somatic Embryo Development in *Picea glauca*
***Derek Albert Law**, University of Manitoba, M. Tahir and C. Stasolla*

P-2038 Direct Shoot Induction from Several Types of Explants of Herbaceous Peony
***Daike K. Tian**, Auburn University, K. M. Tilt, F. Dane, J. L. Sibley, and F. M. Woods*

GENOMES/GENOMICS/BIOINFORMATICS

P-2039 Coupling Functional and Structural Genomics - Expression Level Polymorphisms in Wheat
***Mark C. Jordan,** Cereal Research Centre, Agriculture and Agri-Food Canada, and Daryl Somers*

P-2040 Transposon Transcription and Movement During Maize Tissue Culture
***Shawn M. Kaeppler**, University of Wisconsin-Madison, A. Smith, and Y. Rhee*

IN VITRO TOOLS, TECHNIQUES AND OPTIMIZATION

P-2041 Development of Efficient In Vitro Systems for Peanut (*Arachis hypogaea* L.) Micropropagation and Seed Production
***Benjamin S. Bey**, Tuskegee University, M. Egnin, J. Scoffield, A. S. Williams, D. Mortley, L. S. Crawford, and M. Quain*

P-2042 Regulating Plant Tissue Growth by Mineral Nutrition
***Randall P. Niedz,** USDA/ARS, and T. J. Evens*

Plant Biotechnology Posters

MONOCOT TRANSFORMATION

P-2043 Transgenic Plants of Gladiolus Containing the Coat Protein and Replicase Genes of Cucumber Mosaic Virus
***Kathryn K. Kamo**, US National Arboretum, P. Ueng, J. Aebig, H. T. Hsu, M. A. Guaragna, and R. Jordan*

PLANT TISSUE CULTURE

P-2044 RNA Interference (RNAi) to Control the Soybean Cyst Nematode (Heterodera glycines Ichinohe)
***William Roberto Dall`Acqua**, Kansas State University, Ryan Steeves, Timothy C. Todd, and Harold N. Trick*

P-2045 Genebanking of Vegetatively-propagated Crops - Cryopreservation of Forty-four Mentha Accessions
***David D. Ellis**, USDA/ARS, E. Staats, L. Towill, J. Laufmann, and B. Reed*

P-2046 Embryo Rescue and Meristem Culture Techniques Used in the Development of Oat/Maize Addition Lines
***Mark W. Galatowitsch**, University of Minnesota, P. A. Huettl, M. S. Jacobs, R. L. Phillips, and H. W. Rines*

P-2047 Enhancement of Somatic Embryogenesis and Plant Regeneration in Japanese Larch (*Larix leptolepis*)
***Yong W. Kim**, Korea Forest Research Institute, H. K. Moon and S. Y. Park*

P-2048 Somaclonal Variation and Stability of the GUS Transgene in Somatic Embryogenic-derived Populations of Transgenic Celery
***Guo-Qing Song,** Michigan State University, and K. C. Sink*

P-2049 Genetic Transformation and the Expression of the tCUP Promoter in *Prunus domestica*
***Lining Tian**, Agriculture and Agri-Food Canada, S. Sibbald, X. Wang, and S. Kohalmi*

PLANT TRANSFORMATION

P-2050 *Agrobacterium tumefaciens*-mediated Transformation and Efficient Production of Transgenic Potato (*S. tuberosum* L. ssp.andigena) Plants
***Anjan Kumar Banerjee,** Iowa State University, S. Prat, and D. J. Hannapel*

P-2051 Gene Transfer into Mature Seeds of Some Gramineous Species Via Electroporation
***Takashi Hagio**, National Institute of Aerobiological Sciences A. Sunaga, S. K. Oh, M. Takeda, K. Kakeda, S. Kamachi, K. Konagaya, S. Ando, M. Tsuda, J. Mochizuki, and Y. Tabei*

P-2052 Tangerine Blush: Engineering Soybean to Produce β-Carotene in Seed Cotyledons.
***Blake Lee Joyce**, Unversity of Georgia, P. LaFayette, and W. Parrott*

P-2053 Evaluation of Inducible Cre/lox and FLP/FRT Recombination Systems for Marker Gene Deletion in Rice
***Abhilasha Khattri**, University of Arkansas, and Vibha Srivastava*

P-2054 *Agrobacterium*-mediated Transformation of Tropical Root Crop (*Dioscorea rotundata*) Vital for Food Security In Sub-Saharan Africa
***Marian Dorcas Quain**, University of Ghana, M. Egnin, J. Scoffield, B. Bey, C. Bonsi, and E. Acheampong*

P-2055 High-efficiency *Agrobacterium*-mediated Transformation of Pear (*Pyrus communis* L.) Leaf Segments and Regeneration of Transgenic Plants
***Q. Sun,** USDA/ARS, Y. Zhao, W. Wei, R. W. Hammond, R. E. Davis*

SECONDARY PRODUCTS

P-2056 Hairy Root Cultures Induced by *A. rhizogenes* as a Valuable Source of Known and Unknown Alkamides in Three Species of Echinacea
***Fredy Rolando Romero**, Iowa State University, L. Wu, K. Delate, E. Wurtele, and D. J. Hannapel*

VIROLOGY

P-2057 Functional Role of Potato Virus X Movement Protein Domains in the Virion RNA Translational Activation
***Anna A. Mukhamedzhanova**, Lomonosov State University, M. A. Arkhipenko, S. V. Kozlovsky, O. V. Karpova, N. P. Rodionova, and J. G. Atabekov*

P-2058 In Vitro Assembly of Triple Complexes from Potato Virus X RNA, Coat Protein and the 25-kDa Movement Protein
***Nikolaj A. Nikitin**, Lomonosov State University, O. V. Karpova, O.V. Zayakina, M. A. Arkhipenko, N. P. Rodionova and J. G. Atabekov*

SILENT ABSTRACTS

P-2059 Polyamine Level Modulation Affect to Tobacco BY-2 Cell Growth
***Jose L. Casas-Martinez**, Universidad de Alicante, A. Piqueras, and F. Serrano*

P-2060 Potential Mechanisms for Contaminations of Green Onions with Hepatitis A
***David D. Chancellor**, University of Pittsburgh, Shachi Tyagi, Virginia M. Dato, Sara Bacvinskas, Michael B. Chancellor, and Fernando de Miguel*

P-2061 High Expression Levels of Erythropoietin (EPO) in Plants Cells Using TMV and PVX-based Viral Vectors
***Cristiano Lacorte**, EMBRAPA, J. Shrestha, H. Beenen, D. Lohuis, R. Goldbach, and M. Prins*

P-2062 Polyamines and Ethylene as Stress Indicators During Root Development in Micropropagated Rosa Plantlets Acclimatized to Ex Vitro Conditions
***Abel Piqueras**, Universidad de Alicante, S. Hussein, M. D. Serna, and J. L. Casas*

KS-1

Ever-expanding Horizons. RONALD L. PHILLIPS. Department of Agronomy and Plant Genetics and Microbial and Plant Genomics Institute, University of Minnesota, St. Paul, MN 55108. Email: phill005@umn.edu

World population is exploding—one billion people are added every 14 years. Though we've traveled a long road—from the first hybrid plant, to Gregor Mendel's "Experiments in Plant Hybridization", to the Green Revolution, to the regeneration of plants from cells, to genome sequencing—it's still hard to imagine the road ahead. Genomics offers a new paradigm through knowing the complete genetic code of an organism, the ability to assess gene expression across the entire genome in one experiment, understanding the function of every gene, and the networking of genes and biochemical pathways. Comprehensive gene expression studies will allow the basic understanding of mechanisms that often are the basis of whole industries, such as hybrid vigor. Comparative genome mapping extends the application of new knowledge to an ever-expanding list of species. Large-scale genome projects of major economically important plant species and model systems broaden the horizon for the application of molecular/cell biology. Genetic barriers are being surmounted via embryo rescue techniques, tissue culture, and transformation. Oat by maize crosses have led to strains which have a complete oat genome, plus one maize chromosome. These "addition lines" are useful for many studies including the mapping of DNA sequences, chromosome sorting, cytogenetic studies, and the introduction of unique traits such as C4 photosynthesis into C3 species. Transformation technology involving in vitro techniques has produced useful biotech crop varieties that have been grown on more than one billion acres worldwide. New biofortification traits should lead to improved health for up to one of every three citizens of the world. Golden rice high in pro-Vitamin A may improve the lives of one million children a year. Another application under development is the modification of corn feed that can potentially reduce *E. coli* O157:H7 contamination in the food supply. Just over the horizon, a new era awaits. . . one in which applications of science will lead to improved lives for millions of people.

PS-1

Advances in Molecular Nutrition: Phytochemicals and Antioxidant Response Pathways as Critical Control Points for Chemoprevention of Disease. KALIDAS SHETTY. University of Massachusetts, Department of Food Science, Chenoweth Laboratory, Amherst, MA 01003. Email: kalidas@foodsci.umass.edu

The major chronic diseases such as diabetes, cardiovascular disease, cognition dysfunction and cancer tend to be diet and environment-influenced and oxidation-linked and may manifest in communities around the world, irrespective of income. In light of the role of oxidation dysfunction in diet and environment-related diseases, the conceptual foundation for advances in Molecular Nutrition in addressing the challenges of the above diseases needs a more rationale and integrated model. In this model a significant role for dietary phenolic phytochemicals with antioxidant function (from fruits, vegetables, beverages, herbs and spices and their products) is emerging and could have an important role in preventive management of chronic oxidation and also infectious diseases through modulation of host antioxidant response pathways. A diverse group of phenolic antioxidants from dietary plants are being targeted for designed dietary intervention to manage major oxidation-linked diseases such as diabetes, cardiovascular diseases, arthritis, cognition diseases and cancer. The focus of this integrated model is on how phenolic phytochemicals at the cellular level can counter oxidation-linked biochemical reactions linked to above diseases. The emphasis in particular is on how an alternative mode of energy metabolism through the use of proline may be more important for providing energy and reducing equivalents such as NADPH via pentose phosphate pathway for stimulating protective antioxidant enzyme response. Around this fundamental concept of redox modulation and protective antioxidant response, genetic interactions and cellular signaling related to oxidation-linked disease manifestations can be integrated to develop a more cohesive model for new advances in Molecular Nutrition for better health and preventive management of diseases. The model also has implications for the development of antimicrobial phenolic phytochemicals against bacterial pathogens in an era of increasing antibiotic resistance and for improved strategies for drug discovery and design.

PS-2

Genomic and Proteomic Approaches to Understanding and Manipulating Nutritional Requirements in Mammalian Cell Culture Processes. LAUREL DONAHUE. SAFC Biosciences, 11296 Renner Blvd, Lenexa, KS 66219. Email: ldonahue@sial.com

Mammalian cell growth and protein production are manipulated by biopharmaceutical companies for biotherapeutic protein production. CHO and NS/0 are currently the favored platform cell types for the production of many approved biotherapeutics (various antibodies, growth factors, hormones and enzymes), as well as a majority of those currently in clinical trials. In addition, a variety of human and monkey cell types are used for vaccine production (WI-38, MRC-5 and VERO). Most process development scientists interested in maximizing the growth and production processes use "Design of Experiment" statistical tools to extract data from limited data sets, as well as in process analytical testing to examine nutrients in the culture medium that become depleted during either the growth or production phases. This presentation will describe studies using genomic and proteomic approaches to discover correlations in gene expression and changes in gene expression that relate to the desired process. Examples will be given that (1) demonstrate how this data can be used to successfully replace fetal bovine serum in a cell type used for vaccine production and (2) how patterns of gene expression can mark protein production phases in culture and how this information can be used to improve the culture process.

PS-3

Animal Cell Culture: The Future Beyond Bioreactors and Metabolic Engineering. WEI-SHOU HU, Katie F. Wlaschin, and Joon Chong Yee. Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455. Email: wshu@cems.umn.edu

In the past decade, we have witnessed a tremendous increase in the number of mammalian cell derived therapeutic proteins with clinical applications. The success of making so many of these life-saving biologics available to the public is partly due to engineering efforts to enhance process efficiency. Fedbatch and perfusion cultures in conventional bioreactors are now the industrial norm, vastly increasing production capacity as compared with traditional batch cultures. To further improve productivity, much effort has been devoted to developing metabolically engineered producing cells, which possess characteristics favorable for large-scale bioprocessing. In this presentation, the success of previous efforts future process development opportunities will be discussed. Furthermore, with the availability of genomic data and analytical tools for transciptome and proteome profiling, surveying the physiological landscape of producing cells holds the potential to unveil the secrets of many complex traits, including hyperproductivity. The opportunities afforded by these new research tools and the potential impact on the future of bioprocessing will be a focus of this presentation.

PS-4

Baculovirus Recombinant Protein Production in Insect and Mammalian Cells. THOMAS A. KOST, GlaxoSmithKline R & D, 5 Moore Drive, Research Triangle Park, North Carolina 27709. Email: tom.a.kost@gsk.com

Recombinant baculovirus infected insect cells are used extensively for the production of recombinant proteins. In most instances cells derived from *Spodoptera frugiperda* or *Trichoplusia ni* serve as hosts and the majority of viral vectors are based on *Autographica californica* nuclear polyhedrosis virus. Over the past 20 years many improvements have been made to the baculovirus insect cell expression system (BEVS) including the development of efficient easy to use virus generation methods, a variety of rapid titration assays, and serum free culture media. With these improvements one can readily derive transfer vectors, generate recombinant viruses and scale-up protein production to 100 L volumes within a few months. Scale-up can be readily achieved in a variety of bioreactor vessels including shake-flasks, stirred tanks and wave bags. Growth conditions are relatively simple and in most cases one only needs to monitor and control oxygen levels. Recently modified recombinant baculoviruses containing mammalian cell-active regulatory elements have been developed for mammalian cell gene delivery. These vectors, termed BacMam viruses, have been shown to transduce a wide variety of mammalian cell types and have proven very useful for developing cell based assays for many pharmaceutical targets such as G protein-coupled receptors, ion channels, transporters and nuclear receptors. Recent results also indicate that this approach has potential for the transient production of secreted proteins.

PS-5

Commercial Production of Paclitaxel by Plant Cell Culture. VENKATESH SRINIVASAN, Jennifer D. Alford, Robert R. Rasmussen, Braden L. Roach, Irma Rodriguez, Barbara Schnabel-Preikstas, Beth Slusar-Place, Charles Swindell, Kai Schütte, Michael von Gönner, and Stefan Wilke. Phyton Biotech, Inc., 279 Princeton-Hightstown Road, East Windsor, NJ 08520. Email: phyton@phytonbiotech.com

Paclitaxel production by plant cell culture filled a critical need for the supply of a valuable pharmaceutical entity by en eco-friendly and economical means of production. The challenge of commercial production was set against a background of skepticism about the technology: a) difficulties in expressing whole plant metabolites in the cell cultures, b) ability to successfully improve yields to commercial levels, c) long term stability of metabolite production from plant cells, and d) ability to successfully scale up the production to ensure steady supply. Commercial scale paclitaxel production has been achieved by successfully dealing with these issues in a manner consistent with the experience of other "biopharmaceutical" products. Currently this process has been operating for close to a decade and produces the highest output of paclitaxel among all of the competing sources, viz. from biomass or through semi-synthesis.

PS-6

Cre/*lox*-mediated Site-specific Gene Integration in Plants. VIBHA SRIVASTAVA. University of Arkansas, Department of Crop, Soil & Environmental Sciences, 115 Plant Science Bldg., Fayetteville, AR 72701. Email: vibhas@uark.edu

We studied the utility of Cre-mediated site-specific integration method for streamlining the production of stable transgenic plants using rice as a model. Using this method, we precisely placed a single copy of β-glucuronidase gene (*gusA*) into the designated genomic location. We found that expression variability between transgenic lines produced by this method was greatly reduced. We studied GUS expression in 18 different site-specific integration lines, 11 of which contain precise site-specific integration without illegitimate integration in the background (single-copy lines) and the remaining 7 contain illegitimate integrations in addition to the precise site-specific integration locus (multi-copy lines). We found that *gusA* gene in the single-copy site-specific integration lines is expressed at consistent levels over successive generations, and the gene activity is directly correlated with allelic gene dosage. Segregation analysis of multi-copy lines suggested that in 3 lines illegitimate integrations are genetically linked to the site-specific integration locus. In the remaining 4 multi-copy lines site-specific integration locus was segregated from illegitimate integration locus. Further, the site-specific integration locus derived from silenced multi-copy lines was activated upon segregation from illegitimate integrations. These data suggest that site-specific integration is suitable for streamlining the production of stable transgenic plants.

PS-7

Homologous DNA Integration in Plants. AVRAHAM LEVY. Department of Plant Sciences, Rehovot, 76100 ISRAEL. Email: avi.levy@weizmann.ac.il

Gene targeting, which is homologous recombination-mediated integration of an extra-chromosomal DNA segment into a chromosomal target sequence, enables the precise disruption or replacement of any gene. Despite its value as a molecular genetic tool, gene targeting remains an inefficient technology in higher plants as well as in several eukaryotic species. In species such as chicken, mouse and yeast, earlier reports have shown that the chromatin remodeling activity of Rad54-like proteins, which contain the characteristic ATPase/helicase motifs is important for efficient gene targeting. The genome of plants, like that of other eukaryotes, is organized into chromatin, a compact structure that reduces the accessibility of DNA to machineries such as transcription, replication, DNA recombination and repair. We have developed a new high-throughput assay, based on the use of a fluorescent seed marker, to study the effect of chromatin remodeling on gene targeting in plants. We report that expression of the yeast *RAD54* gene enhances gene targeting in *Arabidopsis* by one to two orders of magnitude, from 10^{-4} to 10^{-3} in the wildtype plants to 10^{-2} to 10^{-1}. We show that the integration events are precise and germinally transmitted. These findings suggest that chromatin remodeling is rate-limiting for gene targeting in plants and improve the prospects for using gene targeting for the precise modification of plant genomes.

PS-8

Using Zinc-finger Nuclease Mediated Homologous Recombination to Manipulate the Mammalian Genome. M. H. PORTEUS. Department of Pediatrics and Biochemistry, UT Southwestern Medical Center, Dallas, TX 75390. Email: matthew.porteus@utsouthwestern.edu

A powerful experimental tool for bacterial and yeast geneticists has been to use homologous recombination to site-specifically manipulate the genome. In addition, homologous recombination has been an invaluable tool in the experimental manipulation of murine embryonic stem cells with the resultant ability to create mice with defined genetic mutations. Unfortunately, the rate of homologous recombination in somatic cells has been too low to be of broad use. In the last decade, several investigators discovered that creating a DNA double-strand break using the I-SceI endonuclease in a reporter gene, stimulated gene targeting by homologous recombination at the reporter by several-thousand fold. We have extended those studies to show that zinc-finger nucleases (ZFNs) can also be used to create double-strand breaks that stimulate homologous recombination. In this session, I will discuss how to design ZFNs, progress we have made in the further development of the biotechnology, and address the nature of the off-target effects of zinc finger nucleases.

PS-9

Chromosome-based Gene Expression Platforms for Cell Engineering. EDWARD PERKINS. Dept. of Biochemistry and Molecular Biology, University of Minnesota School of Medicine, Duluth Campus, Med 249, 1035 University Drive, Duluth, MN 55812. Email: eperkins@d.umn.edu

In comparison to conventional viral-based and nonviral-based gene therapy approaches, chromosome-based gene delivery systems can be stably maintained, autonomous and non-integrating into the host genome. Furthermore, chromosome-based gene delivery systems potentially offer the ability to engineer large genomic regions including distant regulatory elements thereby resulting in sustained transgene expression. Several unique approaches have been put forth towards the creation of next generation chromosome-based systems. These include retrofitting a preexisting small accessory chromosome, truncation of an endogenous chromosome to derive a minichromsomes, *de novo* generation of an artificial chromosome using transfected chromosome elements, and satellite DNA-based amplified chromosomes. Recent advances in satellite DNA-based amplified chromosomes have led to the creation of the ACE system consisting of a platform chromosome that permits rational and tractable site-specific engineering using a modified bacteriophage lambda integrase to deliver transgenes onto the platform chromosome. To date, the ACE platform system has been introduced into a variety of cell types including immortalized, primary and adult-derived stem cells.

Animal Symposia

A-1

The NCI 60 Human Tumor Cell Line Screen: An Information-rich Screen Informing on Mechanisms of Toxicity. ROBERT H. SHOEMAKER. Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, MD 21702. Email: shoemaker@dtpax2.ncifcrf.gov

The NCI 60 human tumor cell line anticancer drug screen was developed in the late 1980's as an in vitro drug discovery tool intended to supplant the use of transplantable mouse tumors in anticancer drug screening. Initially focused on lung cancer drug discovery, it rapidly became clear that some other cell types were necessary as controls. The finding that normal cell types available at the time, i.e. fibroblasts and certain epithelial cell populations such as renal epithelial cells, responded in vitro to anticancer drugs with extreme phenotypes (fibroblasts being pan-resistant and renal epithelial cells being pan-sensitive) in the assay selected for the screen, led to the use of other tumor cell lines as controls. Thus, panels of tumor cell lines were assembled, ultimately representing nine distinct tumor types. While the intent of the screen was to identify compounds with growth inhibitory or toxic effects to particular tumor types (the disease-oriented concept), the patterns of relative drug sensitivity and resistance generated with standard anticancer drugs were rapidly found to reflect mechanisms of drug action. The information-rich character of the screening data provided an additional and unexpected dimension to the screening model which has fueled development of powerful tools for database mining. Indeed, during more than a decade of use, the screen has produced a stream of unexpected discoveries that has impacted fields such as targeted anticancer therapy employing biological agents, virology, pathogenesis of bacterial toxins with bioterrorism potential, and molecular-targeted anticancer drug discovery. In this latter application, the NCI60 profiles of new agents with distinct mechanisms emerging from novel screens can serve to define new mechanistic clusters or to decode previously enigmatic clusters in the existing database. In the case of engineered cell reporter screens, reference to the NCI60 database can help define multiple mechanistic classes within active compounds identified in high-throughput screens.

A-2

High-fidelity In Vitro Modeling of Clinically-defined, Chemically-induced Organ Toxicity in Man Using Adverse Effects of Oncology Drugs as Ethical Learning Sets. RALPH E. PARCHMENT. Laboratory of Human Toxicology & Pharmacology, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland 21702. Email: parchmentr@mail.nih.gov

Innovative chemical structures are important driving forces of R&D effort and product value in the pharmaceutical and chemical industries. Novel structures present a difficult challenge for predicting human safety prior to actual first use, since by definition there is no existing toxicological, SAR or clinical experience to help identify potentially unique organ toxicity profiles and safe dose levels relative to the intended use. Human adverse effects of xenobiotics, whether pharmaceutical or not, are diagnosed and graded in severity by common toxicity criteria. Some of these medical criteria are quantifiable changes in clinical lab values, for example, peripheral blood cell counts and transaminase levels. Others come from medical monitoring (e.g., ECG readings of QTc interval) or physical exam (e.g., mucositis and fatigue). Thus, the goal of non-clinical safety studies of innovator compounds is to predict how these clinical parameters of human organ function will change in response to xenobiotic exposure. In vitro assays are ideally suited to this task if the actual target cell of interest is cultured with retention of its physiological function, so it is capable of responding to xenobiotic exposure in a clinically-meaningful way. The clinical relevance of the cellular response to toxicity can be proven by testing calibrator compounds ("gold reference standards") that are usually anti-cancer agents with known human organ toxicity—exposure profiles coming from the ethical treatment of patients in dose-toxicity clinical trials (Phase I oncology clinical trials). Note the testing is "mechanism-naïve", so safety assessment is not limited to specific known mechanisms of toxicity but rather allows the intact cell to present the integrated response to xenobiotic exposure. The accuracy and value of this strategy was recently demonstrated by an international validation study of a CFU-GM test for predicting acute doses of chemicals that will cause severe, reversible neutropenia. Current work is building on important principles learned from this study about predicting human organ system toxicity—exposure relationships and extending these principles to in vitro assessment of drug-induced mucositis and transaminitis (hepatic toxicity). The CFU-GM study showed that these in vitro systems can be used to predict toxic dose levels in humans, if benchmarked to dose-exposure-toxicity results from rodent or non-rodent animal studies or if combined with toxicokinetic information. Funded by NCI Contract N01-CO-12400. This research was supported in part by the Developmental Therapeutics Program in the Division of Cancer Treatment and Diagnosis of the National Cancer Institute.

A-3

Assessing Hepatotoxicity Through Multiple Endpoints. JAMES M. MCKIM. CeeTox, Inc., 4717 Campus Drive, Kalamazoo, MI 49008. Email: jmckim@ceetox.com

Currently it is estimated that the development a new drug from concept to market can take as long as 12–15 years and cost as much as a billion dollars. When promising new drugs fail in preclinical safety testing, in clinical trials, or are withdrawn from the market due to toxicity a great deal of time and energy are lost. A primary goal of the pharmaceutical industry is to make a more efficient process of drug discovery that evaluates all aspects of candidate selection including toxicity. Developing a process for evaluating New Chemical Entities (NCEs) much earlier in the pipeline for potential adverse properties can dramatically improve the quality of new drug candidates. Safer drugs selected earlier should translate to fewer failures and more efficient use of resources. However, in order for in vitro cell based screening paradigms to work they must be robust enough for scientists to make decisions with confidence. They must have an *in vivo* correlate and they must demonstrate the value of the data sets. Today, approximately 40–60% of NCEs that enter animal safety testing fail due to unanticipated toxicity. Adverse effects in liver, kidney, bone marrow and pancreas are the reasons for these failures. Many of the post market withdrawals of promising drugs are also associated with liver toxicity. The focus of this presentation will be to demonstrate how a tiered systems biology approach to early in vitro evaluations with a proven in vivo correlation can add value to the discovery process. The biochemical profiles of several drugs withdrawn from the market will be shown and special emphasis will be placed on how to make decisions based on in vitro data.

A-4

Eicosanoids in Invertebrate Immunity: An In Vitro Approach. DAVID STANLEY[1] and Jon Miller[2]. [1] USDA, ARS, BCIRL, 1503 S. Providence Road, Columbia, MO 65203 and [2]Dept. Biological Sciences, Northern Illinois University, DeKalb, IL 60115. Email: stanleyd@missouri.edu

Eicosanoids are oxygenated metabolites of arachidonic acid. Two major groups of eicosanoids are prostaglandins (PGs) and various lipoxygenase (LOX) products, all of which exert profound influences in mammals. Eicosanoids also act in insects, where they mediate cellular immune defense reactions to microbial infections and metazoan parasites. In this talk I introduce eicosanoids, review our work on eicosanoid actions in insect immunity, and present new data on cellular resistance to viruses. In our In Vitro studies, *Manduca sexta* hemolymph was collected and diluted. The hemocytes were challenged with bacteria and microaggregation reactions were recorded. Microaggregation reactions increased in challenged preparations. Treating the hemocyte preparations with pharmaceutical inhibitors of eicosanoid biosynthesis disabled the microaggregation reactions. The influence of the pharmaceuticals was reversed by treating the disabled cells with arachidonic acid or with PGH. We infer that hemocytes produce the eicosanoids responsible for mediating cellular immunity in insects. More recently, we considered eicosanoid actions in established insect cell lines. We used two cells lines, one non-permissive to viral infection (HzAM1, a pupal ovarian line from *Helicoverpa zea*) and one permissive (HvAM1, a pupal ovarian line from *Heliothis virescens*). Treating the HzAM1 line with inhibitors of eicosanoid biosynthesis, then challenging the cultures with the AcMNPV baculovirus, resulted in significantly increased proportions of cells producing virus and higher overall extracellular virus concentrations. We infer that eicosanoids affect one or more insect cellular mechanism(s) of viral resistance. We report on similar experiments with the permissive line in a poster presentation.

A-5

Experimental Approaches to Evaluation of Immune Functions in Mosquito Cell Lines. A. M. FALLON, Department of Entomology, University of Minnesota, St. Paul, MN 55108. Email: fallo002@umn.edu

Expression of immune functions in vitro offers an approach to purifying antimicrobial gene products that protect an insect from infection and modulate host-pathogen interactions in vector insects. We have used the C7–10 cell line from *Aedes albopictus* and the Aag-2 line from *Aedes aegypti* to identify several classes of proteins that have antibiotic functions. Synthesis of antimicrobial products can be induced by exposing growing cells to heat-killed bacteria. The cells readily phagocytose dead bacteria, and exhibit associated physical changes including rounded morphology and aggregation. Biochemical changes include secretion of effector proteins, such as transferrin, cecropins, defensins and lysozyme, into the culture medium. Experimental approaches that have been particularly informative include analysis of radiolabeled proteins on polyacrylamide gels, purification of candidate peptides by HPLC, differential display and subtracted cDNA libraries, and protein analysis by tandem mass spectrometry. Our interests now focus on the interaction of the mosquito cell with the obligate intracellular bacterium, *Wolbachia pipientis,* and the means by which *Wolbachia* escapes the cellular immune response.

A-6

Determination of the Effects of Ecdysteroids and JH on Nodulation Responses. V. FRANSSENS[a], G. Smagghe[b], G. Simonet[a], I. Claeys[a], B. Breugelmans[a], A. De Loof [a], and J. Vanden Broeck[a]. [a]Laboratory of Developmental Physiology, Genomics and Proteomics, Department of Animal Physiology and Neurobiology, Zoological Institute, K.U. Leuven, Naamsestraat 59, B-3000 Leuven, BELGIUM and [b]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, BELGUIM. Email: vanessa.franssens@bio.kuleuven.be

Insects have a highly developed innate immune system, including humoral and cellular components. The cellular immune responses refer to hemocyte-mediated processes such as phagocytosis, nodulation, and encapsulation. Nodulation, which is considered the predominant defense reaction to infection in insects, is a complex process influenced by various endogenous factors. However, the precise mechanisms underlying nodulation remain largely unknown. In the present study, we examined the influence of the insect hormones 20-hydroxyecdysone (20E) and juvenile hormone (JH) on the laminarin-induced nodulation reaction in larvae of the flesh fly *Neobellieria bullata*. Treating third-instar larvae of *N. bullata* with 20E prior to laminarin injection enhanced the nodulation response in a dose-dependent manner. The ecdysone agonists RH2485, RH5849 and RH0345 similarly enhanced the nodulation reaction, although they were less active than 20E. In contrast to ecdysone stimulation, supplying larvae with JH or the juvenile hormone analogs (JHA), fenoxycarb and pyriproxyfen, significantly impaired their ability to form nodules in response to laminarin. These findings demonstrate for the first time that 20E and JH play an important regulatory role in the nodulation process.

A-7

Multipotent Adult Progenitor Cells for Vascular Repair. CATHERINE M. VERFAILLIE, Jeff Ross, Fernando Ulloa, Susan Keirstadt, and Aernout Luttun. Stem Cell Institute, University of Minnesota, McGuire Translational Research Facility, 2001–6th St SE, Mail Code 2873, Minneapolis, MN 55455. Email: verfa001@umn.edu

We and others have over the last 5 years demonstrated that cells can be cultured from bone marrow, blood or other tissues that have the ability to differentiate into multiple cell types, not only of mesoderm, but also endoderm and ectoderm. We termed these cells multipotent adult progenitor cells, whereas other groups have named these MIAMI cells, BSSCs, USSCs. We will present evidence that MAPCs can differentiate into endothelium, and be specified to arterial, venous or microvascular endothelium, as well as cells with functional and phenotypic attributes of vascular smooth muscle cells. As such MAPCs could be used to treat vascular insufficiencies. Approaches that will be presented include direct cellular transplantation and generation of bio-artificial vessels form implantation.

A-8

Cell Sourcing for Fibrin-Based Heart Valve-Equivalents. C. WILLIAMS, S. L. Johnson, P. S. Robinson, and R. T. Tranquillo. Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455. Email: tranquillo@cems.umn.edu

Native aortic valve leaflets are composed primarily of valve interstitial cells and myofibroblasts and lined with endothelial cells. However, dermal fibroblasts and smooth muscle cells are often used in tissue-engineered heart valves in attempts to reproduce the native architecture and composition in vitro. For example, we seek to fabricate a bioartificial valve by casting fibrin gel with entrapped cells in the geometry of a valve and controlling the cell-mediated remodeling of the fibrin. Porcine aortic valve cells (PAVCs) and human dermal fibroblasts (HDFs) express different phenotypic markers and perform distinct functions in vivo. In this study we compared PAVCs to HDFs in a fibrin remodeling assay using different basal culture media conditions (DMEM and DMEM/F12) supplemented with 10% fetal bovine serum, transforming growth factor beta (TGF), insulin, ascorbic acid (AA) and aminocaproic acid. We also studied the effect of two growth factors alone and combined on both cell types. Specifically, we compared TGF, fibroblast growth factor (FGF), and FGF with TGF supplementation of DMEM for HDFs and DMEM/F12 for PAVCs. Furthermore, we quantified the effect of different AA concentrations on collagen deposition and mechanical strength. PAVCs cultured in DMEM/F12 were provided with 0, 50 and 150 mg/ml AA and HDFs cultured in DMEM were supplied with 50 and 150 mg/ml AA. In both cases, AA was freshly prepared and added to the cultures 3 times a week. Entrapped cells were cultured in fibrin gels and harvested at 3 and 5 weeks. Collagen and elastin deposition and cell phenotype were evaluated with light level histology and immunohistochemistry, and constructs were assayed for collagen, elastin, cellularity and mechanical properties. There were significant differences among samples cultured in different basal media for a given cell type and between cell types. Significantly greater collagen and elastin deposition and higher ultimate tensile strength and modulus were observed for HDF cultured in DMEM compared to DMEM/F12. HDFs were able to compact the fibrin fibril network more extensively, deposit more collagen, and attain higher ultimate tensile strength compared to PAVCs. PAVC constructs without any AA supplementation contained less collagen compared to the higher AA concentrations, but interestingly, there was no significant difference in collagen deposition between 50 and 150 mg/ml of AA. Replacing TGF with FGF in PAVC constructs caused excessive fibrin degradation and reduced deposition of new extracellular matrix. However, the opposite trend was observed in HDF constructs. These results underscore the importance of cell type and culture media selection and supplementation for the engineering of bioartificial heart valves based on remodeling of fibrin by entrapped cells.

A-9

Engineering Large, Mineralized Bone Tissue Constructs Using Human Mesenchymal Stem Cells. GORDANA VUNJAK-NOVAKOVIC. Department of Biomedical Engineering, Columbia University, New York, NY 10025. Email: gv2131@columbia.edu

Human bone marrow contains a population of mesenchymal stem cells (hMSC) capable of forming several types of mesenchymal tissues, including bone and cartilage. In Vitro expansion and cultivation of hMSC on biomaterial scaffolds could facilitate osteochondral repair, where functional autologous cartilage/bone constructs would be grown and subsequently implanted into the defect site to promote healing. Bone-like and cartilaginous constructs have been made using hMSC and sponges made of biodegradable polyhydroxy-a-esters and silk. However, the construct size and composition were limited by poor mass transfer. We report here that a hydrodynamically active environment of rotating bioreactors markedly improved the structure and mechanical function of engineered cartilage and bone. Culture-expanded hMSCs were seeded onto porous silk scaffolds (8 mm diameter $\times$ 2 mm thick discs, 300–425 μm pores, >95% porosity; 5×10^6 cells per disc). The resulting cell-polymer constructs were cultured for 5 weeks in rotating bioreactors in either control medium (DMEM with FBS), osteogenic medium (control medium with β-glycerophosphate, dexamethasone, ascorbic acid 2-phosphate, rhBMP-2) or chondrogenic medium (control medium with dexamethasone, ascorbic acid 2-phosphate, non-essential amino acids, TGF-β1, insulin). Constructs were assessed for wet weight (ww), dry weight (dw), confined-compression modulus (kPa), DNA, sulfated glycosaminoglycans (GAG), calcium (Ca), alkaline phosphatase activity (AP), histology (von Kossa and safranin-O), and imaged using μCT and contrast enhanced MRI. Bioreactor cultivation of hMSC on silk scaffolds yielded large (8 mm x 2 mm) bone-like tissue constructs with wet weight fraction of Ca approximately 9-fold higher than previously reported, and volume fraction of mineralized tissue within range of values measured for human lumbar verterbral bone. We assume that improved mass transfer in the rotating bioreactor as compared to static culture, in combination with osteogenic effects of BMP-2, were the decisive factors for this highly positive outcome for bone.

A-11

Blood and Endothelial Cell Development from Human Embryonic Stem Cells. D. S. KAUFMAN. Stem Cell Institute and Department of Medicine, University of Minnesota, Minneapolis, MN 55455. Email: kaufm020@umn.edu

Human embryonic stem (ES) cells provide an ideal resource to understand the earliest stages of hematopoiesis (blood cell growth and development). Research on ES cells offers both a unique model to investigate basic developmental biology, as well as a therapeutic cell source to replace or repair cells or tissues damaged by disease or other degenerative processes. Here, we will describe cell culture and animal transplantation models used to elucidate both extracellular protein interactions and intracellular genetic regulation that impact these developmental pathways. Recent work that demonstrates derivation of functional lymphocytes with anti-tumor cell activity will also be discussed, an area of great interest as these results suggest human ES cells may be harnessed as a novel anti-cancer therapy. Finally, other recent studies that demonstrate derivation of endothelial cells and smooth muscle cells from human ES cells as a model of vascular development will also be presented.

A-12

Muscle Stem Cell: Satellite Cell and Sca-1-Positive Cell. ATSUSHI ASAKURA. Stem Cell Institute, Paul and Sheila Wellstone Muscular Dystrophy Center, University of Minnesota Medical School, MN 55455. Email: asakura@umn.edu

Skeletal muscle contains myogenic progenitors called satellite cells and muscle-derived stem cells that have been suggested to be more primitive stem cells. We further investigated the differentiation potential of muscle-derived stem cells and satellite cells to elucidate their biological roles during muscle regeneration. Adult skeletal muscle contains a stem cell population purified as a side population (SP) by FACS on the basis of Hoechst dye exclusion. The muscle SP cells express stem cell antigen-1 (Sca-1) and contain stem cell populations that give rise to skeletal muscle and hematopoietic cell lineages following transplantation in mice. Previous work demonstrates that CD45-positive hematopoietic stem/progenitor cells in the muscle SP population are responsible for the hematopoietic differentiation of the population. Cell transplantation experiments demonstrate that Sca-1$^+$CD31$^+$CD45$^-$ cells in the muscle SP fraction possess the ability to differentiate into endothelial cells (CD31 is an endothelial cell marker). By contrast Sca-1$^+$CD31$^-$CD45$^-$ SP cells express smooth muscle actin and are located perivascularly in muscle. These cells have the potentail to differentiate into myocytes, adipocytes and osteocytes. These results suggest that the major role of muscle SP cells in muscle regeration is to contribute to vascular repair through angiogenesis. In addition, together with satellite cells, muscle SP cells are able to contribute to muscle fiber formation. Taken together, this evidence suggests that muscle SP cells are potentially useful for therapuetic stem cell transplantation for muscle degenerative diseases.

A-13

Nutritional Requirements of Mammalian Cells in Culture: Design and Optimization of the Cell Culture Medium and Processes. PAUL J. PRICE. GIBCO Invitrogen, Grand Island, NY. Email: Paul.Price@invitrogen.com

A classical cell culture medium is made up of a buffered isotonic salt solution supplemented with vitamins, amino acids, a source of energy such as glucose or glutamine and a protein supplement such as serum. As the science of media formulation progressed from serum supplemented to serum-free and then to chemically-defined, substitutes for the serum component and then for serum derived proteins had to be found. Many segments of Industry are presently moving away from media and reagents containing any component of animal or human origin. The outbreak of BSE and CJV in England heightened the awareness of the risk of contamination of therapeutics by prions or viruses and prompted regulatory pressure for companies to address this potential risk. This teaching seminar will cover the basics of a cell culture medium and the advantages and disadvantages of the serum supplement. It will then progress into the construction of serum-free and chemically defined formulations and then to the elimination of all components of animal origin. Ways to optimize both cell growth and productivity will be presented as well as the design of media for specific applications. Emphasis will be placed on reducing apoptosis by controlling osmolality, ammonia, and free radical production and through optimization of the media formulation.

A-14

Cryopreservation of Hepatocytes: Role of Culture Configuration on Survival. ALLISON HUBEL. Department of Mechanical Engineering, University of Minnesota, 1100 Mechanical Engineering, 111 Church St. S.E., Minneapolis, MN, 55455. Email: hubel001@umn.edu

Hepatocytes are being studied for a wide variety of applications. Xenogenic hepatocytes are being used in liver assist devices for temporary liver support. Human hepatocyte transplantation is being used for a wide spectrum of liver diseases. Hepatocytes (human and animal species) are used extensively for in vitro studies of drug metabolism, metabolic and viral diseases of the liver. The supply of fresh differentiated hepatocytes is limited by the ability to induce proliferation of hepatocytes and effectively cryopreserve those cells. Thus, the ability to cryopreserve hepatocytes is essential to insuring supply of these cells. Two distinct challenges exist for preserving hepatocytes: preserving viability and differentiated function. Different approaches to improving post thaw recovery have been used: culture in specialized matrices and culture as spheroids. These approaches have also improved post thaw function as well. Other studies have shown that post thaw apoptosis is a significant factor in cell losses from freezing. Strategies to minimize these losses will be discussed. Finally, advances in our understanding of stem cells (adult and embryonic) may provide new sources of hepatocytes in the future. The importance of developing new sources of hepatocytes and effective methods of preserving them will also be important to the clinical and commercial application of these cells.

A-15

Biophysical and Molecular Changes Associated with Cryopreservation of Sperm. KEN ROBERTS. Medical School University of Minnesota, Departments of Urologic Surgery and Physiology, 6-125 Jackson Hall, 321 Church St. SE, Minneapolis, MN 55455. Email: rober040@umn.edu

The process of cryopreservation invariably induces biophysical changes in sperm, the severity of which depend on the cooling rate, cryoprotectant (CPA) used, and freeze-associated osmotic events. Many of these biophysical events affect the plasma membrane of the sperm and are ultimately dependent upon the plasma membrane's permeability to water during freezing. These biophysical events induce cellular and molecular changes in the sperm, some of which manifest as capacitation-like changes which together have been referred to as 'cryocapacitation'. Optimal cryopreservation parameters (freezing rates, CPA composition and addition, etc) serve to minimize these changes. It is possible that some of these cellular and molecular changes may be minimized, prevented, or reversed by modulation of sperm signaling pathways that lead to physiological capacitation. The biophysical and molecular events of freezing leading to cryocapacitation, and potential mechanisms for prevention of these events, will be discussed.

A-16

Thermal Injury Characterization for Biomedical Applications: In Vitro Model Systems. J. C. BISCHOF. University of Minnesota, Departments of Mechanical Engineering, Biomedical Engineering and Urology, 111 Church St. SE, Minneapolis, MN 55455. Email: bischof@umn.edu

Heat transfer plays a crucial role in many biomedical applications in cryobiology (biopreservation and cryosurgery) and hyperthermic biology (thermal therapies). In these applications, thermal excursions are used to selectively preserve or destroy cells and tissues. Biopreservation is an enabling technology to many biomedical fields including cell and tissue banking, cell therapeutics, tissue engineering, organ transplantation and assisted reproductive technologies. Thermal therapies including cryosurgery are increasingly important in all surgical sub-specialties for minimally invasive thermal destruction of tissues for cancer and cardiovascular disease treatment. In this talk work will be reviewed focusing on in vitro model systems to assess cellular and molecular phenomena that are important in defining outcomes of both cryobiological and hyperthermic biomedical applications. Model systems currently in use in our lab included: cell suspensions, monolayers, engineered tissues (fibrin and collagen based) as well as native tissue explants.

A-17

Three-dimensional Microenvironment and Breast Cancer Progression. PENNEY M. GILBERT[1,2], Nastaran Zahir[1,2], Matthew J. Paszek[1,2], Jonathan N. Lakins[1,2], Kandice R. Johnson[1,2], Gabriela I. Rozenberg[1,2], Alisha Sieminski[1,3], Barbara L. Weber[4,5], and Valerie M. Weaver[1,2]. [1]Institute for Medicine and Engineering, UPENN, Philadelphia, PA, 19104; [2]Department of Pathology, Research Institute, UPENN, Philadelphia, PA, 19104; [3]Department of Biological Engineering, MIT, Cambridge, MA 02139; [4]Abramson Family Cancer Research Institute, UPENN, Philadelphia, PA 19104; and [5]GlaxoSmithKline, Philadelphia, PA, 19101. Email: pgilbert@mail.med.upenn.edu

Stromal-epithelial interactions drive development and maintain tissue homeostasis through a network of soluble and insoluble factors that operate within a three dimensional (3D) tissue. Genetic and epigenetic changes in mammary epithelial cells (MECs) cooperate with a modified tissue microenvironment to drive malignant transformation of the breast. We have been studying how an altered stromal microenvironment contributes to breast tumorigenesis and have specifically focused on understanding the role of changes in extracellular matrix (ECM) composition and organization and integrin expression and activity. Using immortalized normal, premalignant and tumorigenic MECs together with the 3D reconstituted basement membrane (rBM) morphogenesis assay and endothelial co-cultures we could show that the levels, type and activity of integrins change progressively and dramatically during MECs transformation. Recapitulating the altered integrin profile and activity of normal MECs could repress the malignant phenotype of the tumor cells, drive the premalignant behavior of the nonmalignant cells and promote the malignancy of premalignant MECs in culture and in vivo. We also found that tumor progression in vivo is associated with an incremental increase in matrix stiffness that is associated with an increase in integrin expression, focal adhesion maturation and signaling and that increasing ECM stiffness in culture is sufficient to drive similar tumor-like behaviors and alter integrin expression/activity. Accordingly, we have begun to explore how matrix-derived force could drive mammary tumorigenesis and understand what induces ECM stiffness. Interestingly, homeobox genes and force play critical roles in tissue development, are frequently lost in tumors, and can regulate integrin and ECM expression. We showed that HoxA9 is lost in invasive human breast tumors and that re-expressing HoxA9 can revert the malignant phenotype of breast cancer cells in culture and in vivo coincident with BRCA1 induction and normalization of adhesion and integrin expression. Therefore, we demonstrate that proper regulation of the composition and dynamic interplay of cellular and ECM components is essential to the maintenance of tissue equilibrium and evasion of the malignant phenotype in the breast. (Support: DISS0402407, DODW81XWH-05-1-330, NCI CA078731 and DAMD17-01-1-0368).

A-18

3D In Vitro Models Reveal the Invasive, Drug Resistant Phenotype of Metastatic Melanoma. KEIRAN SMALLEY. The Wistar Institute, 3601 Spruce Street, Philadelphia, PA, 19104. Email: ksmalley@wistar.org

Anticancer drug discovery has been long hampered by the lack of predictive models. One explanation for this is that tumors are only drug resistant when grown in the correct tissue microenvironment. Clinically, melanoma is incredibly drug resistant, but this phenotype is rarely seen in tissue culture models. Mouse models, equivalent to human skin, have been difficult to develop due to the differences in anatomy and function of human versus mouse skin. In particular, melanocytes in human skin are aligned on the basement membrane and are dispersed among the epidermal keratinocytes. In mouse skin the melanocytes are situated deep in the hair follicles and dermis. In response to this, our laboratory has developed an organotypic model to recreate the architecture of human skin. However, while these models are histologically equivalent to human skin, they are time-consuming to grow and unsuitable for drug discovery studies. In response to this we have developed a simpler In Vitro model where melanoma cells are grown as spheroids and then implanted into collagen gels. Under these conditions the cells proliferate rapidly and invade into the surrounding collagen in a tumor stage-specific manner. Treatment of the melanoma spheroids with chemotherapy drugs revealed a more drug resistant phenotype than seen in 2D culture. More striking results were seen when the implanted melanoma spheroids were treated with inhibitors of pathways known to be active in melanoma. Cells derived from metastatic melanomas were completely resistant to inhibitors of the PI3/Akt, MEK/ERK and Src pathways when grown as implanted spheroids. However, this resistance was not seen when the same cell lines were grown in adherent 2D culture. The observed drug resistance was dependent upon the presence of serum and the implantation of the spheroids into collagen. Taken together these results demonstrate that culturing the metastatic melanoma cell lines under the correct microenvironmental conditions reveals their drug-resistant nature and that the tumor environment is a critical regulator of drug resistance.

A-19

Reconstructing and Deconstructing the Progression of Human Squamous Cell Carcinoma In 3D Tissue Models. ADDY ALT-HOLLAND. Tufts University, 55 Kneeland St., Room 116, Boston, MA 02111. Email: addy.alt_holland@tufts.edu

The construction of human 3D tissue models of epithelial tissues provides unique experimental paradigms that can trace the complex interplay between multiple cell and tissue types in a biologically-meaningful tissue context. These tissues provide a more global picture of how disease-associated pathways interact in an environment that mimics human tissues and serve as "surrogate" tissues that have set the stage for the accelerated translation of discoveries to the clinic through strategies that will allow target identification and validation. This presentation will describe how our laboratory has developed 3D tissue biology as a portal to discovery of pathways linked to human cancer progression and how these tissue models may serve as a paradigm for clinical translation in the future. We have developed tissue models that mimic distinct stages of squamous cell carcinoma (SCC) in humans including: 1—precancer, 2—low-grade carcinoma and 3—high-grade carcinoma. We have accomplished this by constructing 3D epithelial tissues at an air-liquid interface in which cells have been genetically-modified by suppressing expression of E-cadherin. In light of the emerging view that cancer is a disease of altered tissue architecture driven by abnormal interactions between tumor cells and their tissue microenvironment, we have studied the role of the tumor microenvironment in these stages of SCC progression. We have defined 4 distinct microenvironments that a potentially-malignant cell must encounter as it evolves from precancer to malignancy: 1—Intraepithelial dormancy, 2—Transepithelial migration through the epithelial layers, 3—Attachment to the basement membrane interface and 4—Degradation of basement membrane and stromal invasion. Tissue models that mimic these stages of progression will be described and mechanisms driving them will be outlined. By viewing each microenvironment as a target in the cancer progression pathway, these 3D models have great potential to help close the loop that exists between observations gleaned from rudimentary cell culture systems to those that may be applied in patients-care settings. If this occurs, we will we be able to fully realize the opportunity for translational discovery that these 3D tissue models provide us with.

P-1

Remembering Winter: Vernalization as an Environmentally Induced Epigenetic Switch. RICHARD AMASINO. Department of Biochemistry, University of Wisconsin, Madison, WI 53706. Email: amasino@biochem.wisc.edu

Certain plants, such as biennials or winter annuals, require relatively long periods of cold exposure during winter to initiate flowering the following spring. Cold exposure renders the meristem of such cold-requiring species competent to flower, and this acquisition of competence is known as vernalization. A vernalization requirement ensures that flowering does not occur prematurely before the onset of winter. A similar cold response is bud dormancy; in many species that grow in temperate climates, bud dormancy is not broken until a the plant has "counted" a sufficient number of days of cold to ensure that any subsequent warn weather actually indicates that spring has arrived. Our studies of vernalization in Arabidopsis have revealed that meristem competence is a function of the expression level of certain MADS-box genes such as FLOWERING LOCUS C (FLC) that act as repressors of flowering. Exposure to prolonged cold causes an epigenetic switch of these MADS box genes to an unexpressed state, thus rendering the shoot apical meristem competent to flower. This epigenetic switch is caused by covalent modifications to histones of the chromatin of the flowering repressors.

P-2

Role of miRNAs and siRNAs in Abiotic Stress Responses. JIAN-KANG ZHU. Institute for Integrative Genome Biology and Department of Botany and Plant Sciences, 2150 Batchelor Hall, University of California, Riverside, CA 92521. Email: jian-kang.zhu@ucr.edu

Small non-coding RNAs ranging in size between 20 and 24 nucleotides are important regulators of mRNA degradation, translational repression, and chromatin modification. These small RNAs can be broadly classified as miRNAs (microRNAs) and siRNAs (short interfering RNAs) based on their biogenesis. We found that the expression of some miRNAs and siRNAs in Arabidopsis plants are regulated by abiotic stresses such as drought, soil salinity and cold temperatures. Data on the functional analysis of several miRNAs and siRNAs using mutants and transgenic plants will be presented to support the regulatory roles of small RNAs in plant adaptation to abiotic stresses.

P-3

Diverse Small RNA-directed Pathways in Plants. ZHIXIN XIE. Dept. of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131. Email: zhixin.xie@ttu.edu

Most eukaryotic organisms possess highly conserved RNA silencing machinery that is associated with the formation of 21- ~ 24-nucleotide small RNAs from precursor RNA molecules containing double stranded structures. These endogenous small RNAs, which include microRNAs (miRNAs) and small interfering RNAs (siRNAs) play important roles in regulation of gene expression, maintenance of genome integrity, control of heterochromatin formation, and antiviral defense. Formation or activity of small RNAs requires factors belonging to gene families that encode DICER [or DICER-LIKE (DCL)], ARGONAUTE proteins and, in the case of some siRNAs, RNA-DEPENDENT RNA POLYMERASE (RDR) proteins. Interestingly, unlike many animals, plants encode multiple DCL and RDR proteins. Recent genetic studies in *Arabidopsis* revealed that plants have evolved multiple functionally specialized small RNA pathways that require distinct DCL and RDR factors. Therefore, plants provide a unique system to study the evolution, diversification, and functional adaptation of small RNA pathways. Unique functions associated with distinct DCL and RDR factors in diverse small RNA-directed processes will be presented.

P-4

High Throughput Gene Assembly and Expression Using Viral RNA Replicons Delivered by *Agrobacterium*. Y. GLEBA, S. Marillonnet, and V. Klimyuk. Icon Genetics GmbH, Halle/Saale, D-06120, GERMANY. Email: gleba@icongenetics.de

Plant biotechnology relies on two processes for delivery and expression of heterologous genes in plants, stable genetic transformation and transient expression with viral vectors or with *Agrobacterium*, but only the transient routes provide a speed and a throughput necessary for fast research and development studies. We developed an efficient, versatile and high-throughput vector engineering and expression system based on *in planta* assembly of functional viral vectors from separate pro-vector modules. With this system, we use agrobacteria to deliver various DNA modules that are assembled inside the plant cell with the help of a site-specific recombinase, integrase. The resulting DNA is transcribed, and undesired elements such as recombination sites are spliced out, generating fully functional viral RNA replicons. The proposed protocol allows, by simply treating a plant with a mixture of two or more agrobacteria carrying specific prefabricated modules, to rapidly and inexpensively assemble and test multiple vector/gene combinations, without the need to perform various engineering steps normally required with alternative methods. The process described is very fast (3–6 days); it provides very high protein yield (up to 5 mg per g fresh leaf biomass); and it is based on over 250 prefabricated genetic modules that allow to express a specific protein by adding specific promoters, signal/transit peptides, purification tags, protein fusions, etc., in multiparallel studies.

P-5

Signaling Networks Controlling Disease Resistance Responses in Arabidopsis. J. GLAZEBROOK, Raka Mitra, and Lin Wang. Department of Plant Biology, University of Minnesota, Saint Paul, MN 55108. Email: jglazebr@umn.edu

Plants respond to pathogen attack by activation of a battery of defense responses. Activation is controlled by a complex regulatory network. Sectors of this network are commonly defined based on the identities of needed small molecule signals, salicylic acid (SA), jasmonic acid and related compounds (JA), and ethylene (ET). Genetic dissection of this signaling network in Arabidopsis has identified major regulatory genes involved in these three sectors. A complete understanding of defense network topology and function requires genome-scale analysis. To this end, we have studied genetic perturbations of the network by transcriptional profiling using whole-genome microarrays. Similarity relationships among the transcriptional profiles obtained from key mutants were used to create a basic model of network topology. It is obvious that effective defense requires many regulatory and effector genes which have not yet been identified. Many of these genes are expected to show increased expression in response to pathogen attack. In an effort to identify them, plants with mutations in pathogen-induced genes have been screened for enhanced disease susceptiblity phenotypes. Several important genes have been identified, including a cytochrome P450 monooxygenase required for synthesis of the antimicrobial compound camalexin.

P-6

Profiles in Scourge: Gene Expression Analysis of a Crop Killer. H. CORBY KISTLER and Kye-Yong Seong. USDA-ARS, Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108. Email: hckist@umn.edu

Filamentous fungi are the most common and devastating causal agents of plant disease. Among the diseases of crop plants most important worldwide is Fusarium blight of wheat and barley caused by the fungus *Fusarium graminearum*. With the availability of genome sequences for several filamentous fungi including *F. graminearum*, large-scale functional genomics programs and genome-wide expression analysis is now possible. From the Fusarium sequence we have developed the first Affymetrix GeneChip microarray based on the entire genome of a filamentous fungus. To understand the early infection cycle of the pathogen, we monitored the RNA expression profiles in newly formed spores, in maturing spores and during the early stages of spore germination. We also examined fungal gene expression during infection time-courses in wheat and barley. The ability to detect fungal genes *in planta* is surprisingly sensitive even without efforts to enrich for fungal transcripts. These studies will help to accelerate understanding of pathogen—host interactions by elucidating expression of pathogenicity determinants in the fungus and disease response pathways in the plant.

P-7

In Planta Transcriptional and Functional Patterns of an Agriculturally Relevant R Gene. JAMES M. BRADEEN, B. P. Millett, and D. S. Mollov. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108. Email: jbradeen@umn.edu

Potato late blight disease, caused by *Phytophthora infestans*, is among the most costly crop diseases worldwide. The disease affects both foliage and tubers. Breeding efforts to improve potato late blight resistance have led to the hypothesis that foliar and tuber blight resistance is conditioned by different resistance (R) genes. Previous research also documents changes in foliar blight resistance throughout plant development, suggesting R gene regulation or function is dependent on physiological stage. The cloning of the foliar blight gene *RB* provides tools to test and explore these phenomena. We have developed a highly sensitive RT-PCR assay to examine transgene *RB* expression. *RB*, like many R genes, is one of a cluster of sequence similar but functionally disparate gene copies. However, our assay differentiates not only between *RB* paralogs and *RB* alleles, but even between the *RB* transgene and the allele from which it was cloned. We have also optimized whole plant and whole tuber assays to functionally test for late blight resistance. The potato-*P. infestans* pathosystem is an ideal system in which to test tissue-specific transcriptional and functional regulation of R genes, since *P. infestans* is a natural pathogen of two distinct plant tissues, leaves and tubers. We compared transgene *RB* transcription in foliage and tubers with results of our functional assays. Although the *RB* transgene is expressed in all plant tissues, leaves of transgenic plants are late blight resistant while tubers of transgenic plants are late blight susceptible. In related studies, we are exploring transcriptional and functional regulation of transgene *RB* throughout plant development. We have compared pre-flowering, flowering, and post-flowering transgenics using our RT-PCR and foliar blight resistance assays. Our research provides insight into strategies for the integration of transgene *RB* into potato disease management schemes. Future experiments include exploration of *RB* protein levels and comparison the disease response transcriptomes in various plant tissues and throughout plant development.

P-8

Use of a High-performance, Custom Microarray for Elucidation of Signaling Networks Controlling Plant Defense Responses. M. Sato, R. Mitra, R. van Poecke, J. Glazebrook, and F. KATAGIRI. Department of Plant Biology, University of Minnesota, Microbial and Plant Genomics Institute, St. Paul, MN 55108. Email: katagiri@umn.edu

In systems analysis of a biological system, it is crucial to quantitatively and economically obtain a broad spectrum of information that characterizes the state of the system in detail. mRNA expression profiling could yield data with high information content for the cost. We developed a highly accurate, small-scale, oligonucleotide-spotted microarray ("mini"-array) for the purpose of analyzing Arabidopsis responses to pathogen infection. For a broad spectrum, we selected 464 genes that represent diverse expression patterns captured through many Arabidopsis GeneChip experiments related to biotic interactions. We employed 107 normalization genes with a wide range of expression levels for accurate array-to-array normalization. A hybridization signal from each spot was calibrated by a hybridization signal from the calibration oligonucleotide that was included in each probe solution when the array was spotted. Each probe was spotted by four pens out of total 16 pens. The patterns of four pens for different probes were made overlapping in a symmetric manner, so that the pen effect was removed from the signals by fitting to a linear model. We obtained the correlation between technical duplicates better than 0.98. We conclude that we can omit technical replicates for a miniarray measurement. When the expression ratios calculated from the miniarray data were compared with those calculated from the Affymetrix ATH1 GeneChip data, the correlation was 0.88. This high quantitativity was confirmed by qRT-PCR. We are currently using the miniarray for the purpose of sensitive screening and detailed characterization of reverse genetic Arabidopsis lines and detection, classification, and mapping of naturally occurring alleles in loci controlling defense responses.

P-9

Testing Methods for DNA and Proteins in Transgenic Crops. RAYMOND D. SHILLITO. Bayer CropScience, Research Triangle Park, NC 27709. Email: ray.shillito@bayercropscience.com

The Agricultural, Biotechnology, Grain and Food and Feed Industries test for the presence of transgenic material in plants, seeds, grain and food. Testing starts with confirmation of the transgenic nature of calli, and regenerated plants, and follows the plant through the breeding process until it is commercial. In addition, tests are needed for ensuring purity of commercial seed, and once the crop leaves the farm gate it may be segregated for use in certain markets. Testing is performed in order to satisfy the needs of those involved in trade of grain and foodstuffs to comply with regulatory and labeling requirements that are in force in many countries. Agricultural Biotechnology companies develop methods as part of the product development cycle. These are improved as the product comes to market. Methods are also developed by commercial and government testing laboratories. This presentation will briefly describe some of the tests available for detecting and quantifying DNA and proteins in transgenic plants, seeds, grain and food. Different test methods have different costs and are used to obtain different results. Spraying plants (if they are herbicide resistant) shows if they are sensitive to herbicides. Protein-based methods such as LFS and ELISA can be used in several ways including to measure the expression of the trait (such as the PAT protein). PCR and other DNA-based methods can be used for screening or identify the actual event concerned, and RT-QPCR can estimate the amount present. It is also important to consider the resources required when deciding on the test method to use, as well as whether it needs to be done in the laboratory or the field, and whether the necessary technical skills are available.

P-10

Applications of Testing Methods in the Grain Industry. R. W. GIROUX. Cargill Incorporated, Wayzata, MN, 55391. Email: randal_giroux@cargill.com

Several countries have adopted or are in the process of developing legislation related to the approval of genetically modified grain and grain products and/or the mandatory labeling of foods containing these products. Most countries that have adopted an approval process or mandatory labeling schemes have set tolerances for events or thresholds for the adventious presence of transgenic material in grain products or the final foods based on a %GM content. Once such regulations are enacted, industry and government require analytical methods to monitor supply chains, certify product compliance, and enable enforcement. To test for the presence of transgenic events or to measure the %GM requires validated methods that are fit for the purpose and are suited to the testing environment. To meet this need, the food and feed supply chain are adopting different strategies to make %GM determinations, including protein and DNA-based testing (PCR). During this presentation, several of the approaches that have been implmented will be discussed. In addition, discussion of emerging information on the impacts of genetics, processing, and method performance on these measurements will be discussed.

P-11

A Global Perspective on the Economic Impact of Transgenic Crop Varieties. GREG TRAXLER. Department of Agricultural Economics, Auburn University, AL 36849. Email: traxlgj@auburn.edu

Commercial transgenic crop varieties have been available in the US and other countries since 1996. Twenty-one coutnries grew genetically modified (GM) crops in 2005, but the countries of North and South America accounted for 94%, of world GM crop area. Diffusion has been concentrated among crops and traits as well; four crops (soybean, maize, cotton and canola) and two traits (herbicide tolerance and insect resistance) account for 99% of GM crop area. This presentation reviews the adoption of GM varieties, and surveys studies that have measured the level and distribution of economic benefits from GM crops. The economic benefits of the diffusion of GM crop varieties have been widely shared among farmers, industry, and consumers even though delivery has been through the private sector. GM crops have had a favourable environmental impact by facilitating reduced pesticide use and adoption of conservation tillage. Key institutional factors influencing GM diffusion, and the potential for developing countries to benefit from GM technology are discussed.

P-12

Current Status and Impact of Commercial Plant Tissue Culture. STEVEN MCCULLOCH. Mountain Shadow Nursery LLC, Olympia, WA 98513. Email: steve@mtshadow.com

The present day successes with micropropagation or tissue culture propagation of woody plants are due in part to the early advancements made in the 1960s and 1970s with orchid, tropical and herbaceous plant tissue culture. Since the early 1980s the scope and breadth of commercial woody micropropagation in the United States has continuously expanded. This expansion has been tempered by the higher costs associated with this technology. Commercial woody plant propagation is limited to production of crops that require or benefit from this propagation method. An overview of the history, crops produced and the current and future opportunities of commercial woody plant micropropagation will be discussed.

P-13

Status of Commercial Tropical Foliage Plant Micropropagation. GARY HENNEN. Oglesby Plants International, 2664 SR 71N, Altha, FL 32421-2848. Email: garyh@oglesbytc.com

As the world's appetite for tropical ornamental plants continue to grow, regional production systems are becoming increasingly larger, sophisticated and complex. Micropropagation has been an integral part of tropical foliage production for decades and as the tropical ornamental industry grows so does the requirements for larger volumes of high quality plants. At the same time, large consumers of tropical ornamentals are constantly demanding lower price points, pushing commercial propagators to look for alternative production methods and technologies requiring significant capital investments. This presentation will look at the great success story the micropropagation industry has enjoyed working with tropical ornamentals and some of the challenges facing the industry in the near future.

P-14

Temporary Immersion Bioreactor: An Efficient Technology For Scaling-up Plant Production. M. ESCALONA[1], J. González-Olmedo[1], I. Cejas [1], C. Aragón [1], I. Capote [1], R. Rodríguez[2], M. J. Cañal [2], J. Sandoval [3], S. Roels [4], P. Debergh [4]. [1]Laboratory for Plant Cell and Tissue Culture, Bioplant Centre, University of Ciego de Avila, CUBA; [2]Dept. B.O.S, University of Oviedo, SPAIN; and [3]CORBANA, COSTA RICA; and [4]Dept. of Plant Production University Gent, BELGIUM.

Temporary immersion has been shown to reduce problems usually encountered in liquid culture. Based on this concept, a collective of researcher belong to BioplantCenter adapted a semi-automated system for large-scale propagation of plants. This bioreactor has been named as Twin Flasks system (BIT®) and grouped into the systems with complete immersion by pneumatic driven transfer of liquid medium without medium replenishment. BIT® is relatively simple and easy to use. They enable contact between all parts of the explants and the liquid medium. The culture environment is renewal by forced ventilation during each immersion period. For special type of plants, a forced aeration in the culture recipient can be used. The injection of CO_2 permits to improve the photomixotrophic culture. BIT® has been used for in vitro commercial propagation of a wide range crops: *Ananas, Saccharum sp, Musa sp, Colocasia sp, Araceaes, Eucaliptys sp, Rosaceae, Bromelias, Paeony.* In order to establish a micropropagation procedure and increase the efficacy of BIT®-technology, different parameters should be optimized. Among them, the immersion time, immersion frequency, the volume of nutrient medium, the volume of culture container, the duration of proliferation phase, the use of plant growth retardant, the number of cycle in BIT®. Plants regenerated by BIT® have not showed somaclonal variation detected by molecular probes and evaluations in the field. The simplicity and low cost of BIT® is compatible with large-scale propagation. It permits important lower labor, better biological yield and consecutively reduces production cost.

P-15

Manufactured Seed—An Efficient Method for Delivery of Somatic Embryos to Nurseries. W. C. CARLSON, Weyerhaeuser Technology Center, WTC1B10, PO Box 9777, Federal Way, WA 98063–9777. Email: bill.carlson@weyerhaeuser.com

Manufactured seed can provide a delivery mechanism for cost effective implementation of clonal forestry. Manufactured Seed technology is designed to sow somatic embryos into bare root and container nurseries, enabling seedling growing in conventional facilities. Seed design, automation and seedling cost near that of current orchard seed are all critical to implementing the technology widely in forestry. Weyerhaeuser is in late stages of development for low-cost, automated manufactured seed production processes for its seed design. The seed design incorporates many analogs of natural seed, including biodegradable materials. Manufacturing processes involve automating all stages of the process, from somatic embryogenesis through sowing in the nursery. Demonstration machinery for manufacturing and advanced automation for many of the steps in seed manufacturing are completed and operating.

P-16

Therapeutic Protein Expression in the Plant-based LEX System. VINCENT P. WINGATE, Biolex, 158 Credle Street, Pittsboro, NC 27312. Email: vwingate@biolex.com

Lemna, or duckweed, is a small aquatic plant that can be quickly transformed to produce recombinant proteins in a contained and controlled bioprocessing environment. The benefits of the LEX System™ include a high transformation efficiency, rapid clonal growth in a contained and controlled environment with high expression using a simple and inexpensive production format. To date, over 25 different human therapeutic proteins have been produced by the LEX System™, including several hard-to-make proteins and 10 different monoclonal antibodies.

P-17

Production of Biodefense-Related Proteins in Tobacco. K. WYCOFF. Planet Biotechnology. Email: kwycoff@planetbiotechnology.com

Botulism and anthrax are lethal bacterial diseases whose symptoms are caused mainly by biological toxins. Botulinum neurotoxin and anthrax spores are considered potential bioweapons, and current defenses and treatments are inadequate to protect a large target population that is likely to include civilians. Both therapeutic and prophylactic treatment with monoclonal antibodies have been shown to be more effective and safer than alternatives, but stockpiling sufficient doses is likely to be expensive. Plants as a production system offer the promise of lower costs. We have demonstrated that plant-made monoclonal antibodies to botulinum neurotoxin A are effective and safe in a mouse model. We have also produced in plants a fusion protein based on a human anthrax toxin receptor and shown it to be an effective decoy, blocking the toxic effect of anthrax lethal toxin in vitro.

P-18

Transgenic Expression and Recovery of Biologically Active Recombinant Human Insulin from *Arabidopsis thaliana* Oilseeds. ELIZABETH W. MURRAY[1], Cory L. Nykiforuk[1], Joseph G. Boothe[1], Richard G. Keon[1], H. Joseph Goren[2], Nancy A. Markley[1], Maurice M. Moloney[1] [1]SemBioSys Genetics Inc, 110, 2985-23 Ave NE, Calgary, Alberta. T1Y 7L3 CANADA and [2]Department of Biochemistry and Molecular Biology, University of Calgary, Faculty of Medicine, Calgary, Alberta T2N 4N1 CANADA. Email: murraye@sembiosys.com

With the emergence of new delivery technologies and the rise in incidence of diabetes, the demand for affordable insulin will soon exceed the current manufacturing technologies. To address this potential production shortfall, we have developed a novel expression and purification technology to produce human insulin in oilseed plants. Recombinant human precursor insulin was expressed in *Arabidopsis* oilseeds and was found to accumulate at a level of 0.13% of the total seed protein. The precursor protein was digested with trypsin, in vitro, to produce mature insulin with a mass identical to that of the predicted $DesB_{30}$-insulin product. In addition, we confirmed the biological activity of this plant produced insulin using cell based assays of receptor phosphorylation and insulin tolerance tests in mice.

P-19

An Overview of the Orchid-fungal Symbiosis in Nature, and its Application In Vitro to Promote Conservation. L. W. ZETTLER. Orchid Recovery Program, Department of Biology, Illinois College, Jacksonville, IL 62650. Email: lwzettle@ic.edu

In nature, the orchid life cycle is initiated and closely tied to the availability of fungi in various substrates (*e.g.*, decaying wood, *Sphagnum* moss). For reasons not yet understood, fungi infect orchid seeds forming coils of hyphae (pelotons) within the embryo, protocorm, seedling, and mature plant. Once lysed, these structures serve as a critical energy (carbohydrate) source until photosynthesis is initiated, and give these plants an alternative nutritive strategy (=mycotrophy) into adulthood. In terrestrial orchids, mycotrophy is believed to supplement photosynthesis, but serves as the primary source of carbon for achlorophyllous species. Epiphytic orchids, which may rely on mycotrophy to a lesser extent, utilize fungi as a source of free water to resist desiccation on arboreal substrates. Given the importance of fungi *in situ*, the preservation of orchid seeds alone has raised conservation concerns, and has prompted interest in using fungi in vitro for propagation (=symbiotic seed germination). This presentation will discuss ongoing global efforts to cultivate orchids threatened with extinction using fungi (*e.g.*, *Platanthera holochila*, endemic to Hawaii), and will discuss the potential ecological consequences of this practice.

P-20

Symbiotic and Asymbiotic Orchid Seed Germination as Tools in Conservation. S. L. STEWART. Environmental Horticulture Department, University of Florida, Gainesville, FL 32611. Email: sstewart@ifas.ufl.edu

The loss of orchid-rich habitat worldwide, as well as the restoration of many historical orchid habitats has demonstrated the need for researchers to develop appropriate propagation methods for the Orchidaceae. Traditionally, seed germination has been seen as the most efficient and effective method of plant production for orchids-asymbiotic seed germination historically being the most commonly used method for both conservation or commercial ends. While asymbiotic germination does represent a simple method for the mass production of orchids, it does not take into account the physiological need for fungal mycorrhizae during seed germination and, possibly, during later life stages. Only symbiotic orchid seed germination accounts for this fungal partner in both germination and subsequent development of the orchid plant. Both the asymbiotic and symbiotic methods represent possible avenues in producing orchid seedlings for use in conservation and restoration efforts. The benefits and shortcomings of both methods will be discussed.

P-21

In Vitro Strategies for Conservation of Madagascar's Endemic Orchids. MARGARET MARY FROM. Omaha's Henry Doorly Zoo, Center for Conservation and Research, 3701 S 10th Street, Omaha, NE, 68107. Email: psl@omahazoo.com

Madagascar's orchid species represent some of the most critically endangered members of the family on earth today. The high degree of endemism; estimated at more than 80%, the richness of diversity among Madagascar's orchids, and the continuing threats to the country's remaining natural areas, make the country's conservation action plan imperative if these species are to survive for the future. Ex situ conservation measures present an opportunity to help preserve the island's more than one thousand orchid species. Few orchids are being propagated in Madagascar, and in vitro germination protocols were not previously worked out for the majority of the native orchid species. Reintroduction of orchids to the country's remaining natural areas was virtually non-existent prior to this collaborative effort. Orchid populations are rapidly declining in many parts of the country. A partnership between Omaha's Henry Doorly Zoo in the USA; which provided all in vitro biotechnology training, and the University of Antananarivo, Madagascar, successfully propagated many endemic Malagasy orchids at a great distance from the seed source. A strategy of in vitro culture that allows the orchids to be returned to Madagascar while still in sterile cultures, minimizes the risk of introducing pathogens to the natural environment. The micropropagated orchids are treated to lower nutrient and sucrose levels in vitro prior to their return to protected areas, and are acclimatized to ex vitro conditions when they arrive in Madagascar. Timing the reitroductions to coincide with the wet season helps assure a higher survival rate. Additionally, cryopreservation protocols were developed at Omaha's Henry Doorly Zoo laboratory, which are preserving orchid seed germplasm for long-term conservation purposes. Species representing 14 seperate orchid genera have been successfully micropropagated with more than 600 individual orchids already returned to Madagascar from the zoo, where they have been acclimatized and reintroduced directly into Ranomafana National Park through cooperation with the government and local residents in Madagascar. In some cases no natural seedling recruitment has occurred near the mother plants first observed in 2000, and the seedlings successfully reintroduced in 2004 and 2005 represent the only juvenile specimens for some of the populations being studied.

P-22

Expanding the Utility of Alfalfa. R. A. DIXON, F. Chen, Y. Pang, and G. Peel. Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, Oklahoma, 73401. Email: radixon@noble.org

Alfalfa (*Medicago sativa*) is perhaps the world's major forage legume. Forage quality itself is an important target for biotechnological improvement. Many years of research have identified lignin as an impediment to forage digestibility, and lack of condensed tannins as promoting pasture bloat and limiting nitrogen nutrition. Recent progress on understanding and manipulating the pathways leading to lignin and condensed tannins will facilitate engineering of alfalfa and other forage legumes for reduced bloating potential and improved digestibility and palatability. The above traits involve natural products, which are made by plants for their own health care (ie to ward off pests and pathogens). However, the impact of plant natural products on human health is increasingly recognized. With the advent of genetic and genomic approaches, the synthesis of many plant natural products is now understood at a level to permit their engineering in crop plants. Alfalfa has been engineered as a delivery vehicle for isoflavone phytoestrogens and the antioxidant epicatechin. Many other compounds of nutraceutical or pharmaceutical value could be profitably made in alfalfa. Forage crops with genetically improved quality (output) traits will benefit both the health of the animals that consume them and the environment through reductions in waste excretion and greenhouse gas emission. Furthermore, the same modifications to lignocellulose that improve digestibility may also improve the processing ability of forage crops for biofuel production.

P-23

From Crops to Biorefineries. O. V. SELIFONOVA. Biotechnology Development Center (BioTDC), Cargill Incorporated, Minneapolis, MN 55440. Email: olga_selifonova@cargill.com

Alternative or genetically modified crops have become an integral part of modern biotechnology. Current methodology for introduction of foreign genes into economically important plant species can be used not only for crop improvement and production of novel products in plants, but also for alteration of biomass composition that is tailored for needs of industrial bioprocessing. Biobased industrial chemicals derived from biomass, vegetable oils and carbohydrates have all prerequisites to compete with the scale, flexibility and efficiency of the petrochemical industry. 3-Hydroxypropionic acid (3-HP) is one of the examples of biobased industrial chemicals that can be produced from renewable sugars. The chemical is not commercially available in large quantities, but has the potential to be a new industrial platform chemical. 3-HP has two functional groups and can be readily converted to a range of important chemicals, such as acrylic acid and 1,3-propanediol. Currently, no known organism makes 3-HP as a metabolic end-product. Using various sources of genetic information we designed and constructed several different metabolic routes to 3-HP. We then selected one route for further development based on a series of design criteria, including high theoretical yield from sugars. This route also required significant use of directed evolution to modify and improve enzyme activities. This work provides an example of the use of modern biotechnology to develop sustainable routes to industrial chemicals from crop-based renewable resources.

P-25

Development of Roundup Ready®Alfalfa Varieties. MARK MCCASLIN, Peter Reisen, Holly Deery, Sharie Fitzpatrick, and Stephen Temple. Forage Genetics International, P.O. Box 339, Nampa, ID 83653–0339. Email: mccaslin@forgegenetics.com

Roundup Ready® Alfalfa (RRA) was jointly developed by Forage Genetics International (FGI) and Monsanto Company. In 1998 FGI produced ~ 150 transgenic alfalfa plants expressing the CP4 EPSPS gene, driven by an enhanced version of the Figwort mosaic virus promoter. Alfalfa is a cross pollinated autotetraploid plant. To achieve high trait purity (i.e. >90% Roundup tolerant plants in a RRA variety) a two transgenic event, dihomogenic breeding strategy was adopted. From the initial 150 transgenic plants two commercial transgenic events were selected based on tolerance to Roundup herbicide, agronomic performance, molecular characterization and reproductive stability of the transgene insert. Event-specific PCR markers were developed to genotype Roundup Ready® plants carrying one or both transgenic events. Both Roundup Ready® Alfalfa (RRA) events were introgressed into elite FGI breeding populations using a modified backcrossing breeding strategy (MBCx). Four to six cycles of modified backcrossing were used to develop FD3 to FD9 type breeding populations. Advanced MBCx lines containing the two commercial events (RRA event B and RRA event D) were kept separate in the field and in the greenhouse. Elite parents were selected from breeding nurseries established with seedlings from these advanced MBCx lines. Crosses between elite parents containing event B were crossed to elite parents containing event D. The 1,1 dihomogenic progeny, containing a single copy of each event, were identified using event specific markers. Based on pedigree and agronomic characteristics sets of these dihomogenic plants became Syn0 parents for RRA experimental varieties. In 2003 Syn1 seed of several RRA experimental varieties (FD3-FD8) was produced in isolation near Nampa, ID. Trait purity of these populations ranged from 92.5 to 94.8%, with a mean of 93.8%—very near the theoretical, expected value of 93.7%. In August and September 2003 multiple location variety trials were established to evaluate agronomic performance and crop safety of RRA experimental varieties. Forage yield and quality data collected in 2004 and 2005 showed performance of the RRA experimental varieties, under conventional herbicide treatment, equal to or better than the commercial check varieties. Crop safety of the RRA experimental varieties sprayed with maximum labeled rate of Roundup herbicide was excellent. Following U.S. deregulation of the two transgenic events in June 2005, fifteen RRA varieties were commercially released in August, 2005.

* ®Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC.

P-26

Development and Characterization of Alfalfa Populations Tolerant to Glyphosate. GLEN ROGAN, Sharie Fitzpatrick, Todd Pester, Daniel Kendrick, Michael Horak, Melinda McCann, Karu Karunanandaa, Stephen Temple, and Mark McCaslin. Monsanto Company, 800 N Lindbergh Blvd, St. Louis MO 63167. Email: glennon.j.rogan@monsanto.com

Monsanto Company and Forage Genetics International have developed varieties of Roundup Ready* alfalfa that are tolerant to glyphosate, the active ingredient in Roundup® agricultural herbicides. Roundup Ready alfalfa was developed using *Agrobacterium*-mediated plant transformation to stably incorporate into the alfalfa genome a coding sequence that encodes for a glyphosate-tolerant form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Roundup Ready alfalfa varieties enable growers to apply Roundup agricultural herbicides from planting through five days before cutting, providing an additional tool for improved weed control, excellent crop safety and preservation of yield potential and forage quality. Characterization of Roundup Ready alfalfa plants was performed as part of the food, feed and environmental safety assessment performed prior to regulatory submissions. Key questions addressed were whether the introduced trait or the transformation and regeneration process impacted the phenotype of alfalfa or composition of forage and whether there were any significant environmental impacts associated with the introduction of Roundup Ready alfalfa. Information and data on the introduced trait indicate that the CP4 EPSPS protein is safe for consumption. Other than the introduction of tolerance to glyphosate, there were no biologically meaningful phenotypic differences between Roundup Ready alfalfa populations and the alfalfa control or conventional reference varieties. The levels of key nutrients and components in Roundup ready alfalfa forage were comparable to the control and within the population of commercially available alfalfa varieties. Collectively, these results establish that Roundup Ready alfalfa is safe for use as feed or food and for release into the environment. *®Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC.

P-27

Impact of Yieldguard Rootworm on Corn Rootworm Control. S. C. JOHNSON, G. R. Heck, and T. T. Vaughn. Monsanto Company, Chesterfield, MO 63017. Email: ty.t.vaughn@monsanto.com

In 2003, the Monsanto Company commercialized YieldGuard® Rootworm (YGRW) protected corn hybrids. These hybrids use a variant Bt Cry3Bb1 insecticidal protein which is known to be biologically active against several species within the Coleopteran family Chrysomelidae, including western corn rootworm, *Diabrotica virgifera virgifera* LeConte, northern corn rootworm, *D. barberi* Smith and Lawrence, and Mexican corn rootworm, *D. v. zeae* Krysan and Smith. YGRW expresses the Cry3Bb1 protein in the root system where larvae feed and inflict damage. Thus, the level of expression is critical to the control of the insects that come into contact with the root system. Through a series of experiments, it was shown that while the level of Cry3Bb protein may vary between hybrids and over time, the level of protection is not affected by such variation; that is, the expression level in commercial hybrids is sufficient to provide consistent protection for CRW larval feeding. There are many benefits for growers, consumers and the environment associated with the commercialization of YGRW. YGRW hybrids are more efficacious than soil and seed-applied insecticides in protecting roots from larval feeding damage. The Cry3Bb1 protein produced in the root does not require activation (as many conventional insecticides do), and its performance is unlikely to be impacted by severe environmental conditions. Varieties containing the Cry3BB1 protein are also combined through conventional breeding with other genetically enhanced maize varieties. Combinations with herbicide tolerance and lepidopteran insect protection provide hybrids to growers that offer more complete crop protection from the most economically damaging insect and weed pests. This performance and grower satisfaction has been demonstrated over the past 2 years of commercial experience. YGRW technology was initially launched in 2003 on approximately 400,000 acres. The number of acres have steadily increased over the past 2 years to approximately 4.5 to 5 million acres in 2005. Growers that have adopted YGRW technology have been extremely pleased with the performance and yield protection generated from planting these hybrids. In 2005 for example, most growers realized a significant yield advantage over other control options. This advantage was likely augmented due to the severe drought in 2005 across much of the corn belt. The YGRW technology protected the roots from larval feeding allowing the roots to access water from a deeper zone within the soil profile.

P-28

Wide-cross Whole-genome Radiation Hybrid Mapping in Cotton. DAVID M. STELLY. Dept. of Soil and Crop Sciences, Texas A&M University, College Station, TX USA 7843-2474. stelly@tamu.edu

The development of animal genomics was catalyzed strongly by the advent of whole-genome in vitro radiation hybrid (RH) mapping. In lieu of a comparable in vitro plant system, we opted to develop an in vivo system, based on interspecific hybridization between *Gossypium hirsutum* L. and *G. barbadense* L, using irradiated pollen. The underlying rationale was that [1] an egg cell nucleus could be used to "rescue" the irradiated sperm nucleus, [2] the interspecific nature of hybridization could provide allelic diversity, [3] the gamma irradiation could segment the inherited paternal genome, and [4] the unique origin of each radiation hybrid would greatly reduce and potentially eliminate chimerism within individual panel members. Initial experiments established a workable irradiation treatment and culminated in the establishment of a 5-Krad wide-cross whole-genome radiation hybrid (WWRH) panel of *G. hirsutum*. RH maps were derived by analysis of SSR data using RHMAP software, and were compared to locally available linkage maps and hypoaneuploid cytogenetic stocks. While the 5-Krad (50-Gy) RH panel was capable of detecting synteny among unlinked primitive linkage maps, the map resolution seemed to be too low in some areas. We therefore re-investigated higher dosages, and constructed and characterized a second WWRH panel after 8-Krad segmentation of the *G. barbadense* L. genome. Limited numbers of strategically chosen markers were used to compare WWRH mapping results to the 5-Krad WWRH maps and to linkage maps. The results indicate WWRH mapping can contribute significantly to cotton genomics.

P-29

Evolution of Chromatin Structure and Function. S. M. Kaeppler. Department of Agronomy, University of Wisconsin, Madison, WI 53706. Email: smkaeppl@wisc.edu

DNA packaging into chromatin is necessary for stable chromosomal segregation, and also affects transcription. DNA is packaged around octamers of histones, and histone and DNA modifications determine local chromatin states. Transitions among chromatin states occur via chromatin remodeling proteins. Protein motifs involved in chromatin modification and remodeling are conserved across plants and animals. However, important differences are observed when comparing plant and animal proteins, and monocot versus dicot proteins. In this presentation, I will discuss how our consortium has discovered putative chromatin proteins in plants using queries from diverse species. DNA methyltransferases, SET-domain proteins, and methyl-binding domain proteins will be used to exemplify evolution of chromatin proteins. Functional consequences will be discussed, and patterns of divergence will be highlighted.

P-30

Allium Genomics: Exploiting Model Plants for Analyses of Enormous Nuclear Genomes. M. J. HAVEY. USDA-ARS and University of Wisconsin, Madison, WI 53706. Email: mjhavey@wisc.edu

Enormous genomic resources have been developed for the grasses, culminating with the complete genomic sequence of rice and reduced-representation sequencing of maize. These extensive resources may be applicable to other major groups of monocots outside of the grasses. The order Asparagales (carries the Alliums and asparagus) and the commelinids (carries the grasses) are sister monophyletic groups within the monocots. The Alliaceae (onion, garlic, leek, chive, bunching onion, among others) is the second most economically important family in the monocots, following only the Poaceae. The huge nuclear genomes of the Alliaceae are major constraints to the development of genomic resources. We sequenced asparagus and onion BACs and revealed high densities of retroelements and transposons with few open-reading frames. We also observed little synteny on the recombinational and sequence levels among asparagus, onion, and rice, as might be expected given that the Asparagales and commelinids split at least 130 million years ago. Nevertheless, genomic resources developed for the grasses are useful for translational genomics of the Alliums. Occasionally physically linked sequences in rice show genetic linkage in onion and this microsynteny across shorter genomic regions aids in the identification and mapping of candidate genes. Single-copy expressed regions in the rice genome show significant similarities and share most introns with coding regions in onion, allowing the development of PCR-based markers carrying indels or single nucleotide polymorphisms to evaluate for associations between candidate genes and economically important traits.

P-31

High Efficiency and High Throughput Transformation of Cereals Mediated by *Agrobacterium* for Functional Genomics. T. KOMARI. Plant Innovation Center, Japan Tobacco Inc., 700 Higashibara, Iwata, Shizuoka 438–0802, JAPAN. Email: toshihiko.komari@ims.jti.co.jp

Major cereals joined the list of plants that can be transformed by *A. tumefaciens* a decade ago. Then protocols for rice and maize have been tremendously improved. Overall efficiency has been increased at least 10 times, range of transformable genotypes widened, time for tissue culture shortened, workload for tissue culture lessened, and related techniques developed like elimination of selectable markers from transformants and reduction of vector backbone transfer. The progress in transformation technology, which plays indispensable roles in applications including construction of T-DNA tagged lines, map-based cloning, characterization of cloned genes and large-scale screening for gene effects, is a key factor in current and future advances in cereal functional genomics. Throughput of transformation in functional genomics must be very high. Rice is ideal in this context because of remarkable efficiency, short tissue culture periods, and small workloads for tissue culture. Maize is also high in efficiency of transformation but falls short of rice. Thus a possible option is to screen gene effects initially in rice and to characterize selected genes in maize. Taking advantage of the efficiency in rice, we have initiated a high-throughput process to screen genomic fragments of plants that may cause phenotypic changes. This is an approach in functional analysis of genomic sequences, which has recently been highlighted due to importance of non-protein-coding sequences and of multiple patterns of transcription in studies of eukaryotic genomes. This system was designated as *TraitExplorer*™ because agronomically useful phenotypes identified in the process could be exploited as new traits in crop improvement. Initial characterization of transgenics has indicated that genomic fragments with unique functions may potentially be found by this method.

P-32

Plant Tissue Transformation Using Periodic Arrays of Vertically Aligned Carbon Nanofibers. T. E. MCKNIGHT, A. V. Melechko, G. D. Griffin, and M. L. Simpson. Oak Ridge National Laboratory, Oak Ridge, TN 37831. Email: mcknightte@ornl.gov

Periodic arrays of vertically aligned carbon nanofibers (VACNFs) have been demonstrated as effective vectors for delivery of large molecules, including DNA, into a variety of cell types. Using an approach that combines the massive parallelism of whisker-mediated delivery and the precision of microinjection, arrays of VACNF needles may be surface modified with material and simultaneously pressed into the intracellular domains of large numbers of mammalian and plant cells. While a variety of micromachined materials have been studied as gene delivery arrays for both plant and animal cells, these materials often have been found to have inadequate aspect ratio or lack mechanical strength to effectively penetrate the plant cell wall. In contrast to these micromachined materials, VACNFs feature a covalent bonding structure that provides strong, but flexible, vertical elements well suited to the rigors of cellular interfacing. They also have extremely high aspect ratio, with tip diameters of typically less than 100 nm and lengths up to many tens of microns. Mechanical strength and high aspect ratio provides for effective penetration into cells, including those protected by rugged cell walls such as yeast and pollen. The surface of nanofibers may be modified with adsorbed or covalently attached biomolecules and interfaced into cellular targets, including direct penetration into the nuclear domain. In this overview, we will describe the fabrication and functionalization of VACNFs and the application of these modified VACNFs as massively parallel delivery vectors for both plant and animal cell transformation.

P-33

DNA-coated Nanoparticles Mediated Transgene Expression in Plant Cells. François Torney[1], Brian Trewyn[2], Supratim Giri[2], Victor Lin[2] and Kan Wang[1]. [1]Center for Plant Transformation, Iowa State University, Department of Agronomy Ames, IA 50011 and [2]Department of Chemistry, Iowa State University, Ames, IA 50011. Email: ftorney@iastate.edu

Plant genetic engineering relies mostly on biolistic and *Agrobacterium*-mediated transformation technologies. Both techniques allow DNA delivery into plant cells and subsequent integration into the genome. Recently, the development of nanomaterials such as mesoporous silicate nanoparticles (MSN) was shown to deliver marker genes into animal cells (Radu et al., 2004). The distinct feature of this nanoparticle is that it can both deliver DNA as well as chemicals encapsulated in the particles. Controlled release of the filling substance is also possible using this material (Gruenhagen et al., 2005). Here we show that this material can be used for transforming tobacco mesophyll protoplasts and immature maize embryos. Transgene expression was observed both transiently and stably. In addition, chemicals encapsulated in the MSN can be controlled-released *in planta* when appropriate induction reagents are applied. The use of mesoporous silicate nanoparticles to deliver transgenes and various substances simultaneously into plant cell opens a wide range of applications for future plant genomic study.

Gruenhagen, J.A., Lai, C.Y., Radu, D.R., Lin, V.S., and Yeung, E.S. (2005). Real-time imaging of tunable adenosine 5-triphosphate release from an MCM-41-type mesoporous silica nanosphere-based delivery system. Appl Spectrosc 59, 424–431.

Radu, D.R., Lai, C.Y., Jeftinija, K., Rowe, E.W., Jeftinija, S., and Lin, V.S. (2004). A polyamidoamine dendrimer-capped mesoporous silica nanosphere-based gene transfection reagent. J Am Chem Soc 126, 13216–13217.

P-34

Transfection: A Reliable and Efficient Method for Maize Transformation. M. E. HORN, J. A. Esser and G. Hall, Jr. Agrigenetics/Mycogen, 5649 E. Buckeye Road, Madison, WI 53716. Email: michael.horn@phytonbiotech.com

Maize has historically been amongst the most difficult of plant species to genetically engineer. Currently, particle bombardment (biolistics) and *Agrobacterium tumefaciens* are used to insert foreign genes into maize in a stable manner. Both of these methods are covered by patents making them expensive to practice for commercial purposes. We have developed a third method, one that is free of patent encumbrance, is reasonably efficient, and produces regenerated transformed plants in a reasonable mount of time. Callused immature zygotic embryos are incubated with naked DNA in a sterile cuvette for a given length of time and then electroporated at a specified voltage, capacitance, and pulse length and wave shape. A decay wave was successful whereas a square wave was not. The protocol was optimized for transient expression of the GUS gene and those parameters were used to produce stable transformants at a frequency of ~0.5%. The use of tobacco Rb7 matrix attachment regions (MARs) gave a frequency of nearly 4% using the optimum transient parameters. Further optimization of the electroporation parameters for stable transformation resulted a transformation frequency of nearly 4% even without MARs. Further optimization could raise the transformation frequencies even higher. Without MARs, single copy insertion events composed about 50% of the total. With MARs, the proportion of single copy insertion events was 90% of the total. This data implies that Transfection is a viable transformation system for maize.

P-35

A Novel Plant Transformation Technology—Lipoic Acid. YINGHUI DAN*. Monsanto Company, 700 Chesterfield Parkway, St. Louis, MO 63017 and *Current address: Institute for Advanced Learning and Research, and Departments of Horticulture and Forestry, Virginia Polytechnic Institute and State University, 150 Slayton Avenue, Danville, VA 24540. Email: ydan@vt.edu

For the first time a breakthrough in plant transformation technology has been discovered in the antioxidant, lipoic acid (LA), found in most living organisms. Utilizing LA in *Agrobacterium*-mediated transformation processes across five different plant species has significantly improved the transformation methods, even for previously recalcitrant genotypes. Frequencies of soybean independent plant transgenic events were increased from 0.6 to 3.6%, potato from 3 to 19%, tomato from 28 to 94%, and wheat from 2.9 to 5.4%, and putative transgenic embryo frequency of cotton from 41 to 61%; frequency of escapes was reduced in soybean from 92 to 72%, potato from 50 to 16% and tomato from 91 to 53% under the optimal conditions. This study also demonstrated that the increase of the transformation frequency and reduction of escapes in tomato were accompanied by 2-fold reduction in severity of browning/necrosis of *Agrobacterium*-infected cotyledonary tissues, 2-fold increase in the survivability of the transformed cotyledonary tissues, 4-fold increase in the percentage of transgenic shoots and 3-fold reduction of the percentage of non-transgenic shoots when using LA under optimal conditions. LA application in plant transformation has dramatically resolved the three common problems in plant transformation: recalcitrance, tissue browning/necrosis of the transformed cells/tissues, and escapes, which severely limit the number of transgenic plants that can be regenerated.

P-36

Evaluation of an Automated Image Analysis System for Factors which Stabilize Gene Expression. J. M. CHIERA and J. J. Finer. Department of Horticulture and Crop Science, OARDC/The OhioState University, Wooster, OH 44691. Email: chiera.4@osu.edu

A commonly observed phenomenon after transformation of plant tissues, regardless of delivery method, is variable gene expression. This phenomenon requires the time consuming task of creating a large number of clones in order to increase the probability of obtaining a successful transformation event. The reasons for this variability in gene expression are unknown, but it is likely that protein or message instability, or transgene silencing is involved. With the advent of green fluorescent protein (GFP) and other fluorescent proteins, it is now possible to track transgene expression from the time of DNA introduction to plant recovery. The use of fluorescent proteins in combination with automated image collection and analysis software allows for the continuous monitoring and quantification of *in vivo* gene expression in multiple tissue samples over time. With these tools, we can begin to dissect factors that positively or negatively affect transgene expression in transiently and stably transformed tissues. We have evaluated sequences that appear to stabilize GFP expression in a cotyledon transient expression system. In control bombardments, without the additional sequences, GFP expression peaks 24 hours post bombardment and declines to minimal levels within 72 hours. Use of GFP fused to some sequences led to extended GFP expression through 168 hours. Co-introduction of these same sequences on different vectors extended expression slightly but not to the same extent as the GFP-fusions. These factors may influence gene expression by stabilizing protein, mRNA, or by suppression of host silencing.

P-37

Agrobacterium Mediated Gene Transfer in Plants. MARC VAN MONTAGU. Institute Plant Biotechnology for Developing Countries (IPBO), Universiteit Gent, BELGIUM. Email: mamon@psb.Ugent.be; Website: www.psb.UGent.be

The discovery that some Rhizobiaceae had evolved into pathogens capable to genetically engineer a plant cell became an interesting chapter in the study of plant microbe interactions. The exploitation of this capacity, now a good twenty years ago, to engineer novel traits into crop plants, was a major breakthrough in fundamental and applied plant sciences. The possibility to add and later, thanks to the iRNA-technology, to silence at will a set of genes in most plant species, got molecular plant sciences off the ground. It also allowed the engineering of new traits in some of the major crops. Now, global agriculture requires the extension of this technology to the improvement of barely domesticated crops. Unraveling the molecular base of plant growth and development, of stress response and of biomass production is now becoming possible. Industry already plans the production of new compounds and new materials in plants. To do the research and these applications successfully, we need a substantial improvement of the efficiency of our universal gene vector Agrobacterium. We can ineed not rely on engineering of some model plants. We should be able to engineer the highest yielding cultivars and make that they can be grown in a sustainable way, this means with less water and nutrients than in today's agriculture. It is urgent that specialist in "Plant Cell and Tissue Culture" take up this challenge.

A-1000

Response of Rosiglitazone, UAB 30, and Atorvastatin in the Human Melanoma Prevention Assay. E. ELMORE[1,2], A. Jain[1], L. Kopelovich[3], F. L. Meyskens[2], V. E. Steele[3], and J. L. Redpath[1,2]. [1]Department of Radiation Oncology, University of California, Irvine, CA, 92697, [2]Chao Family Comprehensive Cancer Center, University of California, Irvine, CA, 92697, [3]Chemopreventive Agent Development Research Group, Division of Cancer Prevention, NCI, Bethesda, MD, 20892. Email: eelmore@uci.edu

Due to the increasing incidence of malignant melanoma, which was projected to reach over 59,000 cases in 2005 (http://seer.cancer.gov), and the poor prognosis for patients with late stage disease, we have developed a screening assay for identifying melanoma prevention agents. The assay measures chemopreventive agent induced changes in melanoma-related biomarkers in radial growth phase human melanoma cells (WM3211). We report data on rosiglitazone—a PPARγ agonist used to treat Type II diabetes, UAB 30—a retinoid that is selective for RXRα, and atorvastatin—an HMG-CoA reductase inhibitor used to lower cholesterol. The assay incorporates an exposure to UVB (25 mJ/cm^2) with both pre- and post-treatment with potential preventive agents. Biomarkers used to measure agent efficacy include: induction of annexin V, an early marker for apoptosis; induction of E-cadherin, a biomarker that is expressed in melanocytes and WM3211 cells but is lost in metastatic melanoma cells; inhibition of n-cadherin, a biomarker that is expressed in melanoma cells but not in melanocytes. E-cadherin plays a key role in the communication between melanocytes and keratinocytes, which is important in the control of melanocyte growth *in vivo*. N-cadherin expression allows the fibroblasts to control the growth of melanocytes. The following are some of the important findings from our study. Rosiglitazone was positive for E-cadherin induction and strongly positive for N-cadherin inhibition.UAB 30 and atorvastatin induced E-cadherin at multiple concentrations. All three agents demonstrated a positive effect on the N-cadherin/E-cadherin expression ratios relative to the ratios with cells treated with UV-B alone. Rosiglitazone and atorvastatin were active at clinically achievable concentrations. The activities of these agents in the assay were: rosiglitazone > atorvastatin >UAB 30. The assay data suggests that rosiglitazone, UAB 30, and atorvastatin have potential to prevent melanoma. Supported by NCI contract No. N01-CN-43300.

A-1001

Lineage Specificity and Interspecies Variation in Hematopoietic Toxicity Testing. Carla Pereira, Jackie Damen, CINDY MILLER, and Emer Clarke. Contract Services, StemCell Technologies Inc, Vancouver, BC V5Z1B3, CANADA. Email: emer@stemcell.com

In the search for efficient and cost effective ways to screen lead compounds for hematotoxicity, the use of Colony Forming Cell (CFC) assays has received a great deal of attention. These robust standardized assays allow the detection of toxicity on hematopoietic progenitor subsets (erythroid, myeloid, megakaryocytic) to evaluate potential cytopenic conditions, as well as mesenchymal progenitors to evaluate potential damage to bone and connective tissue. The toxic effects of three antineoplastic compounds were tested on erythroid, myeloid and mesenchymal progenitor growth. Results indicate that each compound displays a unique spectrum of toxicity on each progenitor lineage showing different relative susceptibility to toxicity depending on the compound tested.

IC_{50} Values for Various Bone Marrow Derived Progenitors

Progenitor	5-Fluorouracil	Hydroyurea	Paclitaxel
Erythroid	2.4 ug/mL	75 uM	5 ng/mL
Myeloid	0.5 ug/mL	30 uM	5 ng/mL
Mesenchymal	0.4 ug/mL	148 uM	4 ng/mL

In addition, up to 10-fold differences were seen between human and murine progenitor sensitivity to each compound. Our data highlights the multifaceted nature of primary cells from bone marrow and unique specificity of action of individual compounds.

A-1002

Computer-aided Tissue Engineering: Predicting Self-assembly of Prostate Cancer Spheroids. K. O'CONNOR, H. Song, and S. Clejan. Tulane University and Health Sciences Center, New Orleans, LA 70118. Email: koc@tulane.edu

Computational methods that predict tissue assembly aid in the production of in vitro constructs that mimic native tissue. In particular prostate cancer cells self-assemble on an attachment-limiting substrate into spheroids that resemble micrometastases. These tissue constructs exhibit drug resistance that approaches clinical levels and have application to high-throughput drug testing and design of patient-specific treatments. Two mathematical models of spheroid formation have been developed based on collision theory and Monte Carlo technique. The models accommodate a variety of size populations in the inoculum: single cells, spheroids of different sizes, and combinations of cells and spheroids. Model simulations provide an excellent fit to experimental concentrations of spheroids as measured by the residual error between these two data sets. Collision theory predicts spheroid size distributions over a 5-fold range of cell concentrations in the inoculum. Also it accurately predicts trends in the adhesion properties of DU 145, LNCaP and PC 3 cells, including an up-regulation in the expression patterns of E-cadherin and other adhesion molecules upon spheroid formation. Monte Carlo simulations predict long-range interactions between aggregating cells on the order of several cell diameters. This study provides experimental evidence that cancer cells, which have deficient gap junctions, communicate with intercellular bridges that transport membrane vesicles (1 to 3 microns in diameter) between cells. The bridges contain tubulin and can extend at least 100 microns in length. The computational methods presented here have proven exceptional robust in predicting the physical assembly of spheroids and underlying biological phenomena. Since the composition of spheroids is dependent on their size, the models may be able to predict both spheroid size and composition from the properties of the inoculum. In addition, spheroids may be useful in the study of intercellular adhesion and communication, which have prominent roles in metastatic progression of cancer.

A-1003

Characterization of Neuroblastoma Cells Cultured in Three-dimensional, Microgravity Rotary Bioreactor: Organoid Formation and Free Cell Dynamics. R. A. REDDEN and E. J. Doolin. The Children's Institute for Surgical Science, The Children's Hospital of Philadelphia, Philadelphia, PA 19104. Email: redden@email.chop.edu

Neuroblastoma, one of the most common and deadly pediatric tumors, features clinical, genetic, and biologic heterogeneity that defies simple risk assessments and demands more extensive characterization. The three-dimensional rotary bioreactor offers a unique low-shear, microgravity culture environment in which many cancer cell lines form small tumor-like organoids. The bioreactor allows analysis of inherent cellular characteristics and behavior, without confounding influences seen in animal models and traditional 2D culture. Materials and Methods. A suspension of human neuroblastoma cells (CHP-212, ATCC) was seeded into the rotary bioreactor (Slow-turning lateral vessel, Synthecon) at 5 x 10^5 cells/ml. Two aspects were examined: 1) growth, morphology, and composition of organoids, and 2) the number and viability of 'free' cells. Organoid samples were taken at 12 hours, and at day 1, 2, 5 and 8; and were stained with hematoxylin and eosin. Digital microscopic images were analyzed using SigmaScan software. Media samples for cell counts—both viable and nonviable—were taken hourly for the first 12 hours and daily afterwards. Results/Discussion. Heterogeneous cell aggregates formed spontaneously within 6 hours. Over time, the aggregates became larger homogeneous spheroids composed of hundreds of viable cells, with characteristic inner necrotic regions, presumably due to limited diffusion of nutrients. Furthermore, the thickness of the viable zone likely indicates inherent cellular resistance to hypoxia. The number of free cells decreased exponentially, plateauing at less than 5% of original seeding density by 12 hours. After day 1, nonviable cells began to increasingly appear, presumably due to shedding. The free cell profile curves, both the initial disappearance of viable free cells and the later appearance of nonviable cells, may be an indirect measure of cell-cell adhesive capability and organoid metabolism. Identification of inherent cellular factors intrinsic to specific clinical presentations of neuroblastoma could provide invaluable information regarding characterization and treatment of this devastating disease.

P-1000

RNAi-Mediated Silencing of Maize Chromatin Genes Confer Increased Transformation Efficiency in Maize. M. A. MCGILL, S. M. Kaeppler, and H. F. Kaeppler. University of Wisconsin-Madison, Dept. of Agronomy, Madison, WI 53706. Email: mamcgill@wisc.edu

Chromatin remodeling plays a crucial role in gene regulation and expression. Therefore, we hypothesize that disruption of chromatin-associated genes that function either to regulate nucleosome spacing or to maintain a 'silent' state of transcription will increase transformation frequency in maize. The maize chromatin-associated gene SGB101 is the probable maize ortholog of yeast ARD1, an N-terminal acetyltransferase required for telomere and mating-loci silencing. The maize chromatin gene HON102 encodes a linker histone protein within the Histone H1 homology group. In our study, RNAi-inducing inverted-repeat (IR) constructs targeting the maize chromatin-associated genes SGB101 and HON102 were bombarded in individual, replicated experiments along with the empty vector control, pMCG161, and the stable transformation efficiency was measured. The average stable transformation rate for the IR construct targeting SGB101 was 9.0%, and was statistically significantly higher than the pMCG161 control efficiency of 4.3% over 12 independent, replicated experiments. Similarly, the average stable transformation rate for the IR construct targeting HON102 was 6.9%, and was also statistically significantly higher than the average transformation rate of 3.9% for pMCG161 over 9 independent, replicated experiments. Maize RNA-silenced SGB101 and HON102 T_2 lines are currently being re-transformed with a control vector and stable transformation efficiencies compared between sibling 'null' and RNA-silenced embryos. Methylation status of repetitive sequences in both the individual RNA-silenced T_0 lines and pMCG161 T_0 lines will be presented. Our current interpretation is that RNAi-induced silencing of chromatin-associated genes increase transformation frequency through a repression of transcriptional gene silencing (TGS).

P-1001

Stable Transformation of *Taxus*. HELENA MATHEWS, Nikolaus Matheis, Fira Negru, Karin Connors, Vaka Reddy, Al Lammers, Debra Schuster, Mylavarapu Venkatramesh, and D. Ry Wagner. Email: hmathews@exelixis.com

Paclitaxel (Taxol®), a complex diterpenoid derived from *Taxus* species has stimulated intense research during the past two decades due to its antitumor activity and more recently due to potential additional therapeutic uses. In order to provide reliable, high quality preparations of paclitaxel and other taxanes we have turned to *Taxus* cell culture as a production method for these compounds. As a part of our research and development program we have undertaken the metabolic engineering of the paclitaxel biosynthetic pathway. While significant progress has been made in understanding the biochemical pathways for taxanes biosynthesis, no routine transformation protocol of *Taxus* cells is currently available. In this presentation we will report a successful, stable transformation system for *Taxus* in which we have achieved up to 10% transformation efficiency in experiments to date.

P-1002

A Novel Shoot Organogenesis and Transformation System for *Nicotiana obtusifolia* Accession PI 555573. BAOCHUN LI, Willa (Qingwei) Huang, and Hui Qiu. Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY 40546-0236. Email:bli2@uky.edu

N. obtusifolia accession PI 555573 is of important interest for PMP-oriented plant variety development since it is resistant to *P. tabacina*, the causal agent of the major tobacco disease blue mold, in a hypersensitive fashion. There is a tremendous interest to study this plant using modern genomics tools. To this end, an efficient regeneration and transformation system will be very beneficial. However, *N. obtusifolia* accession PI 555573 is not responsive to tissue culture using its leaf pieces as explants, a common practice for tobacco shoot organogenesis and transformation. We have identified the immature seeds from *N. obtusifolia* accession PI 555573 to be highly responsive for shoot organogenesis. About 48% of the immature seeds produced shoots or calluses four weeks after they were incubated on a shoot induction medium; approximately 88% of those responsive produced shoots with an average number of about 15 shoots after they were subcultured onto the same shoot induction medium for an additional four weeks. An Agrobacterium-mediated transformation system using this shoot organogenesis method plus an infection by *A. tumefaciens* strain GV3850 carrying binary vector pKM24G that harbors an *nptII* gene and a *gusA* gene has also been developed. Approximately 41 putative events have been recovered and grown in the greenhouse for T1 seed production. T1 seeds from 34 events have been collected, and assayed for kanamycine resistance by germinating them on a kanamycine-containing medium. Kanamycine resistance was able to be transmitted to the T1 progeny in 32 events, and was transmitted in a Mendelian 3:1 ratio in 8 of the 32 events. The kanamycine resistant T1 seedlings were also found to be *gusA* positive by histochemical assays. We are now in the process of performing Southern hybridization on the genomic DNA from those T1 plants to characterize transgene integration.

P-1003

Selecting Disease Resistant Transgenic Grapevine for Field Tests. D. J. GRAY, Z. T. Li, S. A. Dhekney, M. Dutt, M. Van Aman, J. Tattersall, and K. T. Kelley. University of Florida/IFAS, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703-8504. Email: djg@ufl.edu

In preparation for field tests of transgenic grapevine, greenhouse screening was conducted to identify and evaluate a lytic peptide (LIMA-1) gene that rendered transgenic plants resistant to the xylem-limited bacterium *Xylella fastidiosa*, which causes Pierce's disease (PD). Concurrently, an endogenous thaumatin-like protein (VVTL-1) gene was re-engineered for constitutive expression and transgenic lines selected for resistance to the foliar fungus *Uncinula necator*, which incites powdery mildew disease. Transgenic plants of *Vitis* cultivars 'Merlot', 'Seyval Blanc' and 'Thompson Seedless' and rootstocks 'Freedom' and 'Tampa' expressing LIMA-1 are now being screened for PD resistance. 'Merlot', 'Seyval Blanc' and 'Thompson Seedless' containing VVTL-1 are being screened for powdery mildew resistance. The commonly-adopted practice of grafting grapevine scions to rootstocks presents unique opportunities to simultaneously evaluate several transgenes and/or control unwanted gene flow. For example, novel biologically active peptides produced by a transgenic rootstock may be carried in xylem sap through a graft union to protect a non-transgenic scion from diseases. Possible composition of a transgenic grapevine field test include the following: 1) PD resistant transgenic rootstock grafted to various non-transgenic scions, which would contain gene flow, 2) PD resistant rootstock grafted to powdery mildew resistant scion, which would allow gene flow of VVTL-1 (limited to pollen for 'Thompson Seedless') but test the concept of resistance to multiple pathogens via grafting, 3) Non-grafted transgenic and non-transgenic plants to serve as controls.

P-1004

The Immunodominant Allergen Ara h2 Is Silenced In Transgenic Peanut Via The RNA Interference (RNAi) Strategy. K. N. KONAN[1], O. M. Viquez[2], and H. W. Dodo[1]. [1]Alabama A&M University, Department of Food and Animal Sciences, Food Biotechnology Laboratory, Normal AL, 35762; and [2]Vanderbilt University Department of Pathology, C3322-MCN, Nashville, TN 37232. Email: koffi.konan@email.aamu.edu; hortense.dodo@email.aamu.edu

RATIONALE: Allergy to peanut is a growing health problem worldwide. It is an IgE mediated hypersensitivity reaction, and the most deadly of food allergies. Yet to date, there is no effective treatment. A transgenic peanut with a significant reduction or total elimination in allergen content would considerably reduce the incidence of allergic reactions in peanut sensitive individuals. The objective of this study was to downregulate and/or silence Ara h2, the most immunodominant allergen from peanut using the RNAi strategy. **METHODS**: Peanut hypocotyls were infected with *Agrobacterium tumefaciens* harboring an Ara h2-specific inverted repeat transgene separated by a pkd intron sequence. Southern blots were performed to determine the presence and integration of this transgene in the genome of regenerated plants, and Western blots were performed using an Ara h2 monoclonal antibody to specifically detect the presence or absence of the Ara h2 protein. **RESULTS:** A total of 51 plants were kanamycin-resistant following transformation. Their phenotype and growth rate were comparable to the control plants. Southern analysis revealed 35 out of 51 plants stably integrated the Ara h2 inverted repeat transgene. Western blot revealed 2 transgenic lines with total elimination of the Ara h2 protein doublet band of about ~ 20 kDa, and 3 other transgenic lines with a reduction in the Ara h2 protein content as compared to the control non transgenic. **CONCLUSIONS:** The immunodominant peanut allergen Ara h2 expression was successfully down-regulated and silenced by RNAi. These data provide proof of concept and support the feasibility of the production of hypoallergenic/allergen-free peanut using the RNAi strategy.

P-1005

Antibiotic Marker Free Approach for Obtaining Salt Stress Tolerant *Vigna mungo* (blackgram). N. B. SARIN[1], P. Bhomkar[1], C. P. Upadhyay[1], S. Deb Roy[1], R. Rajwanshi[1], A. Muthusamy[1], M. Saxena[1], N. Shiva Prakash[1], M. Pooggin[2] and T. Hohn[2]. [1]School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA; and [2]Botanical Institute, University of Basel, SWITZERLAND. Email: ceracon@vsnl.com, hohn@fmi.ch

The production of pulses is facing a number of constraints due to soil salinity, poor soil fertility and moisture retention capacity of marginal and submarginal soils. Blackgram [*Vigna mungo* (L.) Hepper] is an important pulse of India providing a rich source of dietary protein to the largely vegetarian population. Adverse biotic and abiotic stresses limit the yield of *Vigna mungo*. Because of a narrow genetic base in the pure-line selection and limited natural variation for abiotic stress tolerance, a viable option is to genetically engineer blackgram for abiotic stress tolerance. In this investigation, the candidate gene was the Glyoxalase-1 (Gly1), the over-expression of which has earlier been shown to impart salt, drought and heavy metal stress tolerance in a model plant. For antibiotic marker free approach, a novel construct, pnptlox+csm-Gly1, in which the Gly1 gene is driven by the constitutive promoter, *Cestrum* (csm) viral and the selection marker (kanamycin) gene is flanked by lox sites, allowing its removal by the lox-sequence specifice cre-recombinase, has been used. Transgenics of *V.mungo* have been developed, in which the Southern analysis showed single copy insertion of the Gly1 transgene.The transgenic plants showed higher salt and methylglyoxal tolerance as compared to the controls under laboratory conditions. Experiments are continuing to assess the efficacy of this system under contained glass-house conditions.

P-1006

In Vitro Production of Turmeric (*Curcuma longa* L.) Microrhizomes as a Potential Source for Secondary Metabolites. MATTHEW COUSINS[1], Jeffrey Adelberg[1], Feng Chen[2], and James Rieck[3]. [1]Dept. of Horticulture, Clemson University, Clemson, SC 29634; [2]Dept. of Food Science and Human Nutrition, Clemson University, Clemson, SC 29634; and [3]Dept. of Experimental Statistics, Clemson University, Clemson, SC 29634. Email: jadlbrg@clemson.edu

Production of phytochemicals through the use of cell, callus, and hairy-root culture has been studied significantly in the past. In this study, microrhizomes of turmeric (*Curcuma longa* L.) plantlets were developed in vitro in large (6-liter) Liquid Lab vessels. Fresh and dried microrhizomes were processed from four clonal accessions. Methanolic, soxhlet derived extracts were assayed as to their ability to scavenge the DPPH radical and chelate ferrous iron to determine their antioxidant potentials. Paired comparisons were made between extracts of fresh and dried tissue within clones. EC_{50}s of tissue extracts were compared with extracts from commercially produced store bought powdered turmeric. Fresh tissue extracts were significantly more potent than extracts from store bought turmeric powder in all cases for both assays. In the DPPH assay, tissue drying led to a significant decrease in potency in all clones. The effect of tissue drying on ferrous iron chelating ability of extracts was clone specific. DPPH scavenging effects of dried tissue were usually of similar intensity to store bought powders with only one clone showing a significant difference in potency. Extracts from recently dried tissue were significantly more potent than extracts from store bought turmeric powder with regard to the iron chelation assay. Commercial harvest, curing, processing, and storage methods may have negative effects on the antioxidants present in rhizomes of turmeric although genotypic selection can minimize this effect. This research presents an alternative method to acquire quality-assured, pest-free plant material on a schedule irrespective of uncertainty associated with international outsource of seasonal, agricultural products.

P-1007

Castor Seed Development and Storage Lipid Biosynthesis. GRACE Q. CHEN[1], Yeh-Jin Ahn, and Louisa Vang. US Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710 Email: QHGC@pw.usda.gov

Castor seed (*Ricinus communis* L.) produces an oil at 60% of seed dry weight, and its fatty acid composition consists of 90% ricinoleate (12-hydroxy-oleate). Because of the presence of the hydroxy group, the fatty acid has physical and chemical properties that make it desirable for many industrial uses, including lubricants, coatings, plastics, and fungicides. However, castor seed contains toxin ricin and hyper-allergenic 2S albumins detrimental to castor grower and processor. Thus, considerable effort has been directed toward the understanding of the mechanisms of ricinoleate biosynthesis and transgenic production of ricinoleate from temperate oilseed plants. To explore the control points contributing to the ricinoleate accumulation, a series of seed development studies including morphogenesis, fatty acids accumulation and expression profiles of lipid genes involved in castor oil biosynthesis have been conducted. By using quantitative real time polymerase chain reaction analysis, the transcripts levels for 14 lipid genes at various stages of seed development have been determined. The lipid genes displayed various temporal patterns and had maximum induction ranging from 2 fold to 43,000 fold. Furthermore, gene expression clustering analysis using a computer software revealed that the expression pattern of the lipid genes fell into five distinct clusters. These expression-profiling data provides insight into the regulatory networks that coordinate the global response to seed developmental programs and lead to castor oil synthesis.

P-1008

Production of Biologically-active *Acidothermus cellulolyticus* endo-1, 4-β-glucanase (E1) Enzyme in Transgenic Rice Plants for Alcohol Fuels and Cleaner Environment. HESHAM ORABY[1], Balan Venkatesh[2], Bruce Dale[2], Rashid Ahmad[1], Callista Ransom[1], James Oehmke[3], and Mariam Sticklen[1]. [1]Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824; [2]Department of Chemical Engineering and Material Sciences, MichiganState University, East Lansing, MI 48824; and [3]Department of Agriculture Economics, Michigan State University, East Lansing, MI 48824. Email: orabyhes@msu.edu

A great deal of efforts has been exerted towards improving ethanol yield and reducing its production costs. A vision to enhance U.S. economic security has set a target of using plant-derived materials to meet 10% of chemical feedstock demand by 2020—a fivefold increase. The vast majority of ethanol has been produced from maize seeds. The cheap availability of lignocellulosic biomass, the less greenhouse effect and the large quantity of ethanol that could be produced from cellulose compared to maize seeds might have the potential to make the cost of ethanol competitive with the cost of gasoline. For ethanol to be produced from plant biomass sources, enzymatic hydrolysis of cellulose to fermentable sugars is employed. These enzymes are still expensively produced in large-scale microbial fermentation tanks. A cost reduction might be achieved by producing biomass crops that can sustainably and actively self-produce the desired hydrolysis enzymes. While rice seed is the useful portion of this crop, its remaining biomass has limited use. Traditionally, farmers throughout the world burn rice fields after harvest. Burning is inexpensive and mitigates against rice diseases. However, the increased levels of smoke give rise to health concerns such as increased incidence of asthma. These concerns, for example, gave rise to California legislation that limits rice straw burning to its minimum in 2001. Therefore, rice could be recommended for use as a viable bio-based energy crop. The aim of this study was to develop transgenic rice plants that produce a high amount of active microbial (*Acidothermus cellulolyticus*) endoglucanase (E1) and test the enzymatic activity in the cellulose to glucose conversion. The *Agrobacterium* strain LBA4404 was used to transform mature seed derived calli of the rice cv. Taipei 309 to transfer a plasmid construct containing the *e1* gene designed for apoplast-targeting of the E1 enzyme, beta-glucuronidase (*uid*A; *gus*) and the *bar* herbicide resistance selectable marker genes. Molecular analyses of T1 plants confirmed presence and expression of the transgene. The amount of E1 enzyme extracted from T1 plants accounted for up to 4.9% of the plant total soluble proteins, and its accumulation had no apparent deleterious effects on plant growth and development. Approximately 22 and 30% of the cellulose in the Ammonia Fiber Explosion (AFEX)-pretreated rice and maize biomass respectively was converted into glucose using rice E1 heterologous enzyme. As rice is the major crop of the world with minimal use for its straw, the results may suggest an economically successful strategy for producing biologically active hydrolysis enzymes in rice for alcohol fuel while substituting the wasteful practice of rice straw burning with an environmentally conscious technology.

P-1009

High-yields and Extended Serum Half-life of Therapeutic Proteins Expressed as Fusion Glycoproteins in Tobacco Cells. J. F. XU, S. Okada, K. J. Goodrum, J. J. Kopchick and M. J. Kieliszewski. Department of Chemistry and Biochemistry, Ohio University, 350 W. State St., Athens, OH 45701. Email: xuj@ohio.edu

Therapeutic proteins like human interferon α (IFNα) and human growth hormone (hGH) generally possess short serum half-lives due to their susceptibility to serum proteases and small size, hence rapid renal clearance. Chemical derivatization, such as addition of polyethylene glycol groups, overcomes these problems but does so at the expense of bioactivity, which is often greatly decreased. Here we describe a new method that yields biologically potent IFNα2 and hGH in high yield and with increased serum half-life when expressed as arabinogalactan-protein (AGP) fusions in cultured tobacco cells. We designed synthetic genes encoding a secretion signal peptide, IFNα2 or hGH and 2, 10 or 20 Ser-Pro repeats at the C-terminus of the transgenic fusion proteins. As predicted by the Hyp-Contiguous Hypothesis, the proline residues in the Ser-Pro repeats were all hydroxylated and subsequently arabinogalactosylated. The secreted fusion glycoproteins gave 500–1800 fold greater secreted yields than the corresponding non-glycosylated IFNα or hGH controls. Importantly, an increased in vivo serum half-life of up to 13 fold was observed for the fusion glycoproteins, and their biological activity was similar to native IFNα2 and hGH. Thus, the use of arabinogalactan glycomodules may provide a general approach to the enhanced production of therapeutic proteins by plants.

P-1010

Plant Transformation and Horizontal Gene Flow of a Plant ABC Transporter Gene. C. N. STEWART, JR., R. J. Millwood, J. S. Davis, K. P. Burris, A. Mentewab, and J. N. Burris. Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996. Email:nealstewart@utk.edu

Recently the arabidopsis ABC transporter WBC19 was described to be an effective kanamycin selectable marker in tobacco. ABC transporters are membrane-spanning proteins involved in the active transport of substrates across membranes. The putative subcellular target for WBC19 is the tonoplast as kanamycin is shunted into the central vacuole. Through collaborations WBC19 is being tested for efficacy in different plant species. We have produced transgenic canola and have compared the efficacy against the bacterial NPTII gene. In both tobacco and canola, WBC19 seems to be a viable NPTII substitute. One biosafety issue with the NPTII gene and other bacterial resistance genes is the potential threat of horizontal transfer back to bacteria from transgenic plants in the environment, which might create new antibiotic resistance problems. While this threat has not proven to be real, there remains public and regulatory concerns, especially in Europe. To that end, we tested a horizontal transfer scenario by subcloning WBC19 under the control of a bacterial promoter and transforming it into bacteria to test for kanamycin resistance. Even though bacteria harbored the construct in a plasmid, no kanamycin resistance was conferred to the bacterial host, which is consistent with the subcellular target being the tonoplast. Since bactera have no central vacuole, it apparently is mistargeted in bacterial cells. It might be that plant-derived transgenes could be as effective and less controversial than transgenes from other organisms in certain cases.

P-1011

Quantitative Multiplex Real-time PCR as a Screening Tool for Estimating Transgene Copy Number in Transgenic Citrus. A. A. OMAR, M. G. H. Dekkers, J. H. Graham, and J. W. Grosser. University of Florida, IFAS, CREC, 700 Experiment Station Road, Lake Alfred, Fl 33850. Email: omar71@ufl.edu

Quantitative real-time PCR (RT-PCR) was adapted to estimate transgene copy number in transgenic citrus plants. This task is normally achieved by Southern analysis, a procedure that requires relatively large amount of plant material and is both costly and labor-intensive. 'Hamlin' sweet orange (*Citrus sinensis* (L.) Osbeck) transgenic plants were generated using protoplast/GFP transformation system. These transgenic materials represented a range of copy number. The standard curve is the key element for the quantitative assay: since it is based on the standard DNA used, the choice and the preparation of this DNA is extremely important. One of the proposed methods to prepare the standard DNA consists of mixing plant DNA with a plasmid carrying the transgene (Ingham et al., 2001). This procedure, however, introduces several sources of error that cannot be controlled: previous absolute quantification of both plant and plasmid DNA is necessary, together with precise knowledge of the nuclear genome size of the plant species to be assayed. Unfortunately for most plants, only approximate estimates are available. All these problems were by-passed by simply taking the DNA of one transgenic line and using its dilutions to construct the standard curve. From the standard DNA stock solution, accurate four-fold serial dilutions were prepared and utilized to obtain the standard curves necessary for relative quantification of an endogenous gene and transgene. Estimated copy number in the plants using RT-PCR was correlated with the actual copy number found with Southern blot analysis. The results indicated that there was a significant correlation between the two methods. Thus, RT-PCR can provide an efficient means of estimating copy number in transgenic citrus.

Ingham, D.J., Beer, S., Money, S. and Hansen, G. (2001). Quantitative real-time PCR assay for determining transgene copy number in transformed plants. Biotechniques 31, 132–134, 136–140.

P-1012

Abstract has been withdrawn

P-1013

Medium-term In Vitro Storage of Pear (*Pyrus* L.) Germplasm. B. M. REED. USDA-ARS, National Clonal Germplasm Repository, 33447 Peoria Rd, Corvallis, OR 97333–2521. Email: corbr@ars-grin.gov

The United States Department of Agriculture, Agricultural Research Service National Clonal Germplasm Repository (NCGR) at Corvallis, Oregon, preserves genetic resources for pears (*Pyrus* L.). More than 1800 clones and more than 300 seed accessions in 26 *Pyrus* species are maintained in this genebank. The main collections are held as trees in the field. The backup collection includes about 200 in vitro and more than 100 cryopreserved accessions. The in vitro collection is stored at a constant 4°C with 12 h of low light following one week of cold acclimation. Data on plant health and length of storage was taken for *Pyrus* accessions stored at NCGR from 2001 to 2005. Multiple storage cycles were evaluated for 66 accessions and a single cycle for 187 accessions. The mean storage time for 253 *Pyrus* accessions was 30.4 ± 12 mo. Many accessions varied with storage cycle, probably due to medium preparation or handling of shoots. Among the European pear (*P. communis*) accessions, five wild collected accessions stored for an average of 26 ± 2.4 mo, seven clones of 'Bartlett' pear for 3.5 years (42.7 ± 12.5 mo), four clones of 'Doyenne du Comice' for 19.8 ± 9.9 mo, and eight 'Old Home x Farmingdale' clones averaged more than 4 years (50 ± 13 mo) in cold storage. Pear species stored very well under standard conditions. Six *P. amygdaliformis* accessions averaged 38.7± 11 mo, eight *P. calleryana* 36.7 ± 5 mo, and three accessions each of *P. cordata* 44.6 ± 10 and *P. cossonii* 33.7± 15.5 mo. The longest storing accession was *P. gharbiana* No. 4 which remained in storage for over 8 years before requiring repropagation. A few cultivars consistently stored for short times, but no particular trends were observed. Storage at 4° C with a photoperiod appears to be excellent for most *Pyrus* germplasm.

P-1014

Indirect Shoot Organogenesis from Leaves of *Dieffenbachia*: A Method to Produce Somaclonal Variants. X. SHEN, M. E. Kane and J. Chen. Environmental Horticulture Department, University of Florida, P.O. Box 110675 Gainesville, FL 32611. Email: xis300@ufl.edu

Dieffenbachia, one of the most popular ornamental foliage plants in the United States, continuously ranks in the top ten for annual wholesale value. Market demand for new cultivars with novel features put a high demand on the breeding of *Dieffenbachia*. Selection of somaclonal variants produced in vitro can be an efficient approach for new cultivar development. A novel protocol for indirect shoot organogenesis was determined for four *Dieffenbachia* cultivars using leaf explants excised from in vitro shoot cultures. For callus induction, leaf blade explants, 5 × 5 mm^2, were cultured on MS medium supplemented with TDZ at 0, 1, 5, or 10 μM and 2,4-D at 0, 0.5 or 1 μM. For subsequent shoot formation, callus clumps were transferred to MS basal medium supplemented with 2-iP at 0, 20, 40 or 80 μM and IAA at 0 or 2 μM using cv. 'Camouflage'. Optimal callus induction was obtained on MS medium containing 1 μM 2,4-D and 5 μM TDZ for all cultivars tested. A significant effect of cultivar on callus formation and subsequent shoot differentiation was observed. Nodular callus production was induced from 'Camouflage' and 'Camille' leaf explants, whereas compact and friable calli were produced from 'Octopus' and 'StarBright'. The frequency of callus formation was 96 % for 'Camouflage', 62 % for 'Camille', 66 % for 'Octopus' and 52 % for 'StarBright', but no shoot organogenesis was observed on 'Octopus' calli. The maximum shoots regenerated per callus (7.9) was obtained in 'Camouflage' after 16 weeks from calli cultured on MS medium with 40 μM 2 iP and 2 μM IAA. Ex vitro survival of in vitro regenerated plantlets was 100 %. Significant phenotypic variation in leaf variegation was observed and is being evaluated.

P-1015

Micropropagation of *Calopogon tuberosus*, a Native Terrestrial Orchid, by Shoot Production from Corm Explants. P. J. KAUTH, W. A. Vendrame, and M. E. Kane. Environmental Horticulture Department, University of Florida, PO Box 110675, Gainesville, FL 32611. Email: pkauth@ufl.edu

In the United States native orchids have recently become popular landscape plants within a small group of enthusiasts and hobbyists. Production of native orchids by in vitro seed culture is centralized among hobby nurseries and growers. A major obstacle to market advancement of native orchids is the availability of plants, as well as the extensive growth and flowering period of many native orchids. Micropropagation may provide a system to clonally propagate native orchids on a large-scale or more efficient basis than in vitro seed culture. No published protocols exist for successful micropropagation of native terrestrial orchids. Previous experiments by the authors have shown that *Calopogon tuberosus* may be a model native orchid to establish an optimum micropropagation system. Three *in vitro* derived corm explants divided into top, middle, and bottom sections were cultured on 1/2 MS supplemented with 3% sucrose and 0.8% TC agar. Three cytokinins (BA, TDZ, 2iP) at two concentrations (1 and 5 μM) were screened for shoot production. Phyto*Tech* Culture Boxes with 100 ml medium were used as culture vessels with 8 replications per treatment with nine total explants per replication. After 4 weeks culture, explants were subcultured to corresponding medium supplemented with 0.1% charcoal to reduce phenolic browning of tissues. Shoot number was recorded weekly for 8 weeks, and leaf number and length, root number and length, corm diameter, fresh weight, and dry weight was recorded after 16 weeks culture. The highest number of shoots was observed on 5 μM BA derived from the bottom corm explant. In all shoot growth parameters, those on both 1 and 5 μM BA proved to be significantly higher than most other treatments. Additional experiments involving corm explants to determine optimum conditions for shoot induction will be discussed.

P-1016

Comparative In Vitro Shoot Multiplication, Rooting and Ex Vitro Acclimatization of Sea Oats (*Uniola paniculata* L.) Genotypes. M. E. KANE, N. L. Philman, P. Kauth, X. Shen, P. Sleszynski, S. Stewart, and C. Valero Aracama. Environmental Horticulture Department, P.O. Box 110675, University of Florida, Gainesville, FL 32611–0675. Email: kane@ifas.ufl.edu

Coastal dunes serve as a natural barrier against the destructive forces of hurricanes and storms but are continually being eroded. Dune stabilization is usually accomplished by planting native dune species, such as sea oats (*Uniola paniculata* L.). Sea oats are propagated by seed; however, frequent hurricane damage to donor populations has significantly limited seed availability. A complete sea oats micropropagation protocol was developed to complement seed propagation. To maintain genetic diversity, the protocol must be applicable to the production of numerous sea oats genotypes. The in vitro and early ex vitro growth performance of fifteen RAPDs differentiated sea oats genotypes from four Florida populations were evaluated. Shoot explants were cultured for 4 wks on MS mineral salts, 87.6 mM sucrose, 0.56 mM myo-inositol,1.2 μM thiamine-HCL supplemented with 2.2 μM BA and solidified with 0.8% TC agar. Genotypes exhibited significant differences in Stage II shoot and leaf production, morphology and biomass. Microcuttings cultured on 1/2-strength MS mineral salts supplemented with 87.6 mM sucrose, 0.56 mM myo-inositol, 1.2 μM thiamine-HCL, 10 μM ±-naphthalene acetic acid and solidified with 0.8% TC agar exhibited high rooting (84–100%) with 10 of 15 genotypes rooting 100%. Genotype had a significant effect on Stage III microcutting root number and biomass as well as root:shoot ratios. Ex vitro survival (30–93%), shoot production, leaf number and width and plant height varied significantly between genotypes. Although Stage III rooting percentages were very high, 40% of the genotypes exhibited less than 50% ex vitro survival. A genotype dependent negative carry-over effect of in vitro culture on ex vitro survival is suggested.

P-1017

In Vitro Regeneration of Periwinkle. ANDREA SWANBERG and Wenhao Dai. Department of Plant Sciences, North Dakota State University, Fargo, ND 58105. Email: wenhao.dai@ndsu.edu

Periwinkle (*Catharanthus roseus*) is a common bedding and landscaping plant. It is a natural host of phytoplasma pathogens which cause diseases in more than 300 higher plant species, such as X-disease, aster yellows, and ash yellows. Therefore, periwinkle is an ideal experimental plant species for study of phytoplasma disease, including plant resistance and host/pathogen interaction. Tissue cultures of three periwinkle cultivars ('Pacific Coral', 'Sunstorm Rose', and 'Sunstorm Apricot', Syngenta Seeds, Inc., Downers Grove, IL) were established by incubation of sterile seeds in Murashige and Skoog (MS) medium. In vitro plants were proliferated from nodal explants in MS medium supplemented with 2.5 mM benzyladenine (BA). To elongate in vitro shoots, 1.0 mM gibberellic acid (GA_3) was added into proliferation medium. All cultures were maintained at 25 °C under cool-white fluorescent light with a 16-hr photoperiod and subcultured every 4 weeks. Adventitious shoots were regenerated from both leaf segments and internodes when incubated on Woody Plant Media (WPM) containing various concentrations of BA and naphthalene acetic acid (NAA). The effect of genotype, explant, plant growth regulator (PGR) on in vitro regeneration of periwinkle was investigated. More shoots produced from internodal explants than from leaf explants. Internodal explants of 'Pacific Coral' regenerated a mean of 4.9 shoots in the medium with 5 mM NAA and 5 mM BA, while 'Sunstorm Rose' produced a mean of 4.7 shoots in the medium with 10 mM NAA and 20 mM BA. 'Sunstorm Apricot' failed to regenerate shoots in all treatments. Microcuttings were in vitro rooted in half strength MS medium with 0, 0.5 and 5 mM NAA. The treatment with 5 mM NAA had 100% rooting rate and produced more than 10 roots per explant. Rooted plants were transferred to flats filled with Sunshine Mix #1 (Sun Gro Horticulture Canada, LTD,Seba Beach, AB, Canada) and covered with clear plastic tops. The covers were gradually removed in a 3-week period. Surviving plants were potted into Sunshine Mix #1 and grown in the greenhouse.

P-1018

Comparison of Different Callus and Plant Regeneration from Different Explants in Triploid and Tetraploid Turf-type Bermudagrasses. SONG ZHANG[1], Wayne Hanna[1], and Peggy Ozias-Akins[2]. [1]Department of Crop and Soil Science, Coastal Plain Experiment Station, University of Georgia, GA 31793 and [2]Department of Horticulture, Coastal Plain Experiment Station, University of Georgia, GA 31793. Email: szhang@uga.edu

The turf-type bermudagrasses are recalcitrant in tissue culture. The complicated genetic backgrounds make the tissue culture and plant regeneration even more difficult. In this experiment, young inflorescence, nodes and mature embryos were used as explants to induce callus in triploid TifSport, TifEagle, 97-4 and tetraploid 93-132, 93-135, 93-156 and 93-157 on MS medium supplemented with 2,4-D 1–1.5 mg/l + BA 0.01 mg/l + proline 1.16 g/l. There were 4 types of callus observed in callus induction and subculture. Type I was fluffy, soft, and white non-embryogenic callus, commonly observed in all cultures. Type II was globular, transparent, and hard, but sticky callus, which was pre-embryogenic and could be selected for subculture. Type III callus was transparent compact embryogenic and Type IV callus was white opaque and compact. Both Type III and Type IV calluses were embryogenic and regenerative. Embryogenesis was observed in TifEagle and 93-132. Shoots were regenerated from compact calluses in TifEagle, 93-132 and TifSport on MS medium with 2,4-D 0.1 mg/l + BA 0.5–1.0 mg/l.

P-1019

The HBK3 Homeobox of Knox Gene is Responsible for Developmental Control and Shoot Apical Meristem Determination in Maturing Somatic Embryos of *Picea abies*. M. F. BELMONTE, M. Tahir, and C. Stasolla. Department of Plant Science, University of Manitoba, Winnipeg Manitoba R3T 2N2, CANADA. Email: stasolla@ms.umanitoba.ca

Plant homeobox genes have been shown to orchestrate many developmental processes including shoot apical meristem development. The class I homeobox of knox gene, HBK3, has been previously isolated from the conifer *Picea abies* (L.) Karst (Norway spruce). In the present study, the HBK3 gene was used to assess the function of the shoot apical meristem during somatic embryogenesis of Norway spruce. Production of both over-expressing and antisense somatic embryo lines carrying the HBK3 gene was examined and verified using PCR and Northern blot analysis. Somatic lines over-expressing the HBK3 gene were shown to improve the overall number of embryos produced in culture. These lines were also able to convert more readily. This improved quality was a direct result of a larger and structurally competent shoot apical meristem. In support of this suggestion, we have found the HBK3 over-expressing line to transition into stably determined somatic embryos from their pro-embryo mass form more readily then their antisense counterparts. When HBK3 is overexpressed in an *Arabidopsis thaliana SHOOTMERISTEMLESS* (*STM*)—*beta-glucuronidase* (GUS) reporter line, the shoot apical meristem of mature embryos also increases. We show further that HBK3 appeared to promote over-expression of *STM* outside of the central zone of the shoot apical meristem and into the petiole of more mature plants perhaps explaining the more severe and aberrant leaf phenotype. Taken together, these results suggest a direct and positive role for HBK3 in the determination and competence of the shoot apical meristem.

P-1020

Mineral Nutrient Requirements for Regulating the Growth of Plant Tissue. R. P. NIEDZ and T. J. EVENS. USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: rniedz@ushrl.ars.usda.gov

The mineral nutrient requirements of nonembryogenic citrus callus were characterized by breaking the MS salts into the following five factors—NH_4NO_3, KNO_3, Ca-Mg-Cl-Mn-SO_4-PO_4, metals, and Fe-EDTA. A D-optimal response surface experimental design where each factor was varied over a range of concentrations was constructed. Callus was grown on each treatment combination, fresh/dry weights and friability were measured, and each measured response analyzed by ANOVA. Callus growth ranged from 31%–135% MS levels and the resulting polynomial model had an R^2 of 0.98 and a predictive R^2 of 0.92. The model was validated by generating predictions of salt combinations not included in the original design but within the original experimental design space. The responses of callus grown on these new salt combinations were then compared to the predicted values. The implications of this approach in defining the appropriate types and concentrations of mineral nutrients for in vitro responses, including the importance of mineral nutrition, the limitations of traditional methods of defining mineral nutrient formulations, and what it means to "optimize" in vitro responses will be discussed.

A-2000

Preservation of Cells by Freeze Drying. LIA H. CAMPBELL, Kristy Sarver, Sarah Miller, Brian Leman, and Kelvin G. M. Brockbank. Cell & Tissue Systems, Inc., Charleston, SC, 29403. Email: lcampbell@celltissuesystems.com

Cells can be cryopreserved and stored for extended periods in vapor phase nitrogen. However, methods that would allow the storage of cells and tissues at room temperature without the need for special storage equipment and/or shipping conditions could greatly increase the availability of cells and tissues in a variety of settings. Freeze drying is used routinely for processing food and for the sale and distribution of a wide variety of pharmaceuticals and proteins. Preliminary work is being done in our lab investigating freeze drying of cells and tissues utilizing cardiovascular models. Most successful freeze drying protocols incorporate the use of disaccharide sugars like trehalose because of their ability to protect the cell membranes and proteins during drying. In these experiments, two different methods for introducing sugars into cells were investigated, long term exposure of cells to sugars and solution permeabilization using penetrating organic compounds like dimethyl sulfoxide. Additionally, development of solution formulations designed to protect the cells during freeze drying and some work into development of freeze drying protocols were investigated. Each method for introducing sugars into cells demonstrated protection of the cells during both freezing and subsequent drying steps but was dependent on how dry the cells were at the completion of the drying cycle. Similar results were obtained using the solution permeabilization strategy. Due to the complexity of the freeze drying process, much work is still required to freeze dry cells so that they can be stored at room temperature. However, these experiments demonstrated that it is a feasible strategy for storage and that further development will produce successful freeze drying protocols.

A-2001

Use of a Cancer Cell Line Profiling Array to Evaluate the Effect of Chemotherapeutic Agents on Cullin 5 (Cul5) mRNA Expression. K. ZAFFARKHAN, C. Koch, and M. J. Fay. Department of Pharmacology, Midwestern University, 555 31st Street, Downers Grove, IL 60515. Email: mfayxx@midwestern.edu

Cullin 5 (Cul5) is a member of the evolutionarily conserved Cullin protein family that function as scaffolds within E3 ubiquitin ligases that target proteins for ubiquitin-mediated degradation by the 26 S proteasome. Previous research has implicated Cul5 as a putative tumor suppressor in breast cancer since it is located on a region of chromosome 11(q22–23) that is associated with loss of heterozygosity. In support of a role for Cul5 in breast tumorigenesis, we previously demonstrated a decrease in Cul5 mRNA expression in breast cancer samples versus matched normal tissue. Even though Cul5 is a putative tumor suppressor in breast cancer, few studies have addressed the factors that affect Cul5 mRNA expression. The purpose of this research was to determine if chemotherapeutic agents induce Cul5 mRNA expression in human cancer cell lines. To evaluate the effects of chemotherapeutic agents on Cul5 mRNA expression, a Cancer Cell Line Profiling Array containing samples of 26 different cancer cell lines treated with various chemotherapeutic agents was probed for Cul5 expression. Cul5 mRNA was expressed in untreated control cancer cells of various tissue origins (e.g. lung, colon, breast, ovary, cervix, prostate, brain, skin, kidney, liver, and bone). There was variability in Cul5 expression between the different cancer cell lines in response to the various chemotherapeutic agents. Of all the chemotherapeutic agents, the iron chelator desferrioxamine appeared to induce Cul5 expression in the greatest number of cancer cell lines. This research was supported by NIH CA85279, an American Medical Association Seed Grant, and Midwestern University.

A-2002

Calcium Oxalate Crystals in Commercial Fetal Bovine Serum: Implications for Cell Culture, Phagocytosis and Biomineralization Studies In Vitro. C. E. PEDRAZA, and M. D. McKee. Faculty of Dentistry, McGill University, Montreal, QC, CANADA H3A 2B2. Email: claudio.pedraza@mcgill.ca

Mammalian cell culture and organ culture require basal medium supplemented in most cases with fetal bovine serum (FBS). FBS has not yet been fully characterized and its composition varies from lot to lot. Here we report that examination by light microscopy of different lots from different companies (n=17; from HyClone, Invitrogen and Wisent) of commercially available FBS consistently revealed the presence of crystalline material—mainly calcium oxalate dihydrate (COD). COD crystal morphology was visualized by conventional light microscopy and by scanning electron microscopy, and chemical composition analysis by energy-dispersive X-ray microanalysis in a scanning electron microscope revealed strong spectral peaks for calcium and oxygen. Crystal counts per litre of FBS ranged from $13.2 \times 10^5 - 10 \times 10^6$, and colorimetric, chemical-kit assays were used to determine oxalate (0.030–0.060 mmol/L) and calcium (0.1–1.0 mmol/L) concentrations. Detection of crystal-bound proteins by immunoblotting revealed the presence of osteopontin and fetuin—known regulators of COD crystal growth that are found in serum. Western blot analysis for osteopontin showed similar binding to calcium oxalate monohydrate (COM), also occasionally found in FBS. To examine cell responses to these crystals, phagocytosis of synthetic COM and COD crystals was evaluated in the macrophage-like murine cell line J744A.1, and in murine NIH-3T3 fibroblasts. Internalized crystals induced disruption of cell-cell adhesion, release of reactive oxygen species, and also induced membrane damage to cells as assessed by Calcein-AM uptake. Our results demonstrate that the presence of calcium oxalate crystals in FBS have the potential to affect calcium levels in cell culture systems, and may sequester mineral-binding proteins and ions, all of which in turn may affect cell metabolism, cell signaling and other cell responses. The presence of crystals in FBS is particularly relevant to cell culture studies on phagocytosis and biomineralization where they would introduce previously unrecognized experimental variables. *Supported by the CIHR.*

A-2003

Antioxidant and Antiproliferative Effects of Turkish *Rheum ribes* Ethyl Acetate Extract. PEMBEGUL UYAR. Graduate Program of Biotechnology, Middle East Technical University, TURKEY. Email: pembegul@metu.edu.tr

Rheum ribes L. is an annual herb of the Polygonaceae family and found mostly in Eastern Turkey. Its fresh stems and petioles are consumed as vegetables and used as a medicinal plant to promote digestion, improve appetite, treat diarrhoeas, used against hemorrhoids, measles, smallpox. This study was designed to investigate the antiproliferative, and antioxidative properties of *Rheum ribes* young shoots as its edible parts. *Rheum ribes* extracts (RRE) were prepared as dry sample to ethylacetate ratio of 1:12. Antioxidant capacity of the extract was determined by the ability to scavenge 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), and the result was expressed as fifty percent inhibitory concentration (IC_{50}) of 0.098 ± 0.009 mg/ml. Human Myeloid Leukemia (HL-60) cell line was used as a model system for the proliferation studies. HL 60 cells were cultured in the presence of various concentrations of RRE, and exposed over 48 hr. The percentage of cell viability was evaluated by metabolization of the tetrazolium salt XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide). *Rheum ribes* ethyl acetate extracts displayed a dose-dependent inhibition of cell proliferation with an fifty percent effective dose (ED_{50}) of 24.15±0.86 μg/ml. These investigations suggested that the ethyl acetate extract from *Rheum ribes* can be considered as a potent antioxidant and a strong antiproliferative agent. As a result, *Rheum ribes* exerts various activities with dose dependent as well as exposure-time dependent manners; in this sense, it has a potential for cancer chemoprevention.

A-2004

In Vitro Tolerance of Filamentous Fungi to Environmental Pollutants: A Potential for Mycoremediation. NAIZA MAE N. DE LOS SANTOS, Krysta Laureen Palma, and Nelson D. Aclan. University of the Philippines in the Visayas, Iloilo City 5000, PHILLIPPINES. Email: blackdiamond_honey@yahoo.com

There is so much concern and interest on environmental pollution and ecotoxicology nowadays especially the environmental scientists. This study "In Vitro Tolerance of Filamentous Fungi to Environmental Pollutants: A Potential for Mycoremediation" evaluates the response of selected filamentous fungi to chemical pollutants and determine their tolerance and sensitivity effects under varying concentrations of test agents. Four species of filamentous fungi (*Aspergillus flavus, Aspergillus niger, Tricophyton mentagrophytes and Fusarium moniliforme*) are selected and subjected to 3 different concentrations (5ppm, 10ppm, 20ppm) of chemical compounds (lead nitrate, potassium nitrate, potassium dichromate, copper sulfate pentahydrate) using fungal assay. Mycelial growth extensions were measured everyday up to 5 days incubation period. Growth rate responses of metal amended media were compared with the control medium using distilled water. Analysis revealed that the toxicity effects of chemical pollutants do not vary significantly on the growth of selected filamentous fungi. All species are tolerant to the toxicity effects of lead. It was found out that *A. flavus* is sensitive to potassium nitrate and copper sulfate while *A. niger* is tolerant to potassium nitrate and Chronium but not in copper sulfate. *T. mentagrophytes* can tolerate all the metal compounds while *F. moniliforme* can't tolerate chromium and copper sulfate. Based from the results, it is concluded that the selected fungi have specific tolerance to the test chemical toxicants hence, a potential to remove toxic waste in metal polluted sites especially lead and therefore can be used for mycoremediation.

A-2005

Establishment and Characterization of 13 Human Colorectal-carcinoma Cell Lines. JA-LOK KU, Kyung-Hee Kim, Jin-Sung Choi, Sung-Hye Hong, Young-Kyoung Shin, Hong-Sun Kim, Jae-Hyun Park, Il-Jin Kim, and Jae-Gahb Park. Korean Cell Line Bank, Laboratory of Cell Biology, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea. Email: kujalok@snu.ac.kr

We characterized 13 human colorectal carcinoma cell lines (designated SNU-70, SNU-254, SNU-479, SNU-796, SNU-977, SNU-1181, SNU-1235, SNU-1406, SNU-1411, SNU-1460, SNU-1544, SNU-1684, and SNU-1746) established from 13 Korean colorectal-carcinoma patients including the morphology in vivo and in vitro and mutations of *K-ras, p53, APC,* microsatellite instability status in vitro. We have also analyzed the expressions of anticancer drug associated genes such as *MDR1, MXR, MRP1* and *COX2* genes. No lines were contaminated with Mycoplasma or bacteria. All lines were proven to be unique by DNA-fingerprinting analysis using 16 STR (short tandem repeat) markers and 11 cell lines grew as adherent and 2 cell lines as floating aggregates. All lines showed high viability with relatively long doubling times (43.7~120.5hr). Six of the cell lines showed mutations at codons 12 or 13 in the *K-ras* gene, and *p53* gene was mutated in 8 cell lines including 5 missense mutations, 1 nonsense and 2 frameshift mutations. The microsatellite instability was found in 3 lines and the mutations of *APC* gene were found in 4 lines. The high levels of expression in *MDR1*, *MXR* and *COX2* genes were detected in 8, 6, and 6 cell lines, respectively. However, *MRP1* gene was expressed in all cell lines. These well-characterized colorectal cancer cell lines should serve as useful tools for investigating the biological characteristics of colorectal cancer.

A-2006

Animal Component Free T-cell Culture. J. H. MANWARING, B. B. Barnett, and W. G. Whitford. Research and Product Development, HyClone, Logan, UT 84321. Email: john.manwaring@perbio.com

Developments in the understanding of serum-free cell culture methods have increased our ability to more specifically control mammalian lymphocyte cell selection, stimulation, and culture environment. Of the many uses of cultured T cells, those involving the transfer of cells or cell products to patients can pose the most rigorous culture media demands. Safety and regulatory concerns associated with animal-derived media components include lot-to-lot variability and the potential for either immunoactive or infectious contaminants and have driven the scrutinization of culture media raw material sources and manufacturing methods. Furthermore many research applications would be facilitated by limiting the amount and variability of cytokines, antigens, and immunostimulators associated with many animal derived products such as bovine serum. Factors to be considered in developing an Animal Derived Component Free (ADCF) T Cell medium include: 1) The mode of culture—from multiwell plates to bioreactors; 2) The type of cell to be expanded—from immortalized lines to primary autologous cells; and 3) The goal of culture—from providing simple cell mass, to specifically activated sub-populations, to accumulation of secreted product; and 4) the regulatory and safety concerns involved in the application. Presented here are data from HuT-78 on the development of a high-efficiency ADCF human T cell culture medium.

A-2007

Signal Transduction Targets of Modeled Microgravity. ALAMELU SUNDARESAN (Lalita)[1], Kamleshwar Singh[1], Neal R. Pellis[2], and James DuMond Jr.[1] [1]Texas Southern University, Houston, TX and [2] Human Research Program, NASA Johnson Space Center, Houston, TX. Email: sundaresana@TSU.edu

Lymphocyte signal transduction is of paramount importance for an adequate mounting of the immune response. Defects in this area could leave the host prone to infection etc. From activation to the final function be it cell proliferation, locomotion or apoptosis, the signal transduction cascade is critical. Previous experiments demonstrated that both lymphocyte activation and locomotion were inhibited in spaceflight and in ground based modeled microgravity (MMG) cultured human lymphocytes. Calcium signaling pathways were functional in MMG. In T cell activation at the membrane level, Phospholipase C g1 (PLC-g1) facilitates hydrolysis of phosphoinositide di phosphate (PIP_2) to DAG and a molecule called IP_3 (inositide tri phosphate). DAG activates the PKC signaling cascade and IP_3 drives the calcium pathways. While examining human lymphocytes for status of different signaling molecules by immunoprecipitation and, western blotting, deficiencies were found in activation of PLC-g1, this being the predominant isoform active in T cells, is activated by tyrosine phosphorylation. The activated form of the enzyme was found to be down-regulated by more than two fold in MMG cultured lymphocytes Calcium independent isoforms of PKC such as PKC delta and epsilon were also downregulated by more than 50% in MMG compared to ground cultured lymphocytes. This was observed both at the transcriptional and translational levels by immunoblotting and RT-PCR. The IP_3 receptor, whose expression is indicative of calcium mobilization, however did not change in MMG cultured cells. This corroborates evidence using ionomycin that calcium signaling might be unaltered in MMG. MMG thus affects certain signaling cascades selectively. Mouse lymphocytes cultured in modeled microgravity also demonstrated decreased activation compared to ground controls. IP_3 receptor expression in MMGcultured mouse lymphocytes also was not significantly different as compared to ground cultured lymphocytes. IP_3 receptor levels were found to increase in expression as seen by immunobloting in MMG cultures. Nucleotide augmentation in MMG cultured mouse lymphocytes both In Vitro (culture media) as well as *in vivo* as nutrition supplements in antiorthostaically (AOS) suspended mice, was able to recover activation both in lymphocytes isolated from control mice and from. Experiments outlining signal transduction in human vs mouse lymphocytes in MMG would go a long way in delineating immune suppression related phenomena both in space an here on earth. Nucleotide supplementation experiments would be instrumental in design of countermeasures against immunological problems incurred in space. This is especially relevant when long-term space travel is being contemplated. Results from signal transduction experiments in human and mouse lymphocytes as wells as nucleotide supplementation experiments are presented in this study.

A-2008

Formation of Intact Membrane Structures with High Transepithelial Electrical Resistance in Culture Inserts with Mouse Embryonic Stem Cells. R. Konsoula and F. A. BARILE. Laboratory of In Vitro Toxicology, St. John's University, Jamaica, NY 11439. Email: barilef@stjohns.edu

Mouse embryonic pluripotent stem cells, ES-D3, were induced to form membrane structures in cell culture inserts, using combinations of extracellular matrix components and growth factors (GF). The ability of the stem cells to form intact membranes and create tight junctions (TJ) was assessed by measuring transepithelial electrical resistance (TEER) and passage of TJ markers. Stem cells were initially inoculated on inactivated 3T3 mouse fibroblasts in the presence of LIF factor to maintain the undifferentiated state. Subconfluent ES-D3 monolayers were then passaged in the absence of feeder layers and LIF factor to stimulate the formation of embryoid bodies (EB). After 5 days, EB were transferred to 24-well culture inserts coated separately with 1 of 4 extracellular matrix components (collagen I, collagen IV, fibronectin and laminin). Induction of TJ was further facilitated by treating EB on the inserts with 1 of 4 different growth factors (amphiregulin, epidermal GF, keratinocyte GF and transforming GF β-1). After 10 to 14 days, TEER was measured at 500–800 Ω-cm^2, with the combination of fibronectin and KGF yielding highest values. Paracellular permeability (PP) and acute cytotoxicity studies were conducted with confluent monolayers in 96-well plates or culture inserts, repectively. After exposure to 1 of 19 chemicals for 24-h, cell viability and PP was measured using the MTT assay and PP markers, respectively. The data indicate that, at equivalent concentrations, cell viability decreases before the integrity of the membrane is compromised. Also, TEER measurements inversely correlate with increased passage of tight junction markers for most chemicals. We conclude that mouse ES cells can be induced to form TJ in coated culture inserts after treatment with appropriate GF as evidenced by progression of high TEER values comparable to those seen with Caco-2 cells. Support for TJ formation is demonstrated by the lack of high transepithelial resistance in undifferentiated ES cells, and by prevention of transport of PP markers in the differentiated monolayers. However, the extent and direction of differentiation is currently under investigation. ES cells therefore can be induced to differentiate into intact confluent cell membrane-like structures with support for the formation of TJ. The culture system thus has potential as a pharmacokinetic or toxicokinetic in vitro model.

A-2009

BEAS-2B Cells as a Model for the Reductive Activation of Hexavalent Chromium in Human Lung Epithelial Cells. CHARLES R. MYERS, Griselda R. Borthiry, and William E. Antholine. Department of Pharmacology & Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226. Email: cmyers@mcw.edu

Inhalational exposure to hexavalent chromium [Cr(VI)] compounds can cause cytotoxic, mutagenic, and carcinogenic effects. The intracellular reduction of Cr(VI) yields cytotoxic Cr(V) and reactive oxygen species. Bronchial epithelial cells are the primary site of pulmonary exposure to inhaled Cr(VI) and are the primary cells from which Cr(VI)-associated human cancers arise. However, studies with normal human bronchial epithelium (NHBE) have received little attention. BEAS-2B cells are used here as a model of NHBE for studies on the reductive activation of Cr(VI) and toxicity. In clonogenic assays, the cells were very sensitive to soluble (Na_2CrO_4) and particulate Cr(VI) ($ZnCrO_4$, $CaCrO_4$, $PbCrO_4$). A steep decline in clonogenic survival was observed, with estimated 50% survival at 3.63 μM Na_2CrO_4, 0.359 μg $ZnCrO_4$ per cm^2, and 0.206 μg $CaCrO_4$ per cm^2. For $PbCrO_4$, the decline in clonogenic survival was more gradual with 50% survival at 8.60 μg/cm^2. Trypan blue exclusion was a much less sensitive indicator of cell viability. Cells exposed to 400, 200, or 100 μM Na_2CrO_4 for 5 min exhibited a sharp Cr(V) ESR signal (g = 1.9793). A signal at g = 1.9849 was also observed which could represent a Cr(V)-thiol complex. In studies using $Na_2{}^{53}CrO_4$, both of these signals were split into four lines as expected for the nuclear spin (I = 3/2) of ^{53}Cr. Therefore, both the g = 1.9793 and g = 1.9849 signals represent Cr(V) species. Studies with human microsomes and proteoliposomes have demonstrated that cytochrome b_5, in cooperation with P450 reductase or b_5 reductase, has pronounced activity in the reductive activation of Cr(VI) to Cr(V) (ESR g = 1.9793, matching a signal generated in BEAS-2B cells). In human lung, these enzymes are localized almost exclusively in the bronchial epithelial cells. While the expression of several microsomal enzymes typically declines markedly in cell culture, immunofluorescence demonstrated that BEAS-2B cells continue to express b_5, b_5 reductase, and P450 reductase in the nuclear membrane and perinuclear region (smooth endoplasmic reticulum).

A-2010

Comparative Extraction Profiles of Medical Devices Using a Cell Growth Inhibition Assay. DAVID TAN, Alana Renaud, Alicja Sills, Ann M Wright, and Mary Mowrey McKee. CIBA Vision Corporation, Duluth, GA 30097. Email: amy.wright@cibavision.com

To evaluate the cellular viability of mammalian cells exposed to extracts of biomaterials using in vitro cell culture methods. Extracted biomaterials were evaluated using the USP General Chapter and ISO 10993–12 guidelines for preparation of samples for extraction. Extractions were conducted at 121 C and 50 C in saline or water and at 37 C in supplemented MEM media. The USP and ISO 10993–5 testing methods for biocompatibility were followed using the elution assay (EA) and cell growth inhibition (CGI) methods described in the guidelines. Following a designated incubation period, murine fibroblastic cells (L929) were exposed to the biomaterial extracts. Cellular viability was evaluated by either vital dye or by cell counts. Benzalkonium chloride (BAC) was used as a positive control for the analysis of dead cells. All biomaterials passed the EA for both saline and supplemented MEM extraction at 121 C and 50 C for saline and 37 C for MEM. Four polymeric biomaterials passed the CGI assay 121 C and 50 C for saline and 37 C for MEM. One polymeric biomaterial failed the criteria for CGI at 121 C in saline and 37 C in supplemented MEM media. Cells exposed to BAC were cytotoxic in both of the assays (1.25 and 12.5 ppm for EA and 1.25 ppm for CGI, final concentrations). Components of polymeric biomaterials need to be evaluated at specific temperatures and use extraction vehicles which best serve the chemical makeup of the device. The use of the extraction parameters described in the USP and ISO guidelines allow for specific extraction temperatures and vehicles which can be used for differing polymeric biomaterials.

A-2011

Up-regulation of Inducible Nitric Oxide Synthase by Rosiglitazone in Sinusoidal Endothelial Rat Liver Cells In Vitro. MIGUEL REYES[1], Claudia Reyes-Estrada[2], Brissia Lazalde[1]. [1]Faculty of Medicine UJED, Durango, Dgo., MEXICO and [2]Faculty of Medicine UAZ, Zacatecas, Zac., MEXICO. Email: mareyes@ujed.mx

Currently it is believed that inducible nitric oxide synthase (iNOS) has a role in the pathophysiology of fibrotic liver disease through nitric oxide production, since it contributes to the regulation of peripheral vascular tone in patients with cirrhosis and ascites; nevertheless its precise role remains to be elucidated. iNOS expression in sinusoidal endothelium of liver has been poorly studied. The nuclear hormone receptor peroxisome proliferator activated receptor g (PPARg) is a modulator of of iNOS and nitric oxide (NO) production; PPARg can be activated by thiazolidinediones, drugs employed in the treatment of type 2 diabetes mellitus. The aim of this work was to study the effect of thiazolidinediones on iNOS expression at transcriptional level in sinusoidal endothelial rat liver cells in vitro. A sinusoidal endothelial rat liver cell line (CD-31 and von Willebrand factor positive) developed in our lab was used. Confluent monolayers were incubated by 24 h with the thiazolidinedione rosiglitazone (Avandia™) at 0.1 and 1 μM; controls without the drug were included. Total RNA was isolated with Trizol™ reagent and the steady state level of iNOS mRNA was evaluated by semiquantitative RT-PCR using published primers and the SuperScriptT One-Step RT-PCR kit (Invitrogen, Inc., Carlsbad, CA). GAPDH was used as constitutive gene for normalization. Experiments were done in triplicate. Control cultures did not show basal transcription of iNOS; cultures with rosiglitazone showed a clear amplicon similar in intensity at both concentrations of the drug. These results show that sinusoidal endothelial liver cells are able to transcribe iNOs and suggest that rosiglitazone could affect hepatic blood flow through iNOS up-regulation. Since thiazolidinediones are widely prescribed, this issue warrants further studies.

A-2012

Integrin Regulation of Mouse Embryonic Stem Cell Self-renewal. J. DENRY SATO[6], Yohei Hayashi[1], Miho Furue[3,4], Kiyoshi Ohnuma[1], Yasufumi Myoishi[5], Takanori Abe[2], Ryu-ichiro Hata[3,4], Tetsuji Okamoto[5], and Makoto Asashima[1,2,7]. [1]Department of Life Sciences (Biology), Graduate School of Arts and Sciences and [2]Department of Biological Science, Graduate School of Science, Tokyo University, Tokyo, JAPAN; [3]Department of Biochemistry and Molecular Biology and [4]Oral Health Research Center, Kanagawa Dental College, Yokosuka, JAPAN; [5]Department of Molecular Oral Medicine and Maxillofacial Surgery, Division of Frontier Medical Sciences, Graduate Science School of Biomedical Sciences, Hiroshima University, Hiroshima, JAPAN; [6]Mount Desert Island Biological Laboratory, Salisbury Cove, ME; and [7]International Cooperative Research Project (ICORP)/JAPAN Science and Technology Agency (JST), Tokyo, JAPAN. Email: dsato@mdibl.org

We provide evidence that extracellular matrix and integrins play an important role in ES cell self-renewal and subsequent differentiation. We have recently developed a serum-free medium, designated ESF7, in which leukemia inhibitory factor (LIF) stimulated murine ES cell proliferation. When ES-D3 mouse embryonic stem cells were cultured on type I collagen in ESF7 medium, they remained undifferentiated. On the other hand, ES-D3 cells cultured on laminin or fibronectin began to differentiate as indicated by altered morphologies with decreased alkaline phosphatase activity, increased Fgf5 expression, and decreased Nanog expression. We hypothesized that LIF-induced signal transduction differed in mouse ES cells depending upon the ECM components on which they were cultured. Our analysis using western blotting showed that the activation of STAT3 and PKB/Akt was down-regulated in ES-D3 cells on laminin or fibronectin compared to those on type I collagen. In contrast, the activation of ERK1/2 in ES-D3 cells on laminin was up-regulated compared to that in cells on type I collagen. These results together with the observation that ES-D3 cells differentiated on laminin or fibronectin substrata suggested that ECM components contributed to ES cell self-renewal or differentiation by modulating signaling pathways downstream of LIF. ES-D3 cells cultured in ESF7 medium did not express collagen receptors. By contrast, the laminin receptor integrins, and the RGD receptor integrins were expressed in ES-D3 cells cultured in ESF7 medium. These results suggested that integrin signaling was inoperative in ES-D3 cells on collagens but active in the cells on laminin and fibronectin. We conclude that ECM-integrin outside-in signaling initiates differentiation in mouse ES cells. This work was supported by grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan; an International Cooperative Research Project grant from the Japan Science and Technology Agency; a grant from the Smoking Research Foundation (Japan); grants P20-RR016463 and P30-ES03828 from the National Institutes of Health (US); and by a short-term fellowship from the Japan Society for the Promotion of Science.

E-2000

Use of Lettuce Tissue Culture For Developing Transformation Techniques in High School. C. A. HARRISON[1], M. Egnin[2], J. Scoffield[2], and B. Bey[2]. [1]Booker T. Washington High School, Tuskegee, AL; and [2]Plant Biotechnology and Genomics Lab, Tuskegee University, Tuskegee, AL. Email: megnin@tuskegee.edu, cahar313@aol.com

Tuskegee University Southern AgBiotech Consortium for Underserved Communities (SACUC) in collaboration with Booker T. Washington High School has strengthened K-12 science education, bridging the bioscience-divide and implementing classroom biotech activities. In this hands-on activity high school students are introduced to greater biotechnological techniques including tissue culture, micropropagation, cloning and transformation. In this activity, using Carolina's Lettuce Cloning Kit as a basis of tissue culture exposure, students grew surface sterilized lettuce seeds on four different Media [lettuce germination medium (LGM), MS basal medium supplemented with 5mg/L GA3 (MM) or Kinetin (MMK), and Hoagland medium (HO)]. After germination, using aseptic technique, cultures were initiated with cotyledon explants on shoot induction media. The precultured explants were transformed with the *Agrobacterium* strain EHA101/pIG121-Hm with *-uid-A*–intron (GUS), or EHA105 harboring the binary vector pBI121-*mgfper* containing the GFP gene and kanamycin selectable marker, and cocultivated for 3–6 days at 260C ±2. Four student groups were each assigned a reporter gene with a cocultivation period, after which each student group transferred the explants on lettuce regeneration media with carbenicillin and kanamycin for selection. Students obtained the highest germination rate (95%) on MM followed by MMK (80%) and HO (70%) than LGM (40%). Cocultivated explants were subjected to either GUS histochemical assay or UV light for transgene expression. Students will screen for putative transformants based on cocultivation periods. PCR test will be performed on putative transgenic plantlets upon root formation. Students will perform GFP purification using BioRad kits to set a protocol for other schools to follow. Work Supported by USDA/IFAFs, SACUC and Tuskegee University/GWCAES.

E-2001

Resistance of *Serratia Marcescens*. MARY ELIZABETH CROWTHER KENT. Birmingham, AL 35215. Email: hrhqme88@aol.com

Over the previous years, bacteria have become resistant to antibacterial agents and antibiotics used in cleaners to destroy them. Oftentimes, bacteria strands become resistant to their antibodies because of genetic mutation, known as exogenous resistance. However, other forms of resistance occur, such as acquired resistance. Acquired resistance is when a strand develops resistance after contact with an antibiotic. This can occur when a cleaner is used incorrectly or too frequently. Inherent resistance is when the bacteria naturally has its own defense against a certain strand of antibiotics. Acquired resistance is the type of resistance that will be tested for in this experiment. Many examples of resistant bacteria exist today—such as how penicillin has little or no effect on infections today. *Serratia marcescens* is a prominent bacteria that is very common, and easy to obtain for testing. Most forms of bacteria form their own antibiotics. However, due to of time restrictions, *Serratia* cannot build its own antibiotic to be used against itself for this experiment. Therefore, a common bathroom cleaner, Lysol, will be used. This experiment dictated if *Serratia marcescens* would build up resistance to Lysol if treated with it randomly. There were two solutions made with water: 1M and .3M. From research, it was hypothesized that *Serratia marcescens* would most likely build up resistance to the highest molar solution before the lesser one because of the aggressiveness of the solution onto the bacteria growing in contact of it. The *Serratia marcescens* culture treated with 1M Lysol solution did show resistance during the second and third experiments, as seen by the decrease in the zone of inhibition from twenty millimeters to fifteen. However, the third experiment showed an unusual formation of the Lysol on the blood agar disks; sort of like a figure eight. My guess is that there was too much liquid on the filter disk when I placed them on the agar. Then, when I turned them over to go into the incubator, the liquid smeared down the plate. Because of this, the disk no longer contained any Lysol solution, and therefore the bacteria grew to the disk. However, where the Lysol had streaked, there was a zone of inhibition. Thus, on experiment three, the zone goes from fifteen to twenty, then to fifteen again. However, the bacteria grew under the level of blood agar treated with Lysol, showing that such a cleaner does not go underneath the primary surface level. (This growth occurred about twenty millimeters below the primary level measured from outside of the plate. The .3 M solution had no resistance, as seen by the steady ten millimeters throughout most of the experiment. However, there was the "figure eight" mark on them as well. The .3 M treated *Serratia* seemed to be normal, no changes that were unusual. There was growth under the level the Lysol did not reach as well. Occasionally, the zone of inhibition jumped up to fifteen millimeters, but my prediction is that is because I accidently had too much solution on the culture disks, perhaps a drop or two. Withal, I was surprised at how quickly the *Serratia marcescens* grew within two days. This was unlike the *Enterococci* that I tested with last year; that took about three to five days to grow to the level the *Serratia* was. Unfortunately, all did not run so smoothly. The original blood agar plates that I was going to use were contaminated when I opened them. I have no idea what the bacteria on them was, but they were yellow streaks in obvious dots in curvy, flowing lines. There were two plates within the package that did not appear infected, but their performance compared to the performance of the other agar dishes leads me to infer that they were infected as well. Thus, I had to get more blood agar, and lost time between obtaining the agar to conduct my project. If given a chance to continue this project, I probably would have conducted further tests on the interesting event of contaminated agar. I also would have cultured more *Serratia marcescens* from various places, such as my bathroom. I did attempt to do so, but I never got any growth in the beaker that it was stored in. Another source of error could be that my incubator was stored in an area where the temperature outside the incubator was not entirely constant, and therefore could have altered the temperature inside the incubator by a degree or so. In addition, instead of using distilled water, tap water was used to create the Lysol solutions. (This is because in everyday situations, tap water is used to mix with the concentrate.) Certain ions could have effected the results slightly, but this does not seem to be immense.

E-2002

The Effects of Road De-icer ($MgCl_2$) on Plant Germination. CAREN COLLINS[1], Jay Ingram[1], and David Muirhead[2]. [1]North Garland High School, Garland, TX 75044, and [2]Texas Scottish Rite Hospital. Email: cmcollins_06@yahoo.com

Road de-icers are put onto roads throughout the winter season in order to prevent ice-related automobile accidents. However, after the ice has melted, the water evaporates under the heat of the sun and the chemicals of the road deicers are left on the road. These chemical residues are eventually washed off into the ditches, and then into the nearby bodies of water. Radish seeds were germinated in varying concentrations of a popular road de-icer, $MgCl_2$, to test the effects of road salts on plant life. Radish seeds were germinated in 1.5% agar plants with $MgCl_2$ solutions from 1–10% in 1% increments. After 5 days of growth, roots were measured, and roots were dissected. The zone of elongation was removed and fixated using standard plant tissue protocols. Radish seeds that were germinated in the control solution had the second longest average root length. Between the 1% and the 4% deicer solutions, the average length of the radish seed root was dramatically shorter than that of the control. This could be caused by the Chloride ions in the $MgCl_2$, stunting the growth of the seed. The seeds germinated in the 5% deicer solution had the longest average root length. The average length of the radish seeds decreased again, after 5%, which was probably due to a hyper-osmotic environment. The structure of the root also changed as the percentage of the deicer increased. The control radish seed magnified at 400 times magnification has square, normally constructed cells. The 5% radish seed magnified at 400 times magnification has compressed cells that are damaged. As the amount of road deicer present increases, the structure of the root cell of the radish seed changes. The $MgCl_2$ appears to have a positive affect on the seeds because the length is increased, yet the cells are damaged. The radish seeds benefited from the Magnesium in the deicer, but overall, the deicer solutions contained too many Chloride ions, and basically dehydrated the cells of the seeds.

E-2003

Herbal Alternatives to Antibiotics and Their Effects on *E. coli* k12. SIMRAN S. GREWAL. Huron High School, Ann Arbor, MI, 48105. Email: sgrewal89@yahoo.com

It has been reported by National Institutes of Health and the Center for Disease control that the over use of antibiotics in medicine has created an epidemic of "antibiotic resistant bacteria." In the United States, about 10 billion dollars were spent on antibiotics within the last year alone. More and more research has shown that the continuous use of antibiotics will further increase the risk of having more potent, drug resistant bacteria. With this growing threat, there has been a keen interest in the medical community about the natural antibiotic properties of certain herbs. Herbs are shown to be more effective antibiotics, because they are composed of a variety of complex antibiotic compounds, as opposed to many synthetic antibiotics, which are composed only of a few compounds. With more complex antibiotic compounds, the probability that a strain of normal bacteria will mutate into a drug resistant strain is very slim. In my project, I have further supported the hypothesis that natural herbs can be effective antibiotics, by proving that specific herbs, such as turmeric and garlic are just as effective as normal antibiotics such as penicillin, against the *E. coli* k12 strain of bacteria. My hypothesis in relation to this experiment was that if a culture *E. coli* k12 is exposed to the natural extracts of Turmeric and Garlic and then bacterial growth will cease. The *E. coli* k12 culture was maintained on LB Agar plates and the bacterial growth was checked by the disk diffusion method after 24 hours of incubation. Results from the data in my experiment showed that garlic and turmeric were both effective in preventing bacterial growth in the spots that the extracts were positioned. In conclusion, natural herbs have a great potential to become popular, antibiotic treatments; however, further research needs to be done on other strains of normal and resistant bacteria. There are yet a number of herbs to be explored and discovered, and the possibility of finding natural treatments to many deadly diseases is very much in the realm of possibility.

Education Poster and High School Award Winning Silent Abstracts

E-2004

Microbiology Evaluation of Toothbrushes. V. H. FERNANDES and D. L. César. Fundação Educacional Montes Claros, Departamento de Química, Montes Claros, MG, BRAZIL. Email: fernndeshz@hotmail.com

The toothbrushes, used for thousand of people in the whole world, are generally kept in the bathroom—humid and dark place with little ventilation—that is propitious for the growth of fungi and bacteria. The bad hygiene of the places where the toothbrushes are kept, associated to the lack of personal hygiene, compromises the buccal health of the toothbrush users. The present paper has as its main objective to verify the presence of microorganisms in the dental brushes by means of a qualitative microbiological analysis. Its secondary objective is to quantitatively analyze the dental brushes with a more significant sampling rate, providing us statistics data to alert the population on the importance of the personal hygiene, emphasizing the existing relation between microorganisms detected in the toothbrushes and the possible illnesses they can cause. Studies made by the authors of this project with the plastic boxes that come with some toothbrushes, had shown that these boxes work as incubators for the development of microorganisms such as: *Streptococcus*, *Staphilococcus*, *lactobacillus* and *Candida albicans*. As they are in the gram rods morphology—positive isolated and gram short chain clusters of cocci—positive and negative. Some species of these microoganisms are highly pathogenic, being able to cause Pharyngitis, laryngitis, dental decay (very common illnesses of high level of relapse) desmineralization of teeth, serious gingivitis, bad Candidiasis and others. Based on these studies, we propose the development of a sterilizing container to store the toothbrushes in use, substituting the traditional plastic boxes.

E-2005

Proteomics Analysis of Osteosarcoma Cells. M. M. POLMEAR and L. K. Polepeddi. Cell and Structural Biology, University of Colorado at Denver and Health Sciences Center, Denver, CO 80111. Email: Palmers84@aol.com

Osteosarcoma is the most prevalent tumor in dogs and a devastating human cancer, but its pathogenesis is unknown. The objective of this project is to explore the role of a known tumor suppressor, beta-catenin, in tumorigenesis and progression. It was hypothesized that beta-catenin function may be compromised in osteosarcoma cells and that this may be reflected in a decrease in beta-catenin level in these cells. To test the hypothesis, cell homogenates from canine osteosarcoma and stroma tissue samples were prepared and subfractionated into a cytosolic division and a phospholipid-enriched pellet. The proteins in both fractions, from the tumor cells and the control cells, were resolved by SDS-polyacrylamide gel electrophoresis, blotted onto membranes, and probed with a beta-catenin antibody. Quantitative multi-modal analysis with revealed that beta-catenin was reduced by about 1.54 times in the supernatant of cancer cells compared to the supernatant of control cells. Beta-catenin immunoprecipitation revealed discrepancies in binding complexes to this beta-catenin in osteosarcoma versus normal cells. Immunofluorescence localization of beta-catenin revealed a nuclear and membrane accumulation opposed to a more uniform distribution in fibroblast cells. Additional experiments will assess methods for treating cancer by using polymeric encapsulation and viral therapy. The obtained results are consistent with the hypothesis that changes in beta-catenin level/function are involved in osteosarcoma pathogenesis.

E-2006

Determining the Effects of Vitamin A on Short-term Memory Ability. AMANDA M. THOMAS. School of the Osage, Lake Ozark, MO 65049. Email: CgirL13@excite.com

Recent studies have determined that vitamin A can have an impact on memory capacity (Etchamendy, et al, 2001). It has also been determined that vitamin A supplementations can add to memory ability as well as overall health. This is especially true for adults over the age of thirty with problems relating to dementia. The purpose of this experiment is to determine whether or not there is an actual correlation between vitamin A and short-term memory ability. It is hypothesized that the vitamin A supplementation will help the subjects overall memory capacity as measured by a memory test administered by the researcher. A group of sixty subjects were randomly selected from the School of the Osage faculty and staff to participate in both the control (thirty subjects) and experimental (thirty subjects) groups. The control group underwent memory tests both before and after the three week supplementation period; however, they did not undergo supplementation. The experimental group was subjected to the memory test both before and after the three week supplementation period, and underwent vitamin A supplementation. All participants took, under the supervision of a medical doctor, a 10,000 IU softgel capsule each day for a period of twenty-one days. The test results determined that there is a significant change between the memory test scores of the experimental group ($P=0.023$), but there was not a significant change in the test scores of the subjects in the control group ($P=0.263$). The results of this study provide data that vitamin A improves short-term memory abilty.

E-2007

The Development of a Novel Gene Knockout Technique Utilizing RNA-interference. JASON C. ZHANG. Yorktown High School, Yorktown Heights, New York 10598. Email: jason.c.zhang@gmail.com

Gene knockout has become vital in the development of drug related pharmaceuticals, with many institutions focused solely on the application of related methodologies. However, current gene knockout technology is limited and consists of crude deletion techniques and nonspecific pathways. RNA interference, with high specificity and variability, offers an alternative for gene manipulation. Integration of miRNA and siRNA interference pathways allows for optimized processing and knockout. In this investigation, mutant siRNA-coding let 7a-3 miRNA constructs and Firefly-Luciferase-mRNA coding target were transfected into P19 cell cultures. Transfected cells were analyzed with two luminescent makers: Renilla-Luciferase (targeted gene expression) and Firely Luciferase (transfection efficiency control). Levels of gene expression were variable but consistent, the highest being 40%. Following system affirmation, optimization of construct design is required to produce the traditional 90–98% gene knockout. Future introduction of recombinase sites or effector switches will allow for conditional knockout. Using the data derived from the present work, RNAi can be utilized to provide a new degree of gene regulation, controlling gene expression levels at will. Further optimization of this system is essential.

I-2000

Eicosanoids Influence Insect Cell-viral Interactions. C. L. GOODMAN, A. McIntosh, D. Stanley, USDA, ARS, BCIRL, 1503 S. Providence Road, Columbia MO 65203. Email: goodmanc@missouri.edu

Eicosanoids are oxygenated, enzymatic metabolites of arachidonic acid, including prostaglandins and an array of lipoxygenase products. These signaling compounds are important in vertebrate and invertebrate biology. In insect immunity, eicosanoids mediate and coordinate specific cellular actions responsible for clearing infections (including bacterial, fungal, protozoan and parasitic) from circulation. The signaling mechanisms responsible for the widely varying insect resistance to viruses, however, remain unknown. In this poster we present an overview of eicosanoid structures and report on experiments designed to test our hypothesis that eicosanoids also exert important actions in the interactions between insect cells and viruses. To test our hypothesis, we used two cells lines, one non-permissive to viral infection (HzAM1, a pupal ovarian line from *Helicoverpa zea*) and one permissive (HvAM1, a pupal ovarian line from *Heliothis virescens*). Treating the HzAM1 line with inhibitors of eicosanoid biosynthesis, then challenging the cultures with the AcMNPV baculovirus, resulted in significantly increased proportions of cells producing virus and higher overall extracellular virus concentrations. We infer that eicosanoids influence one or more cellular mechanism(s) of viral resistance. Similar experiments with the permissive HvAM1 line resulted in significantly decreased proportions of cells producing virus early in the infection process although this reduction was overcome later in the infection. Inhibitor-treated cells also produced significantly reduced levels of extracellular virus. Our data on a permissive and a non-permissive cell line indicate that eicosanoids influence cellular events required for either permitting or not permitting viral entry and replication.

I-2001

Stable Transformation of a Tick (*Ixodes scapularis*) Cell Line with the *Sleeping Beauty* Transposon System. T. J. KURTTI, R. F. Felsheim, J. T. Mattila, G. D. Baldridge, N. Y. Burkhardt, and U. G. Munderloh. Department of Entomology, University of Minnesota, Saint Paul, MN 55108. Email: kurtt001@umn.edu

A continuous *Ixodes scapularis* cell line (ISE6) was stably transformed using the *Sleeping Beauty* (SB) transposon in the presence of a helper plasmid expressing SB transposase. The transgenesis markers were a red fluorescent protein (DsRed2) gene driven by a CAGGS promoter and the neomycin phosphotransferase (NEO) gene driven by an SV40 promoter. The NEO transposon plasmid was used to counterselect against random recombinants in medium containing the antibiotic G418. Effective counterselection required 30 to 45 days incubation of transfected cells in medium containing G418. Genomic integration of the DsRed2 gene in stably transformed cells was confirmed by Southern blots and inverse PCR. Cloning and sequencing of the integration site demonstrated that insertions of the DsRed2 gene into the genome occurred through the action of the SB transposase and that a TA nucleotide pair was inserted between SB inverted/direct repeat sequences and putative tick genomic sequences. The results demonstrate that the SB transposon system can be used for transgenesis of tick cells.

I-2002

RNAi-mediated Silencing of a DsRed2-expressing *Ixodes scapularis* (Acari: Ixodidae) Cell Line. J. T. MATTILA, G. D. Baldridge, R. F. Felsheim, N. Y. Burkhardt, U. G. Munderloh, and T. J. Kurtti. Department of Entomology, University of Minnesota, St. Paul, MN 55108. Email: matti014@umn.edu

RNA interference (RNAi) is a promising tool for studying host-pathogen interactions at the cellular and organismal levels through targeted disruption of gene expression. We transformed an *Ixodes scapularis* cell line with the gene for DsRed2, a red fluorescent protein, and used a cationic lipid-based transfection reagent to introduce DsRed2-specific double-stranded RNA into the cells. Change in fluorescence was monitored by fluorescence microscopy and quantified by flow cytometry over eight weeks. Decreased fluorescence was seen by seven days post transfection (PT), and fluorescence declined through 21 days PT. Recovery of fluorescence was seen on 35 days PT and fluorescence was nearly restored by 50 days PT. Our study demonstrates RNAi is effective in a tick cell line and we believe it can be a tool to gain useful insights into basic tick biology and tick interactions with pathogenic organisms.

I-2003

Electroporation of *Crithidia ricardoi* with pNUS-GFPH. Moses A. McDaniel, Gieira Jones, Margaret M. Young, Gary L. Harmon and RONALD H. BLACKMON. Department of Biology, Elizabeth City State University, Elizabeth City, NC 27909. Email: rhblackmon2@mail.ecsu.edu

A series of expression vectors have been constructed by E. Tetaud *et. al.* (2002) that allow the expression of biologically active proteins in *Crithidia fasciculata* and *Leishmania sp.* These proteins can be expressed as a fusion protein with a poly-histidine sequence at either the N- or C-terminus or fused with green fluorescent protein (GFP). *Crithidia ricardoi* (a close relative of *C. fasciculata*) is a monogenetic parasite found in the digestive tract of mosquito, *Culex saltanensis*. Here we report the first successful transformation of this species of *Crithidia* using the vector pNUS-GFPcH. In this investigation, optimization of conditions for expression in *C. ricardoi* was performed. The results were confirmed through immunofluorescence microscopy, western blot analysis using anti-GFP and PCR analysis.

I-2004

Functional Analysis of Nictaba in Insect Midgut Cells. G. Vandenborre[1,2], T. Soin[1], L. Jacobsen[3], G. Caputo[4], E. J. M. Van Damme[2], and G. SMAGGHE[1]. [1]Lab Agrozoology and [2]Lab Biochemistry and Glycobiology, Ghent University, BELGIUM; [3]Roche Diagnostics, Indianapolis, IN 46250; and [4]National Resources Canada, Canadian Forestry Service, Sault Ste Marie, ON, CANADA. Email: guy.smagghe@ugent.be

Using feeding trials with insects we have shown that plant lectins can provoke toxic effects or disturb insect development and fecundity, making them putative candidates as insecticidal proteins. At present the mode of action of lectins on insects is still poorly understood. However, there is good evidence that plant lectins can interact with the insect midgut, and by doing so, interfere with insect growth. Recently we have discovered an inducible lectin from tobacco, Nictaba (*Nicotiana tabacum agglutinin*) that consists of 19 kDa subunits that specifically interact with oligomers of N-acetylglucosamine. In this study, we report the effect of Nictaba on the growth of midgut insect cells, FPMI-CF-203, from the eastern spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). Spruce budworm is the most destructive native insect defoliator of northern spruce and balsam fir forests in Canada and the eastern United States. In a dose-response assay, Nictaba caused a 50% inhibition of cell proliferation at a concentration of approximately 10 mg/l. Similar results were obtained with a recombinant Nictaba produced using the Rapid Translation System from Roche Applied Science. This recombinant Nictaba contains a six histidine tag at its C-terminal end which allowed immunocytochemical localization of the lectin in cultured CF-203 cells using an anti-His-FITC monoclonal antibody. Fluorescence and confocal microscopy were performed to check lectin binding to membrane receptor(s) and/or cellular internalization. The interaction of Nictaba with receptor proteins is discussed in relation to possible signal transduction pathways that can lead to inhibition of the growth of insect midgut cells.

I-2005

Validation Analysis of an Ecdysteroid Receptor Agonist Assay Using Intact Cultured Lepidoptera Cells. G. SMAGGHE[1], H. Mosallanejad[1], L. Decombel[1,2], C. Goodman[2], T. Soin[1]. [1]Lab Agrozoology, Ghent University, Ghent, BELGIUM; [2]Presently: Hogeschool Gent, BELGIUM; and [3]USDA-ARS-BCIRL, Columbia, MO. Email: guy.smagghe@ugent.be

In this study we report on the ecdysteroid-responsiveness of the insect cell line Se4 (BCIRL/AMCY-SeE-CLG4) from embryos of the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae). The cells express an ecdysteroid receptor (EcR) activity as indicated by their response to the insect molting hormone 20-hydroxyecdysone (20E). The effect of this hormone includes a 50% inhibition of cell proliferation with the addition of ~1 μM, and morphological changes characteristic of this hormone (i.e., cell aggregation and process formation). With bisacylhydrazine tebufenozide, these typical effects were also induced, leading to the conclusion that this non-ecdysteroid compound displayed a true EcR agonist activity. Moreover, a competition binding experiment with [^{3}H]-ponasterone A demonstrated that tebufenozide showed a similar affinity as 20E, with 50% competition for EcR binding at ~1 μM. The presence of the EcR in the Se4 cells was also confirmed using the monoclonal antibody 9B9; with the molecular weight of the EcR being around 57 kDa. In another series of experiments, three analogues of tebufenozide were tested against Se4 cells and also against beet armyworm last instar (5th) larvae. These results demonstrated a good correlation between in vitro cellular proliferation inhibition and in vivo larvicidal toxicity. As determined in the last decade, N-tert-N-dibenzoylhydrazine and its derivatives are known to be non-steroidal EcR agonists that possess insecticidal activity; the method that we describe here with insect cell cultures allows for the screening of novel insecticidal compounds based on the interaction between the 20E hormone and its receptor EcR.

I-2006

Biologically Potent Broad Spectrum Antibiotics Obtained from the Tetrodotoxin Rich Organs of Puffer Fishes. JOEY D. MANGADLAO. College of Engineering, University of the Philippines, Diliman, Quezon City, PHILIPPINES. Email: jodacks16@yahoo.com

In the Philippines, puffer fish locally known as "butete" is abundant and caught wild by fishermen; it is usually considered "trash" when spotted in fishing nets. Tetrodotoxin is a well studied toxin present in puffer fishes and being explored as a pain killer at present. This study investigates the antimicrobial potential of extracts derived from the liver, gonads, intestines and skin of three identified species (*Arathron hispidus, Arathron manillensis* and *Chelenodon patoca*) of puffer fish. Crude acidified methanol extracts through RotaVap were treated to eight selected microorganisms namely *Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae, Escherichia coli, Trycophyton mentagrophytes, Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae* using filter paper disc assay. Zones of inhibition (mm) were measured and computed in terms of microbial index and compared with the standard antibacterial drugs Chloramphenicol, Erythromycin and antifungal drug Nystatin. DPPH assay was also conducted to test the antioxidant or free radical scavenging activity of the extracted Puffer fish organs. Data analysis revealed that there were no significant differences between the antimicrobial effects of the crude extracts of the three identified species of puffer fish and Chloramphenicol, Erythromycin as well as Nystatin. Therefore, the antibacterial and antifungal activities of the organ extracts were significantly comparable to commercially available antibiotics. DPPH assay result showed only 24% inhibition. Findings concluded that tetrodotoxin-rich organ extracts from puffer fish possess potent antimicrobial activities that can be used as an alternative means of curing pathogenic infections thus, can be an abundant natural source in the production of commercial antibiotics.

P-2000

Elicitation of *Pueraria lobata* (Kudzu) Cell and Root Cultures for Radiolabeling of Isoflavones. ADAM REPPERT, G. Yousef, R. B. Rogers, and M. A. Lila. Division of Nutritional Sciences, University of Illinois Urbana-Champaign, 449 Bevier Hall, 905 S. Goodwin Ave., Urbana, IL 61801. Email: reppert@uiuc.edu

Isoflavone C-glycosides have superior stability relative to isoflavone O-glycosides, and therefore have potential benefits for glucose homeostasis. In vitro cultures of *Pueraria lobata* (kudzu) were elicited to improve this desired source of isoflavones, particularly the C-glycoside puerarin. *P. lobata* seedlings were germinated in vitro, from which solid callus cultures were generated on a B5 medium supplemented with 1 mg/mL 2,4-D, 1 mg/mL NAA, and 0.5 mg/mL kinetin. Cell suspension cultures were derived from solid callus and maintained on a liquid medium of the same composition, with subculture intervals every two weeks. Untransformed root cultures were developed from roots of in vitro *P. lobata* plantlets and were grown in liquid MS medium (supplemented with 1 mg/mL NAA), with subculture intervals every three weeks. *P. lobata* cell suspension cultures were elicited with the abiotic elicitor methyl jasmonate (MJ) (100 micromolar, 500 micromolar) and both cell suspension and root cultures were also elicited with the biotic elicitor yeast extract (YE) (1 mg/mL). While YE elicitation did not improve the isoflavone content of either cell or root cultures, preliminary results indicated that treatment of cell suspension cultures with 100 micromolar MJ stimulated a consistent 24% increase in both puerarin and daidzein accumulation over the control (5.04 mg/g and 2.17 mg/g vs. 4.06 mg/g and 1.72 mg/g, respectively). Root cultures of *P. lobata* accumulated significantly higher levels of puerarin (9.88 mg/g) than elicited or non-elicited cell suspension cultures, and therefore are a preferred system for producing radiolabeled isoflavone C-glycosides for metabolic tracking studies on isoflavone metabolism.

P-2001

Copper Chloride Elicitation of In Vitro Red Clover Isoflavones. NANCY ENGELMANN, Randy Rogers, Padmapriya Vattem*, Jeevan Prasain*, and Mary Ann Lila. Department of Natural Resources and Environmental Sciences, 1024 Plant Sciences Laboratory, University of Illinois, Urbana, Illinois 61801 and *Department of Pharmacology and Toxicology, Room #456 McCallum Building, University of Alabama- Birmingham, Alabama 35294. Email: nengelma@uiuc.edu

Red clover (*Trifolium pratense*) cell cultures have been previously shown by our lab to be a significant source of bioactive isoflavones which may benefit bone density, cardiovascular health, and cancer prevention. The objectives of this study were to determine if elicitation with abiotic agents could significantly increase accumulation of these valuable secondary metabolites in plant cell cultures, and provide a useful resource for product recovery. Red clover seeds were surface sterilized and 21 d old in vitro germinated seedlings were used as a source of vegetative explants. Petiole explants were used to initiate callus on Gamborg B5 medium with 2 mg/L NAA, 2.25 mg/L 2,4-D, and 2.12 mg/L kinetin, and callus was maintained by subculturing at 4 wk intervals. Suspension cultures were induced from callus, grown in the dark, and treated with the elicitor copper chloride ($CuCl_2$) at one of three concentrations (0.005, 0.05, and 0.5 mM) for 1 or 3 d prior to the harvest at 14 d. Harvested cells were extracted with methanol and analyzed for isoflavone content using HPLC. Preliminary data shows that while no increases in isoflavone concentrations were seen in any 1 d treatments, the greatest accumulations for formononetin and genistein were achieved with a 3 d-0.05 mM $CuCl_2$ treatment (approx. 135% and 65% increase compared to control respectively), and the 3 d-0.005 mM $CuCl_2$ treatment led to the greatest biochanin A accumulation (approx. 25% increase). Based on fresh media volume, the peak isoflavone concentrations for formononetin, biochanin A, and genistein were 15 mg/L, 6 mg/L, and 3 mg/L respectively.

P-2002

Biosynthesis of Avenanthramides in Chitin Elicited Oat (*Avena sativa*) Suspension Cultures. MITCHELL L. WISE[1] and Heidi Kaeppler[2]. [1]USDA, ARS, Cereal Crops Research, Madison WI 53726 and [2]University of Wisconsin, Department of Agronomy, Madison WI 53706. Email: mlwise@wisc.edu

Avenanthramides are polyphenolic alkaloids produced uniquely in oat. These metabolites stem from the phenylpropanoid and anthranilic acid biosynthetic pathways. Although numerous avenanthramides have been described, the three principle forms found in oat are conjugates of 5-hydroxy anthranilic acid and either *p*-coumaric, ferulic or caffeic acid (termed avenanthramide A, B and C respectively). Recent studies have shown that these phytonutrients can reduce exercise induced inflammation of muscle tissue in rats and that they also possess anti-atherosclerotic properties. In whole plants the avenanthramides are found primarily in leaf tissue, in response to fungal infection by *Puccinia coronata*, and in the grain. In most oat cultivars all three avenanthramides are found in the grain; however the proportions and absolute quantities are highly variable depending on cultivar and growth environment. To investigate the signaling mechanisms and metabolic flux of this novel biosynthetic pathway we have developed a cell suspension system that is responsive to chitin elicitation. Hydroxycinnamoyl-CoA: hydroxyanthranilate N-hydroxycinnamoyl transferase (HHT) is the final enzyme in the biosynthetic pathway. Here we demonstrate the dynamics of HHT mRNA production, HHT enzyme activity and biosynthesis of avenanthramide A in response to elicitation with crude crab shell chitin in these oat cell cultures.

P-2003

Cell-free Expression of the Tobacco Lectin. D. Breite[1,2], E. Daniel[1,3], G. Vandenborre[4], N. Lannoo[4], L. Jacobsen[1], G. SMAGGHE[4] and E. J. M. Van Damme[4]. [1]Roche Diagnostics, Indianapolis, IN 46250; [2]presently: Embedded Concepts, Indianapolis, IN 46278; [3]presently: Indiana University, Bloomington IN 47405; and [4]Ghent University, BELGIUM. Email: guy.smagghe@ugent.be

Previously we have shown that tobacco (*Nicotiana tabacum*, cv Samsun NN) plants exposed to methyl jasmonate accumulate a lectin in their leaves that is absent from untreated plants (Chen et al., 2002). Characterization of the induced lectin revealed a protein composed of 19 kDa subunits that specifically interact with oligomers of N-acetylglucosamine. Cloning of the cDNA revealed that the lectin is synthesized as a 165 amino acid precursor which undergoes no processing except for the cleavage of the N-terminal methionine residue. Treatment of tobacco leaves with jasmonic acid methyl ester for 3 days results in the accumulation of 200 to 300 µg lectin per gram fresh weight, depending on the age of the plants. Since these lectin concentrations are rather low huge amounts of plant material are needed to purify milligram quantities of the protein. In addition the purification of the lectin from tobacco leaf material is hampered by the tannins present in this tissue, often resulting in a brownish lectin preparation. In an attempt to obtain reasonable amounts of a recombinant lectin, an in vitro coupled transcription/translation system based on an *E. coli* lysate was used to express the lectin gene. The lectin sequence was cloned into the pIVEX2.3d vector which adds a six histidine tag on the C-terminal end of the protein. The plasmid was expressed in the Rapid Translation System (RTS), a cell-free expression format from Roche Applied Science. The soluble protein was captured and purified by nickel metal affinity chromatography and then characterized. Mass spectrometry of the recombinant lectin revealed that the N-terminal methionine was not removed. However, further characterization of the recombinant lectin revealed that the His tagged lectin is fully functional. Native and recombinant lectin show similar agglutination properties, and exhibit the same carbohydrate binding specificity.

Chen, Y., Peumans, W.J., Hause, B., Bras, J., Kumar, M., Proost, P., Barre, A., Rougé, P., Van Damme, E.J.M., 2002. Jasmonic acid methyl ester induces the synthesis of a cytoplasmic/nuclear chitooligosaccharide-binding lectin in tobacco leaves. FASEB J. 16, 905–907.

P-2004

Rapid Screening of Silent Mutations for Cell-free Production of the Plant Lectin Nictaba. E. DANIEL [1,2], D. Breite [1,3], L. Jacobsen [1], E. J. M. Van Damme [4], and G. Smagghe [4]. [1] Roche Diagnostics, Indianapolis, IN 46250; [2] presently: Indiana University, Bloomington IN 47405; [3] presently: Embedded Concepts, Indianapolis, IN 46278; and [4]Ghent University, BELGIUM. Email: emdaniel@indiana.edu

The Nictaba lectin is a chitin-binding protein produced in the tobacco (Nicotiana tabacum) plant upon exposure to the plant hormone jasmonic acid methyl ester [1]. Nictaba, in its native form, consists of a homodimer with subunits of approximately 19 kDa each. This tobacco lectin lacks a signal peptide and undergoes no co- or post-translational processing after synthesis in the cytoplasm, and is proposed to have a role in certain regulatory and cell signaling pathways in plants [1], as well as interaction with insect midgut tissues [2]. As isolating the protein from natural sources is a labor intensive process that yields only a small quantity of protein, a recombinant approach is desired. Recombinant expression levels were optimized by first designing constructs containing silent mutations using the Proteoexpert algorithm software. Rapid Translation System (RTS) technology from Roche Applied Science was then used to quickly screen the linear template PCR products generated based on suggestions provided by the software calculations. The sequence which yielded the most robust expression upon analysis with Western blot was then cloned into pIVEX2.3 d vector. Continuous-exchange cell-free (CECF) RTS technology was then utilized to produce a more significant quantity of protein. The resulting protein was purified, biochemically characterized [3] and found to be biologically active [2]. Protein produced in the Rapid Translation System is histidine tagged, and can be biotinylated or made using amino acids labeled with ^{15}N, ^{13}C or ^{2}H. This allows further investigation of the biological mechanisms in which this protein plays a role in both plants and insects either at the cellular or whole organism level.

1. Chen, Y., et al., Jasmonic acid methyl ester induces the synthesis of a cytoplasmic/nuclear chito-oligosaccharide binding lectin in tobacco leaves. The FASEB Journal, 2002. 16: p. 905–907.
2. Vandenborre, G. et al. Effect of Plant Lectins on Growth of Insect Midgut Cells. in In Vitro Biology Meeting. 2006. Minneapolis, Minnesota.
3. Breite, D. et al. Cell-free Expression of the tobacco lectin. in In vitro Biology Meeting. 2006. Minneapolis, Minnesota.

P-2005

Tissue Culture of *Nerium oleander* Possesses Cytotoxic Activity for Human Cell Lines In Vitro. N. A. HOVHANNISYAN. Department of Microbiology and Plant Biotechnology, Yerevan State University, Alec Manoogian str., 1, Yerevan, 375025, ARMENIA. Email: physiol@ysu.am

The plant *Nerium oleander* (L) is known in the folk medicine. Hot water extracts of oleander patented as Anvirzel [1] were shown to be cytotoxic for human cancer cells in vitro. A technique of oleander callus culture was developed earlier [2]. The aim of this work was to study the cytotoxicity of extracts of cultured oleander tissues for transformed cells in vitro. The following cell lines were used: HT-1080 (human fibroblastoma), K-562 (human chronic myeloid leukemia), Jurkat (human T-lymphoblastic leukemia), PC-12 (rat pheochromocytoma). The results obtained suggested that callus extracts were toxic for all human cells used but not for the rat cells. This species-specific activity was earlier described for extracts of oleander plant and for Anvirzel. The cytotoxic activity of callus extracts was demonstrated to be concentration-dependent and to be increased with tissue sub culturing (from 4th to 14th passages). It could be likely explained by the accumulation of cytotoxic secondary metabolites in cultivated plant tissues. Thus, the oleander callus tissue extracts maintain the cytotoxic activity specific for the intact plant extracts. The results obtained may promote the development of new chemotherapeutics of plant origin.

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2. Profuno P. et al. 1994. Plantes Medicinales et Phytotherapie. V. 26. P.340–346.

P-2006

Marker Gene Removal During Gene Transfer In*Vitis* spp.: A Technological Approach Toward an Improved Science-society Communication. L. MARTINELLI, L. Dalla Costa[1], I. Vaccari[1], V. Poletti[1], and F. Guzzo[2]. [1]Istituto Agrario San Michele all'Adige, 38010 San Michele all'Adige (TN), ITALY and [2]Dipartimento Scientifico e Tecnologico, Università di Verona, ITALY. Email: Lucia.Martinelli@iasma.it

Proper communication of scientific activity is an essential tool for right managing conflicts among the various actors of the debate on transgenic plants, while the adoption of a bioethical approach is becoming more and more critical. Thus, the scientific community is considering increasing social demand for safety in plant gene-transfer techniques by exploiting "sustainable" practices based on technological progress and risk-management expertise. We are developing a multidisciplinary research on this topic. In the laboratory activity, we are assessing potential approaches for exogenous gene transfer into grape based on "clean" tools. Embryogenic calli and somatic embryos of *V. vinifera* (cvs. Chardonnay, Brachetto), 110 Richter (kindly provided by dr. I. Gribaudo), and *V. rupestris* were co-cultured with *A. tumefaciens* carrying the chemically-inducible site-specific *cre/loxP* pX6 vector with the Green Fluerescent Protein (GFP) and the NPTII genes, where the expression of the *cre* recombinase is regulated by the 17-β-estradiol. (Construct kindly provided by The Rockefeller University of New York, prof. Nam-Hai Chua). Putatively transgenic cultures were selected on kanamycin, and individual somatic embryos were isolated and converted into plantlets. Preliminary molecular assays showed the transfer of the GFP gene into the plant genome. Inductions of the cultures on 17-β-estradiol were performed for obtaining marker gene self-excision and GFP gene activation. The fluorescence stereomicroscope (Leica MZ16FA) observations gave encouraging results, showing the expected GFP gene expression. Project EcoGenEtic.Com supported by Trento PAT.

P-2007

Transformation of Anthurium with Transgenes for Bacterial Blight and Nematode Resistance. M. FITCH[1], T. Leong[2], H. Albert[1], S. Schenck[2], P. Moore[1], and D. Gonsalves[3]. [1]Pacific Basin Agricultural Research Center, ARS, USDA, 99-193 Aiea Hts. Dr., Aiea, HI 96701. [2]Hawaii Agriculture Research Center, 99-193 Aiea Hts. Dr., Aiea, HI 96701, [3]Pacific Basin Agricultural Research Center, ARS, USDA, 99 Aupuni St., Hilo, HI 96720. mfitch@pbarc.ars.usda.gov

Anthurium transformation was undertaken to engineer plants for resistance to bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* and to the nematodes *Radopholus simile* and *Meloidogyne javanica*. *Agrobacterium tumefaciens* transformation of embryogenic calli of 'Marian Seefurth' was shown to be highly efficient but 'Midori' was more difficult to transform. Three different *A. tumefaciens* strains, LBA4404, EHA105, and AGLØ, were used for delivery of 6 different gene constructs. The constructs were *Arabidopsis* NPR1, attacin, T4 lysozyme, and attacin+T4 lysozyme for bacterial blight resistance, and cystatin and cystatin+cowpea trypsin inhibitor for nematode resistance. Each construct resulted in transgenic plants. LBA4404 was used in most experiments. Selectively growing sectors of putative transgenic calli were observed 5 weeks after co-cultivation. An efficiency level of 9 lines per 100 mg FW of co-cultivated embryogenic callus clumps was obtained for 'Marian Seefurth' but <2 lines per 100 mg FW was obtained for 'Midori'. More than 600 lines were selected from 'Marian Seefurth' but only 42 lines from 'Midori'. PCR, NPTII ELISA, and Southern hybridization were used to confirm the presence of the selection and/or bacterial resistance transgenes. Bioassays for resistance to the pests will be conducted on regenerated plants.

P-2008

Genetic Transformation of *Cyamopsis tetragonoloba* (GUAR). SANCHITA VAGHCHHIPAWALA and Richard A. Dixon. The Samuel Roberts Noble Foundation, Ardmore, OK 73401. Email: svaghchhipawala@noble.org

Cyamopsis tetragonoloba, commonly known as Guar or "cluster bean" is a large seeded, drought-tolerant annual legume. Within the United States, this species is primarily grown in northern Texas and southwestern Oklahoma. Guar seeds produce a high molecular weight polysaccharide known as Guar gum. This endosperm-derived gum contains more than 90% galactomannan and has the ability to absorb a very high volume of water resulting in a viscous gel which has found wide use in the food, pharmaceutical, cosmetic, paper, textile and oil industries. Slowly, Guar is attaining the status of an industrial crop, and there is a high potential for genetic modification to introduce industrially important traits in this crop. To date, there is only one published protocol for genetic transformation of Guar. Like any other large seeded legume, Guar is also very difficult to transform and a high efficiency transformation protocol is urgently needed. We will describe protocols for the genetic transformation and regeneration of transgenic guar plants. Rooting of transformed shots has previously been a problem with this species, and we describe ways around this difficulty. These studies form the basis of a program to facilitate genetic improvement of guar for better industrial applications.

P-2009

Agrobacterium-mediated Cotton Transformation and Regeneration—Using Sucrose as Carbohydrate Source and Selecting of Transgenics with Kanamycin Selection. S. SUBBARAO, J. Layton, N. Sidorova, L. Tan, J. Washam, E. Jakse, and D. Duncan. Monsanto Company, St. Louis, MO 63017. Email: shubha.subbarao@monsanto.com

Cotton (*Gossypium hirsutum* L.) is an important crop worldwide for textile production. The introduction of insect resistance genes (Bollgard® II Cotton) and herbicide resistance genes (RR Flex® Cotton), has made it an increasingly valuable commercial crop. *Agrobacterium*-mediated transformation of cotton cultivar Coker130 has been achieved via somatic embryogenesis and in vitro regeneration from selected callus tissue. Studies have indicated carbohydrate sources such as glucose and maltose have been widely used in callus induction of cotton, with sucrose being used during regeneration. The use of sucrose in callus induction of cotton and production of transgenic plants using Kanamycin (Kan), as the selectable marker, has been tested at Monsanto, St. Louis. A range of sucrose levels (from 0.1 g/l to 30 g/l, in addition to 30 g/l glucose) have been tested. Sucrose levels of 0.1 g/l up to 1.0 g/l, in addition to the glucose, were shown to be optimal for callus production, with 5 g/l to 30 g/l sucrose (along with the glucose) being detrimental. Kanamycin levels of 40, 50 and 60 mg/l were tested with 40 mg/l showing no negative effects on callus proliferation and selection. As a carbohydrate source, at the optimal levels, sucrose has shown no detrimental effect on callus induction or on regeneration. Healthy, green, GUS positive plants, with a single copy for the gene of interest have been obtained. Fertility is being evaluated in the greenhouse.

P-2010

Development Of An Efficient *Agrobacterium*-mediated Gene Transfer System for Multiple Sweetpotato Cultivars. J. SCOFFIELD, M. Egnin, B. Bey, M. Quain, C. S. Prakash and D. Mortley. Plant Biotechnology and Genomics Research Lab (NASA), Tuskegee University, Tuskegee, AL 36088. Email: megnin@tuskegee.edu.

Factors enhancing *Agrobacterium*-mediated transfer of foreign genes to sweetpotato [*Ipomoea batatas* L., Lam] cells and in vitro regeneration of transgenic plants via somatic embryogenesis were investigated. An intron -containing beta-glucuronidase (*gus-A, uid-A*) gene, under the transcriptional control of CaMV 35S promoter, served as a reporter. Transformation frequency was evaluated by scoring the number of blue sectors expressing GUS activity on leaf explants of Beauregard, Jewel, Rojo Blanco, NCC-58, J6-66, Mogamba, TU-82-155 (TIS) and PI-318846-3. The use of cocultivation media containing high auxin [2,4-D at 2.21 mg/L (CP1G) or 2.5 mg/L (CP1N)] in combination with low cytokinin (BAP at 0.25 mg/L) levels promoted higher transformation rates than either hormone-free or 2,4-D only media. Pre-culture of explants in MS basal medium prior to infection drastically decreased the number of transformed zones. Explants cocultivated on CP1G showed moderate to high GUS activity compared to those cultured on CP1N, which showed very high levels of GUS activity. Ninety two percent of PI-318846-3 and 90% of Beauregard explants displayed significantly ($p < 0.005$) higher levels of GUS activity compared to cultivars tested (Jewel, Rojo Blanco, NCC-58, J6-66, and TU-82-155) with EHA101 and C58. TIS had the lowest GUS expressing areas. The disarmed *Agrobacterium tumefaciens* strain EHA101 was superior (60%) in facilitating the transfer of *uid-A-intron* gene to sweetpotato cells than strain C58. The optimized protocol was used to obtain transient transformation frequencies ranging from 12% to 87% for leaf explants of all cultivars tested. About 58% of the recovered calli were transgenic for both *uid-A* and *nptII* genes in a PCR assay. Resulting kanamycin resistant regenerants will be subjected to PCR, Southern and RT-PCR analyses to test for the presence of the *uid-A*-intron transgene and confirm its integration and expression in these sweetpotato cultivars. Work supported by Tuskegee University, NASA and USDA.

P-2011

Assessment of *Agrobacterium*-mediated Transformation Methods: Simplifying the Induction Process and Factors to Consider in Plant/Strain Competence Selection. ANWAR A. ALSANABANI, Caula A. Beyl, and Anthony Ananga. Plant and Soil Science, Alabama A&M University, Normal, AL 35762. Email sanaban@yahoo.com

Agrobacterium-mediated transformation is the most common method currently in use for plant genetic transformation. However, transformation methods are as many as reports of induction and some are more complicated than others. To simplify an induction protocol, we investigated whether the bacterial medium (Murashige & Skoog, Agrobacterium Mannitol Medium (AMM), and deionized distilled water(DDW)) used to for *Agrobacterium rhizogenes* strain CIP prior to inoculation would impact its virulence as measured by hairy root induction on leaves of *Ziziphus spina-christi*. Root induction frequency was not significantly different as a function of the pre-inoculation culture medium. When bacterial cells were used directly from solid AMM medium compared to bacterial cells transferred into DDW using two stains, MT232 and CIP, we found that the optimal pre-inoculation medium depended heavily on the strain of *A. rhizogenes* used. Strain CIP was more effective (53% frequency of hairy root induction) if transferred into water and less effective (7%) if taken directly from solid medium, whereas strain MT232 worked better if used directly from solid medium (67%) and worked poorly if used from liquid medium (13%). We propose that induction frequencies reported in the literature for various *A. rhizogenes* strains could be misleading if the strains were not given preferable conditions to achieve optimal virulence prior to inoculation. Moreover, ecological information concerning the strains could provide clues to the optimum protocol for induction as well as the matching and selection process for strain type/plant species competence.

P-2012

In Vitro Propagation of Silver Maple. VICKI MAGNUSSON and Wenhao Dai. Department of Plant Sciences, North Dakota State University, Fargo, ND 58105. Email: wenhao.dai@ndsu.edu

Silver maple (*Acer saccharinum* L.) was micropropagated using axillary buds from a mature silver maple tree (> 25 years old) grown in Fargo, North Dakota. Shoot tips (~1.0 cm) were excised from fast-growing shoots and surface sterilized in 70% ethanol for 30 seconds followed by immersion in 10% Clorox (0.6% sodium hypochlorite) for 15 min. Tips were rinsed three times in sterile distilled water and blotted dry with sterile paper towels. Surface-sterilized shoot tips were inserted into the initial medium [Murashige and Skoog(MS) supplemented with 2.5 mM BA]. Tissue cultures were maintained at 25 °C under cool-white fluorescent light with a 16-hr photoperiod and subcultured every 4 weeks. All media (initial and proliferation described below) were supplemented with MS vitamins, 3% sucrose and 0.65 % agar and adjusted to pH 5.8 before autoclaving. To control contamination, PPM (plant preservative mixture, PCT, Inc., Jefferson Place, Washington D.C.) was added to the medium in the first four subcultures. Tissue cultures appeared stable and pathogen free after subculturing in the aforementioned medium for 4–6 months. The effect of basal medium and plant growth regulator (PGR) on silver maple proliferation was investigated. The maximum proliferation rate (~3.0) was achieved by being cultured in MS containing 1.0 μM BA + 0.1 μM thidiazuron (TDZ) + 0.1 μM indole-3-butyric acid (IBA). Microcuttings were in vitro rooted in auxin-free half strength MS medium after being pulsed in half strength MS medium with IBA or naphthaleneacetic acid (NAA). In vitro rooting rate was influenced by auxin concentration and pulsing time. The maximum rooting percentage (75%–100%) and root number (2–5 roots per cutting) resulted from 3-day pulsing with either 5 to 20 mM IBA or NAA. Rooted plants were transferred to flats filled with Sunshine Mix #1 (Sun Gro Horticulture Canada, LTD, Seba Beach, AB, Canada) and covered with clear plastic tops. After one week, the covers were gradually removed during the following one-week period. Surviving plants were potted into Sunshine Mix #1 and grown in the greenhouse.

P-2013

In Vitro Specificity Exhibited by Fungal Mycobionts of *Spiranthes floridana* (Orchidaceae) within the Congener *Spiranthes brevilabris*. S. L. STEWART and M. E. Kane. Environmental Horticulture Department, University of Florida, Gainesville, FL 32611. Email: sstewart@ifas.ufl.edu

Fungal specificity within the Orchidaceae has been considered controversial for many years. Differences in fungal specificity during germination in vitro versus in situ have lead some to consider orchid fungal specificity as generally low; however, others have suggested that specificity, especially in vitro, is surprisingly high. It appears that fungal specificity may be genus or species specific. An in vitro symbiotic seed germination experiment was designed to examine fungal specificity of an endangered Florida terrestrial orchid, *Spiranthes brevilabris*, using fungal mycobionts isolated from the endemic congener *S. floridana*. In a screen of four fungal mycobionts, isolates Sflo-305 (75.1%), Sflo-306 (73.1%), and Sflo-308 (81.3%) (all originating from *S. floridana*) supported higher percent germination, whereas isolate Sbrev-266 (66.7%) (originating from *S. brevilabris*) supported lower percent germination. However, only isolate Sbrev-266 supported protocorm development to Stage 5 (leaf expansion) after 50 d in culture. All other isolates only supported Stage 1 germination. These findings suggest *S. floridana* may be specific for a certain microflora, and may be indicative of the endemic status of *S. floridana* to two sites in north-central Florida.

P-2014

Optimization of Regeneration in *Pongamia pinnata (L.)Pierre*—a Potential Biodiesel Plant Using Taguchi Approach. B. SRINIVAS and S. Ramgopal Rao. Department of Biotechnology, Sreenidhi Institute of Science and Technology (SNIST), Yamnampet, Ghatkesar, Hyderabad—501301, AP, INDIA, Email: bsrinivas@snist.com

The potential of seed derived oil of *Pongamia pinnata (L.) Pierre* (Fabaceae) was rediscovered and recently has gained tremendous importance as an excellent source of Biodiesel. When used as Biodiesel, *Pongamia* oil is oxygen-rich, leaving cleaner exhaust with very little particulate matter and hydrocarbons. Most remarkable feature of this oil is that it adds no net carbon dioxide to the atmosphere. *Pongamia* oil is converted into Biodiesel by transesterification and is blended with diesel for its use as fuel. Owing to its huge market potential as a source of Biodiesel, enormous planting material is required to raise *Pongamia* plantations. Although conventional methods of propagation will contribute to some extent, in vitro propagation will supplement to a major extent. To identify high oil yielding genotypes of *Pongamia*, germplasm has been collected from various locations, and plantations are being raised. Its biochemical and molecular characterization pertaining to the oil yield and quality is in progress. Present study reports the results of optimizing in vitro regeneration in *Pongamia* using Taguchi statistical approach. Regeneration influencing parameters viz., type & age of the explants, its orientation onto the medium, type of medium and phytohormone concentration were studied. An L_{18} orthogonal array (QUALITEK-4) was used to observe the interaction effects and to identify the vital factors governing the regeneration system. An efficient and reproducible regeneration system in *Pongamia* using immature embryo derived cotyledonary explant was standardized. The results obtained and the interaction effects will be discussed.

P-2015

Callus Establishment and Shoot Proliferation in *Jatropha Curcas*: A Biodiesel Plant Through Nodal Explant Culture. S. K. TIWARI, P. K. Shukla**, Amit Pandey, and M. P. Goswami. Forest Genetics, Plant Propagation & Biotechnology Division State Forest Research Instituite, Polipathar, Jabalpur, MP, INDIA and ** Director SFRI, Jabalpur. Email: drsktiwari1963@rediffmail.com; sdfri@rediffamail.com

Callus establishment through nodal explants and shoot proliferation through callus in *Jatropha curcas* is difficult due to lot of problem related to endogenous contamination and presense of phenolic compounds in explants. However, an in vitro protocol has been successfully developed, when the nodal explant was asepitically inoculated in MS culture medium. Various combinations and concentrations of cytokinins and auxins helping in inducing callus formation and shoot proliferation from explants were tried. However, massive and profuse green callus was induced when MS culture medium was supplemented with BAP 4.0 mg/lt with 2,4-D 1.0 mg/lt within 3 to 4 weeks of culture period. The callus was fragile in nature. Shoot proliferation from callus was induced when MS culture medium was supplemented with BAP 5.0 mg/lt and IAA 3.0 mg/lt. On an average, 5 to 6 green and healthy shoots were induced from callus initailly. When the callus was subcultured on the same composition, the shoot multiplication rate increased and on an avarage, 7 to 8 shoots were induced within four weeks of culture period. Green and healty shoots with 3 to 4 nodes were harvested and shifted to polyhuts under high humid condition for *ex vitro* rooting. The plant establishment rate was 62 percent.

P-2016

Recovery from Gross Apical Damage in Dicotyledonous Seedlings. V. GABA[1], S. Amutha[1], K. Kathiravan[1], S. Singer[1], L. Jashi[2], I. Shomer[2], and B. Steinitz[3]. Depts. of [1]Plant Pathology, [2]Food Science, and [3] Plant Genetics, AROVolcaniCenter, POB 6 Bet Dagan 50250, ISRAEL. Email: vpgaba@volcani.agri.gov.il

Normally decapitation of a dicotyledonous seedling at the cotyledonary stage *ex vitro* results in seedling death, as there are no other shoot apical meristems to resume growth. Only a few dicotyledonous species can recover from complete decapitation and regenerate a new shoot. However, if at the cotyledon stage, following removal of the apex and a cotyledon, a cotyledon is left attached to the hypocotyl, after a few days regeneration of a new shoot occurs from the damaged area at the top of the hypocotyl. Histological and scanning electron microscope studies show that there is no pre-existing meristem in the area from which regeneration is observed. The regeneration response usually requires the presence of at least half of a remaining cotyledon, and has been found in a wide range of dicotyledonous families (Annonaceae, Convolvulaceae, Papaveraceae, Chenopodiaceae, Compositae, Umbelliferae, Solanaceae, Linaceae, Malvaceae, Cruciferae), of several major clades (Magnoliales, Laurales, Ranunculales, Caryophyllales, Asterids, Rosids). Similar regeneration has not been observed from monocotyledonous plants. Organogenesis is always from the same small area at the center of the apical part of the hypocotyl. Curiously, leaf primordia are regenerated prior to the production of a shoot apical meristem. Most species regenerate a number of abnormal leaves (1–4) prior to the resumption of normal leaf production. Most of the apically damaged plants regenerate a single shoot, enabling resumption of a monopodial growth. Ploidy level is not changed in the newly regenerated shoot. The relevance and implications of our results to in vitro systems of adventitious shoot regeneration will be discussed.

P-2017

In Vitro Propagation of Rare and Disappear Plants. O. I. Isaeva[2], M. P. MUMINOVA[1], I. N. Grigina[1], and Sh. S. Khamrakulov[2]. [1] Institute of Genetics &PEB AS of Uzbekistan and [2]Samarkands' Botanical Garden, REPUBLIC OF UZBEKISTAN. Email: mmuminova@yahoo.com

The monograph "Flora of Uzbekistan" listed about 4500 kinds of wild supreme plants. Among them about 400 are rare, endemic and relic plants. The more desirable species are slow and expensive to propagate by natural or/and traditional offsets. The goal of this investigation was to develop reliable procedures for multiplying *Allium sativa* by tissue culture. Bulbs were surface sterilised with commercial bleach ACE for 7 to 20 min. The sterile explants were placed on hormone free Murashige and Skoog basal medium (MS) or on MS supplemented with growth regulators (5.0 to 10.0 mg I -1 adenin, 0.1 to 0.5 mg I -1 kinetine, 0.1 to 0.5 mg I -1 3-indoleacetic acid) and cultivated at 24 °C with a 16 h photoperiod. The greatest shoot formation was obtained from the highest level of adenin with IAA. The medium stimulated optimal shoot and bulblet formation.

P-2019

Effects of Auxin Inclusion During Indirect Shoot Regeneration in Model Plant Species. M. J. BOSELA. Department of Biology, Indiana University-Purdue University at Fort Wayne, 2001 E. Coliseum Boulevard, Fort Wayne, IN 46805-1499. Email: boselam@ipfw.edu.

In general, the effects of cytokinins and auxins during in vitro organogenesis in plants are antagonistic, with cytokinins stimulating shoot regeneration and inhibiting root differentiation, and with auxins having an opposite effect; i.e., promoting root formation at the expense of shoot formation as first established by Skoog and Miller in their classic research on morphogenesis in tobacco callus cultures (1957). However, in the case of direct shoot regeneration, where cell dedifferentiation is required prior to organogenesis, shoot yields and growth (elongation) are generally improved by the inclusion of small quantities of auxin. In fact, for some plant taxa, direct shoot regeneration appears to require that the regeneration medium is spiked with auxins. In contrast, the roles of exogenous auxins during indirect shoot regeneration; i.e., from undifferentiated cultures, have been less well studied. Towards this end, we have recently initiated a series of experiments to assess the effects of auxin spiking during shoot regeneration from callus in tobacco and aspen. In preliminary experiments auxin spiking has been associated with the induction of shoot vitrification (waterlogging) in both species. In aspen, auxin inclusion has also been observed to antagonize shoot morphogenesis, favoring the regeneration of non-elongating rosette shoots with small swollen leaves. However, the initial experiments evaluated only a limited number of auxin types and concentrations, and beneficial treatment combinations may have been overlooked. The current experiments include a greater number of auxin types and doses (concentration). The effects of β-lactams, such as carbenicillin and penicillin, which are spontaneously degraded to phenylacetic acid, a naturally produced plant auxin, in plant tissue culture media, on adventitious shoot regeneration are also being assessed.

P-2020

Time Course Study of Turmeric (*Curcuma* longa L.) Microrhizome Development in Large and Small Vessels of Liquid Media with Varied PGR Concentrations. JEFFREY ADELBERG and Matthew Cousins. Dept. of Horticulture, Clemson University, Clemson, SC 29634. Email: jadlbrg@clemson.edu

Turmeric was grown on MS liquid medium with 6% (m/v) sucrose in small (180-ml) jars for 6 weeks and large (6-l) rectangular vessels for 23 weeks with three concentrations each of BA (0, 0.32 and 1 μM) and Me-Ja (0, 5 and 16 μM) in nine (3x3) factorial combinations. Optimal multiplication rate was 5.2× for 28 days regardless of PGR combination. After 28 days, sucrose concentration of medium was about 5% and began to rapidly decrease. There were 95% linear correlations between biomass (dry and fresh) and media use (sugar and water) in small vessels. Growth rates for leaf and root increased over 6 weeks, but were delayed by Me-Ja. In small vessels, the mass of primary microrhizomes increased at a constant rate regardless of PGR treatment. At all PGR concentrations biomass (fresh and dry) at 6-weeks in large vessels was 50% greater than the small vessels (adjusted proportionately by medium volume) although there was little shoot bud multiplication (1.2×) in large vessels. After 6 weeks, 27% and 18% of the sugar, and 47% and 37% of the medium remained, in small and large vessels, respectively for all PGR combinations. Long-term growth in large vessels was maintained by periodic media supplementation. After 14 weeks, leaf and rhizome growth was unaffected by PGR and root growth was inhibited by Me-Ja. During the 23-week period, leaf and root growth rates declined, but rhizome mass increased at a constant rate. The rate of rhizome mass increase was a consistent 26 mg fresh and 4.1 mg dry mass per explant per day for small jars over 6-weeks and large vessels over 23 weeks. At termination, leaves were 6.4%, roots—5.9%, and rhizomes—16.0% dry/fresh mass, indicative of active accumulation of stored carbohydrates. Most of the original explants developed secondary rhizome "fingers" by the 23rd week. Microrhizome development was dependent on time in culture, sugar and water uptake, but not influenced by the PGR's tested.

P-2021

Miniature Sensors and LED Lighting for Advanced In Vitro Experimentation. L. K. TUOMINEN, D. J. Smith, A. G. Vermaak, J. C. Vignali, M. J. Mischnick, and R. C. Morrow. Orbital Technologies Corporation, Madison, WI 53717. Email: tuominenl@orbitec.com

While much experimental work in plant biology includes known and tightly controlled environmental parameters, tissue culture experiments have largely been exempt from this requirement due to the difficulty of aseptically introducing sensors into the culture volume. As part of the development of a prototype Aseptic Plant Culture System, we have worked to develop and/or modify miniature environmental sensors for use in sterile culture vessels. Testing was conducted to verify that light, humidity, temperature, and gel moisture sensors could stand up to repeated autoclave cycles without detrimental effects such as moisture intrusion or reductions in sensor performance. High-density LED arrays have also been developed for use with small (Magenta-sized) culture vessels. A single Science Light Engine can provide up to six different peak wavelengths ranging from near-ultraviolet (400 nm) to near-infrared (730 nm), and each wavelength can provide a maximum output of 300 micromol/m^2/s. Internal, aseptic sensors and adjustable LED lighting will provide the foundation for a novel, versatile experimental system that can bring increased precision and knowledge of environmental conditions to tissue culture experimentation.

P-2022

Germination and Plantlet Regeneration of Encapsulated Somatic Embryos from Transgenic Grape (*Vitis vinifera L*). N. K. NIRALA[1,3], D. K. Das[1], M. K. Reddy[2], P. S. Srivastava[3], S. K. Sopory[2], and K. C. Upadhyaya[1]. [1]School of Life Sciences, JNU, New Delhi 110 067, INDIA; [2] ICGEB, Aruna Asaf Ali Marg, New Delhi 110 067, INDIA, and [3]Jamia Hamdard, New Delhi 110 062, INDIA. Email: nk_nirala@yahoo.com

Grapes are vegetatively propagated through cutting (nodal) but the cuttings cannot be stored even for a day due to infection. Thus synthetic seeds appear to be a better alternative for rapid clonal multiplication and for genetic improvement of grapes. The rice chitinase gene construct under the CaMV 35S promoter was transferred to grape (*Vitis vinifera L*) cv. Pusa seedless using Agrobacterium mediated transformation system. The transformed plants were tested for the presence of the chitinase gene using PCR and Southern blotting. One of the transgenic plants was selected for the production of synthetic seeds. The cotyledonary-stage somatic embryos (5–7 mm in length) originating from leaf explants of grape were encapsulated individually in 2% alginate gel mix with 0.6% activated charcoal. Artificial seeds were germinated successfully on agar medium containing B5 macrosalts (half strength), MS microsalts (full strength), 3% sucrose and 2.9 mM GA. The percentage of germination of encapsulated somatic embryos (ESEs) showed higher than that of non-encapsulated somatic embryos (NSEs) of the same size on the same medium. The percent germination of ESEs showed an increase when the medium was supplemented with quarter strength B5 macrosalts and L-glutamine. It was also found that ABA had no significant influence rather resulted in 4-week delay in germination. Growing the embryos on the full-strength B5 medium containing sucrose + ABA for 4–6 weeks prior to encapsulation helped the encapsulated embryos to store up to 90 days without loss in their germination potential and the capacity to regenerate into plantlets. Well-developed plantlets regenerated from ESEs were successfully established in soil.

P-2023

Identification and Cloning of RAPD Markers Linked to WA CMS in Rice (*Oryza sativa* L.). A. AHMADIKHAH[1,2] and G. I. Karlov[1]. [1]Timiriazev St., No 44, Dept. of Biotechnology, Russian State Agricultural University, Moscow, 127550, RUSSIA and [2]Beheshti St., Gorgan University of Agriculture & Natural Resources, IRAN. Email: ahmadikhah@narod.ru

The cytoplasmic male sterility for wild-abortive (CMS-WA) is widely used for the development of new CMS lines to use in hybrid rice breeding programs. CMS has been assumed to result from the interaction of cytoplasmic components with the substituted alien nucleus. Numerous studies have focused on differences in mitochondrial genomes between CMS lines and their maintainer counterparts. However, the high degree of polymorphism between the CMS line and its maintainer mitochondrial genomes makes it difficult to identify the mitochondrial factor controlling male sterility. Mitochondrial DNA fragments of two nearly isogenic lines of rice (Neda A & Neda B) were amplified by RAPD primers. Two polymorph markers were cloned, sequenced and, on their sequence several SCAR primes were synthesized and tested on the DNAs from several CMS lines (A lines) and their corresponding maintainer lines (B lines). Results of PCR amplification with the newly designed primers showed that one SCAR marker finely could detect the polymorphism between all CMS lines and their corresponding maintainer lines.

P-2024

Marker-assisted Progeny Test for the Use in Mapping Experiment. A. AHMADIKHAH[1,2], G. I. Karlov[1], and V. S. Sheveloukha[1]. [1]Timiriazev St., No 44, Dept. of Biotechnology, Russian State Agricultural University of Timiriazev, 127550, Moscow, RUSSIA and [2]Beheshti St., Gorgan University of Agriculture & Natural Resources, IRAN. Email: ahmadikhah@narod.ru

Molecular markers are useful tools in improvement of plant varieties and provide new opportunities for marker-assisted selection (MAS). One important step in molecular mapping experiments is having a statistically large power; that is a large sample size including two border recessive and dominant individuals in the studied population. Since usually the genotype of one of them, for example recessive group directly is measurable from phenotypic tests, but that of another stay still unknown, thus to overcome this problem we propose a marker-assisted progeny test so that one can obtain the genotype of any segregating plant from the molecular expression of its progeny with high decision. The decision and the number of progeny to be tested are based on the marker nature (dominant or co-dominant), the phase (coupling or repulsion) and the most important one, the distance of the marker to the gene to be mapped. Assuming that our segregating population is a F_2 cross, we have to identify a molecular marker tightly (<5 cM) co-segregating with the given gene in recessive mapping population, then using the tight marker identified at first step we must genotype the ~10 F_3 progenies of remaining individuals in the population. Therefore, from the F_3 progeny genotype it is possible to deduce the genotype of F_2 individuals. The approach will be discussed later.

P-2025

Characterization of *Populus tremuloides* COMT, 4CL1 and 4CL2 Gene Promoters to Identify Regulatory Elements. E. ANINO, S. Blumer, P. Pechter, S. Harding, and C.-J. Tsai, Biotechnology Research Centre, Michigan Technological University, 1400 Townsend Dr., Houghton, MI 49931. Email: eoanino@mtu.edu

The phenylpropanoid pathway supplies a wide range of secondary compounds that play important roles in plant growth and development. Two major classes of phenylpropanoid products are lignin and flavonoids. Lignin serves as a structural component of cell walls and plays a major role in plant defense. Flavonoids form a large class of phenolic compounds associated with stress-induced responses, signal transduction, symbiotic interactions and reproductive-related functions. The diverse functions of the phenylpropanoid products make this pathway a target for genetic engineering. Our study aims at identifying *cis* regulatory elements that are important for spatiotemporal regulation of phenylpropanoid gene expression. Promoters of three aspen genes, encoding caffeic acid *O*-methyl transferase (COMT), and 4-Coumarate: CoA ligase 1 (4CL1) and 2 (4CL2), are targeted for this research. 4CL activates the hydroxycinnamates to their corresponding high-energy CoA-esters, with the two isoforms, 4CL1 and 4CL2, differentially involved in lignin and flavonoid biosynthesis, respectively. COMT, on the other hand, catalyzes the *O*-methylation of 5-hydroxyconiferaldehyde specifically for syringyl (S) lignin biosynthesis. We have generated independent lines of transgenic aspen, harboring serial promoter deletions fused to a GUS reporter gene for studies aimed at identifying the putative regulatory elements. Histochemical analysis of the transgenic aspen have revealed various tissue-specific GUS expression patterns. *COMT* and *4CLI* promoters show xylem and phloem specific expression patterns, while 4CL2 promoter display an epidermis-specific expression. The *4CL1* promoter is strongly induced by nitrogen stress treatment.

P-2026

Suppression of Phospholipase D in Soybean Seed. JUNG-HOON LEE[1], William T. Schapaugh[1], Xuemin Wang[3], and Harold N. Trick[2]. [1]Department of Agronomy and [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66502, and [3]Donald Danforth Plant Science Center, St. Louis, MO 63132. Email: hnt@ksu.edu

Post-harvest degradation of phospholipids deteriorates the nutrient quality of oil during the long-distance shipment or long-term storage of soybean seed. Phospholipase D (PLD) may be involved in the first step of phospholipid degradation. To preserve the amount of phospholipids in soybean seed, we have made a RNA interference (RNAi) and an antisense constructions using the soybean phospholipase D (SPLD) gene. A 1300 bp SPLD sequence was used to construct a RNAi and an antisense structures under control of soybean seed specific 'beta-conglycinin' promoter. Soybean transformation was performed via the particle inflow gun (PIG) on three cultivars, 'Fayette', 'Flyer', and 'Jack' and transformed cultures were selected on hygromycin. We have produced ten independent PLD suppressed soybean lines. Based on western blot analysis and enzyme assay, transgenic soybean seeds were observed to express lower level of SPLD protein and enzyme activity than non-transgenic controls. Ninety-two lines of $T_{3:4}$ transgenic soybeans (pSPLDi) and $T_{1:2}$ transgenic soybeans (pSPLDanti) were planted at KSU Ashland farm, Kansas, on 21st of June. Using PCR and southern blot analysis to identify the gene of interest, we have selected homozygous lines (8 lines), segregating lines (24 lines), and non-transgenic lines. The *Hin*dIII digestion pattern is similar to the T_0 plant digestion pattern, however, some different banding patterns were observed among the T_4 soybean plants. We will continue to analyze these transgenic soybean seeds from the field test and determine 5 major fatty acid contents, phospholipids profile, SPLD enzyme assay as well as western blots. The results of further research will also be discussed.

P-2027

Abstract has been withdrawn

P-2028

Development of Transgenic Rice Using Electroporation Technique after the Vacuum Treatment. SEA-KWAN OH[1,3], T. Hagio[2], A. Sunaga[2], K. Konagaya[2], S. Kamachi[2], S. Ando[2], M. Tsuda[2], J. Mochizuki[2], Y. Tabei[2], and H.-Y. Kim[3]. [1]National Institute of Crop Science, NARO, Tsukuba, JAPAN; [2]National Institute of Agrobiological Science, Tsukuba, JAPAN; and [3]National Yeongnam Agricultural Research Institute, NICS, RDA, Milyang, KOREA. Email: ohsk7@affrc.go.jp

We have applied novel transformation technique that can directly deliver foreign genes into mature seeds of rice using electroporation after the vacuum treatment. This technique was invented by Dr. T. Hagio et al.(2004) (Patent Application No.: PCT/JP03/08937). Two early-maturing varieties of rice were used. They were 'Kitaake'(Japanese variety) and 'Milyang 208'(Korean variety). Mature seeds were soaked in water overnight at 25–28. Then the seeds were soaked in electroporation buffer for about 3 hours under the condition of reduced air pressure. Electroporation was carried out with a CUY-21 (NEPA GENE Co., Ltd, Chiba Japan) in a 1.0 cm-wide cuvette or 2ml-microtube containing 1.0 ml of electroporation buffer. The electroporation buffer basically contained spermidine, calcium chloride, PVP, Silwet L77 and 100μg/ml plasmid DNA which was pWI-H5K harboring *npt*II gene (Ugaki et al. 1991). In some experiment cellulase concentration was investigated to raise the transformation efficiency. Fifty times of electric pulses (50 v/cm, 50 or 75 ms/pulse) were applied in one experiment. After the electroporation the seeds were incubated in water supplemented with 0.5% PVP for several days, and they were transferred to selection medium containing geneticine sulfate. In our first trial, five geneticine-resistant plantlets were regenerated out of 500 seeds in 'Kitaake', and four geneticine-resistant plantlets were regenerated out of 500 seeds in 'Milyang 208'. Presence of *npt*II gene was confirmed by PCR analysis in some plantlets. The putative transgenic plants are grown in the greenhouse in order to prepare for Southern blot analysis and advance to the next generations (T_1). We will report the details in our poster.

P-2029

Plant Transcriptional Responses to RDX (Royal Demolition Explosive). MURALI R RAO. University of Tennessee, Department of Plant Sciences, Knoxville, TN 37996. Email: mraghave@utk.edu

Nitroaromatic compounds such as RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine, Royal Demolition Explosive or Research Department Explosive), TNT (2,4,6-trinitrotoluene) and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) are important contaminants of the environment and are released into the biosphere almost exclusively from anthropogenic sources. These compounds are generally recalcitrant and remain in the biosphere, where they constitute a source of pollution due to toxic, mutagenic and carcinogenic effects on humans and other biological systems. Among these compounds, RDX is currently one of the most prominent explosives in the United States. RDX is being used more extensively and is becoming a common soil and groundwater contaminant in the United States. Current remediation technologies include incineration, land filling and composting, which are not efficient or cost-effective and are physically challenging. Phytoremediation is an emerging non-intrusive, aesthetically pleasing and low cost technology. Before plants can be fully utilized for phytoremediation or phytosensing, a better understanding of the molecular biology, especially genomics, is necessary. This study focuses on the transcriptional regulation of plant genes upon RDX exposure. Furthermore, the function of various genes and promoters will be studied in response to RDX exposure. Arabidopsis thaliana oligonucleotide microarray technology will be employed to elucidate the genetic responses of plants to RDX and plant genetic engineering approaches will be applied for the development of potential phytoremediation and phytosensor technologies.

P-2030

Modified Leaf Phenolics and European Corn Borer Herbivory in Oxalate Oxidase Transformed Corn. J. SIMMONDS[1], J. Mao[2,3], K. Hubbard[1], I. Altosaar[3], and J. Arnason[2]. [1]Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario, K1A 0C6, CANADA; [2] Dept Biology, University of Ottawa, Ottawa, Ontario, K1N 6N5, CANADA; and [3]Dept Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, Ontario, K1H 8M5, CANADA. E mail: simmondsja@agr.gc.ca

There is compelling evidence that H_2O_2 is a central signalling molecule in stress and wounding responses, pathogen defense, and regulation of cell cycle and cell death. A very early response to insect attack is the transient production of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2). Hydrogen peroxide generated in response to wounding can be detected at the wound site and in distal leaves within an hour of wounding. Activation of defense genes result in the accumulation of proteinase inhibitors and a range of secondary metabolites which interfere with herbivore feeding, growth, fecundity and fertility. To investigate effects of enhanced steady state production of H_2O_2 in corn leaves on European corn borer herbivory, a gene for a H_2O_2 generating enzyme, wheat oxalate oxidase (OxO), under the control of a constitutive promoter was introduced into inbred lines. Steady state H_2O_2 levels in leaf tissue were increased 2–3 fold in OxO lines which exhibited significantly improved resistance to insect feeding in laboratory and field evaluations. Transcriptional activity of 13-lipoxygenase (13LOX) a key regulatory enzyme of phenylpropanoid metabolism, encompassing a wide range of biologically active compounds, was significantly enhanced in OxO lines. The OxO lines exhibited modified secondary metabolism resulting in a significant reduction of hydroxamic acids and elevated levels of hydroxycinnamic acids; total soluble phenolics were elevated three-fold. Ferulic acid, the most prominent intracellular soluble phenolic acid, significantly reduced European corn borer larval development when included in artificial diets at concentrations similar to that in OxO leaf tissue.

P-2031

WHISKERS™-Mediated Transformation of Cotton. J. R. BERINGER, L. W. Baker, C. Clifford, A. Miller, A. M. Palta, D. Pareddy, T. Strange Moynahan, L. Schulenberg, and J. F. Petolino. Dow AgroSciences, LLC, 9330 Zionsville Rd., Indianapolis, IN 46268. Email: jrberinger@dow.com

The adoption of transgenic cotton varieties expressing insect and herbicide resistance genes has steadily increased in the decade since their commercial introduction. *Agrobacterium* has been the most common method of introducing these genes into cotton; though, alternative methods of transgenic production may still be desirable. Microparticle bombardment has also been used for cotton transformation; however, WHISKERS™-mediated gene transfer, by virtue of its simplicity and potential for scale-up, could represent yet another means of delivering DNA to plant cells. This report focuses on the development of a transgenic production system based on WHISKERS™ transformation of embryogenic tissue cultures of cotton.

P-2032

A Novel Plant Transformation Enhancer. Y. DAN*, C. L. Armstrong, J. Dong, X. Feng, J. E. Fry, G. E. Keithly, B. J. Martinell, K. A. Rayford, G. A. Roberts, C. Rommens, L. A. Smith, L. Tan, and D. R. Duncan. Monsanto Company, 700 Chesterfield Parkway, St. Louis, MO 63017 and *Current address: Institute for Advanced Learning and Research, and Departments of Horticulture and Forestry, Virginia Polytechnic Institute and State University, 150 Slayton Avenue, Danville, VA 24540. Email: ydan@vt.edu

We have demonstrated for the first time that lipoic acid (LA) has significantly increased the percentage of transgenic plants per explant from 41 to 179% and the percentage of independent transgenic plant events per explant from 28 to 94% in *Lycopersicon esculentum* cv. MicroTom. In addition, the frequency of escapes has been reduced from 91 to 53%. This study also demonstrated that the increase of the transformation frequency and reduction of escapes in MicroTom were accompanied by 2-fold reduction in the severity of *Agrobacterium*-infected tissue browning/necrosis, 2-fold increase in the survivability of the transformed tissues, 4-fold increase in the percentage of transgenic shoots and 3-fold reduction of the percentage of non-transgenic shoots when using LA under optimal conditions. Following the success in MicroTom, LA was successfully applied in potato, soybean, cotton and wheat *Agrobacterium*-meditated transformations. Frequencies of soybean independent transgenic plant events were increased from 0.6 to 3.6%, potato from 3 to 19% and wheat from 2.9 to 5.4%, and putative transgenic embryo frequency of cotton from 41 to 61%; regeneration of escapes was reduced in soybean from 92 to 72% and potato from 50 to 16% under the optimal conditions. Utilizing LA in plant transformation has achieved significant improvements over the previous transformation methods for MicroTom, potato, soybean, cotton, and wheat at Monsanto. The detail information on the transformation using LA in these plant species will be discussed in this poster.

P-2033

In Vitro Regeneration and Transformation in Chilli Pepper (*Capsicum annuum* L.). KARAMPURI SUBHASH, Peddaboina Venkataiah, and Thamidala Christopher. Plant Biotechnology Research Group, Departments of Botany, Kakatiya University, WARANGAL-506 009, A. P., INDIA. Email: subhash_shashi@yahoo.com

The effective protocol for plant regeneration and genetic transformation, which can be used for the improvement of chilli pepper (*Capsicum annuum* L.) quality, was established. High frequency of plant regeneration was observed when cotyledon, hypocotyl and leaf explants were cultured on MS medium added with IAA (1.0 mgl^{-1}) and BAP (3.0 mgl^{-1}). The development of high frequency shoot regeneration from cotyledon, hypocotyl and leaf explants in chilli pepper provided impetus for the present investigation. *A. tumefaciens* strain LBA4404, harbouring pCAMBIA-1301 or pCAMBIA-2301, was used for co-cultivation with cotyledon, hypocotyl and leaf explants from three genotypes (CA-960, G4, and X-235). Different types of explants viz., cotyledon, hypocotyl and leaf were immersed in bacterial suspension by giving various time intervals (10–60 minutes) and co-cultivated for two to five days on regeneration medium. The explants after co-cultivation were transferred to regeneration medium containing cefatoxime (500 mgl^{-1}) and selection agent hygromycin (25 mgl^{-1}) or Kanamycin (100 mgl^{-1}). Histochemical staining for GUS activity (X-gluc) provided preliminary evidence for successful transformation. In the present investigation, the selection agent (hygromycin or kanamycin), explant source and pre-culture are important factors in recovering high frequency transformants. The putatively transformed shoots were rooted on MS medium containing 1.0 mgl^{-1} IAA and 10 mgl^{-1} hygromycin or 50 mgl^{-1} kanamycin. Currently, experiments are under way to test the heritability of transgenes in genetically transformed pepper.

P-2034

Microarray Analysis of Gene Expression in Barley During *Fusarium graminearum* Infection HATICE BILGIC, Seungho Cho, Lexingtons Nduulu, Kevin Smith, and Gary J. Muehlbauer. Department of Agronomy and Plant Genetics, University of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle St. Paul, MN 55108. Email: bilgi001@umn.edu

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is one of the most significant cereal fungal diseases around the world. Seed quality is negatively impacted by the accumulation of deoxynivalenol (DON), a mycotoxin produced by *F. graminearum* during infection. To improve our understanding of FHB resistance in barley and to develop genetic strategies for effective breeding, we analyzed allele-specific gene expression at QTL regions on barley chromosomes 2(2H) and 3(3H) using two near-isogenic line (NIL) pairs. The chromosome 2(2H) QTL was associated with FHB resistance and heading date. The chromosome 3(3H) QTL was associated with reduced DON accumulation. Our objectives were to identify genes associated with resistance to FHB and DON accumulation on two barley chromosomes. We used the Barley1 GeneChip to monitor differential regulation of barley genes after inoculation with *F. graminearum* in two NIL pairs containing either a resistant or susceptible allele for the chromosome 2(2H) and 3(3H) QTL regions, respectively. We sampled the respective NIL pairs at two time points, 48 and 96 hours after inoculation with water or *F. graminearum* and will present a description of the GeneChip analysis.

P-2035

Expression and Stability of the *Respiratory Syncytial Virus*-F Gene in Advanced Generations of Tomato. JOANN LAU and Schuyler S. Korban. Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801. Email: joannlau@uiuc.edu

Transgene stability and consistency of expression of the antigenic protein(s) are important parameters for the development of a plant-based oral vaccine against the human *respiratory syncytial virus* (*RSV*). The integration, expression, and stability of the *RSV*-F protein were evaluated in the T_3 generation of transgenic cherry tomatoes (*Lycopersicon esculentum* Mill cv. Swifty belle). Expression of the *RSV*-F antigen under the control of the E8 fruit-specific promoter was investigated in the third generation of transgenic tomatoes lines, transformed via *Agrobacterium tumefaciens*. Transgene integration of the *RSV*-F gene in the T_3 generation was initially determined by polymerase chain reaction (PCR). PCR analysis revealed that by the third generation, plants were homozygous for the transgene. Southern blot analysis was used to determine stable integration and transgene copy number of the four highest expressing plants. Southern blot analysis of the high-expressing plants revealed that they all contained a single copy number of the *RSV*-F transgene. Enzyme-linked immunosorbent assay (ELISA) was also used to determine *RSV*-F protein expression. ELISA results revealed that protein expression ranged from 0–14 mg/g of fresh weight, with an average of 2 mg/g of fresh weight. The implications of these findings will be discussed.

P-2036

Transgenic Apple Lines Expressing an Antigenic Protein Against the Human *Respiratory Syncytial Virus*. JOANN LAU and Schuyler S. Korban. Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801. Email: joannlau@uiuc.edu

Respiratory infections present serious risk to people throughout the world. One of the major pathogens responsible for bronchiolitis and pneumonia in infants worldwide is the *Respiratory syncytial virus* (*RSV*). Past clinical trials with formalin-inactivated *RSV* vaccine did not protect against infection, but rather exaggerated lower respiratory disease. In recent years, alternatives to using inactivated viruses have been identified. Among those, subunit vaccines, relying on production of antigenic proteins that induce neutralizing antibodies yet do not carry the replicating machinery or the infectious component of the disease agent, have been deemed as viable alternatives. Recently, plants have emerged as reliable and cost-efficient "bioreactors" for production of subunit vaccines. Moreover, antigen production in plants can lend itself for oral delivery of these subunit vaccines by stimulating the production of both mucosal and serum antibodies eliciting the immune response. Previously, we have developed transgenic tomato lines expressing an RSV-F protein, and found that oral delivery of tomato fruits to test mice induced the appropriate immune response (Sandhu et al., 2000). In this study, we have introduced constructs carrying the gene coding for the RSV-F protein, driven by the CaMV35S promoter, into apple (*Malus domestica* Borkh. cv. Gala) leaves via-*Agrobacterium*-mediated transformation. Nine putative transgenic lines have been identified, and the presence of the RSV-F gene has been confirmed by polymerase chain reaction (PCR). A total of 25 plants from these nine transgenic lines have been rooted, acclimatized, and transferred to the greenhouse. Stable integration of the transgene is confirmed by Southern blotting, and expression of the RSV-F has been determined using both Northern and Western blots. As expected, different levels of expression of the transgene are observed among the different transgenic lines. This is a first step in our effort to assess the efficacy of using apple for developing a plant-based vaccine against RSV.

P-2037

Molecular Characterization of *PgAGO*, a Novel Conifer Gene of the ARGONAUTE Family Expressed in the Apical Cells and Required for Somatic Embryo Development in *Picea glauca*. D. A. LAW, M. Tahir and C. Stasolla. University of Manitoba, Department of Plant Science, Winnipeg, MB, CANADA R3T 2N2. Email: umlawda@cc.umanitoba.ca

A new member of the ARGONAUTE (AGO) family of proteins was isolated from conifer and designated as *Pg*AGO (Gene Bank Accession No. DQ068741; protein ID. AAY67884). The complete coding sequence of *PgAGO* was obtained through screening of cDNA libraries generated from white spruce (*Picea glauca*) somatic embryos. The *PgAGO* gene has an open reading frame of 2880 bp and encoded a protein of 960 amino acids. The predicted protein has an isolectric point of 9.17, a molecular weight of 107 kD and lacks prominent hydrophobic domains, which makes its cellular location inconclusive. The novel protein contains the two conserved regions (the PAZ and the PIWI domains) which are typically found in all members of the AGO family. The PAZ domain of *PgAGO* is composed of 117 amino acid residues and it shares a low degree of homology with similar domains in other species. The C-terminal PIWI domain is composed of 86 amino acids and is more conserved. Localization and transformation studies suggest that PgAGO is required for embryo development, specifically for proper shoot and root apical meristem differentiation. RNA in-situ hybridization shows that PgAGO transcripts are preferentially localized in cells of the shoot and root apical meristems from the early phases of embryo development. RNA-mediated suppression of PgAGO also results in pronounced structural abnormalities of the apical meristems. In embryos with suppressed PgAGO expression the root meristems lack the group of mitotically inactive central cells, whereas the shoot apical meristems are poorly organized and lack a defined layer of apical initials. These abnormalities result in poor post-embryonic performance culminating in meristem abortion and growth cessation.

P-2038

Direct Shoot Induction from Several Types of Explants of Herbaceous Peony. D. K. TIAN, K. M. Tilt, F. Dane, J. L. Sibley and F. M. Woods. 101 Funchess Hall, Department of Horticulture, Auburn University, Auburn, Alabama 36849. Email: tiandai@auburn.edu

The peony is a very important plant based on its medicinal and ornamental use. However, some barriers still exist regarding commercial production via tissue culture. In this study, shoots were successfully induced from three herbaceous peonies 'Xishi Pink', 'Radiant Beauty' and 'Fenlin Hongzhu' in half strength MS medium with different concentration combinations of BA+GA_3, BA+TDZ, BA+TDZ+GA_3 or TDZ. After an initial treatment of 2 min, 15 min or 1 hr in 90 μM TDZ, explants from nodal stem or petiole sections of 'Xishi Pink' formed shoots in MS medium without PGR. Shoots were easiest regenerated from nodal stem tissue (100 % success rate in 10 days), while the success rate of petioles generating shoot primordia or shoots after one month of culture was reduced (50–80% success rate depending on PGR combinations). The shoots or shoot cluster formed at meristematic regions only: at axillary bud eye of nodal stem, junction of rachis and petiolules, and junction of petiole and leaflets. After shoot initiation, explants were transferred to MS medium with GA_3, BA+GA_3 or without PGR. Shoots from the explants in the initial media with BA +TDZ or TDZ, were stronger and shorter than shoots from the initial medium with BA+GA. Shoot subculture was most efficient in medium containing BA +GA_3. Stem and petiole explants also produced callus at the cut surface (100%) after one or two days of culture and more callus generated on stem cuttings. Only limited amount of callus formed on leaf cuttings. However, no shoots were obtained from callus.

P-2039

Coupling Functional and Structural Genomics—Expression Level Polymorphisms in Wheat. MARK C. JORDAN and Daryl Somers. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd., Winnipegg, MB, CANADA R3T 2M9. Email: mcjordan@agr.gc.ca

The study of the genetic basis of complex traits via gene expression analysis using microarrays will uncover many hundreds of genes which will mainly be genes downstream in signalling cascades. For use in plant breeding the key elements regulating these downstream genes must be identified. Combining gene expression and QTL analysis (expression level polymorphism (ELP) analysis) has the potential to link trait analysis and phenotype with gene identification. In order to provide proof of concept for ELP analysis in wheat it was necessary to determine the extent of variation observed in gene expression under field conditions and to determine if the expression of genes and gene clusters could be associated with mapped genetic markers. A subset of a mapping population was grown in each of two years in three locations with three replications per location in a lattice design. Immature seed from each line was collected 5 days after anthesis and immediately frozen in liquid N. RNA was isolated for expression analysis using the Affymetrix wheat GeneChip. A preliminary experiment was carried out to determine the extent of variation within a location. For all three locations the rep effect was not significant. Subsequent analysis was performed on a single rep from one location with all 42 lines subjected to expression analysis. Genes with highly significant differences in expression between genotypes were identified and subjected to clustering using K-means. The expression level of each of these genes was correlated with 330 markers mapped on the population. In many instances groups of genes which clustered together on the basis of expression analysis could be mapped to a specific chromosomal region. Preliminary results indicate a location on chromosome 5B which may regulate the expression of hardness and/or storage protein related genes. The results are encouraging that ELPs in wheat can be used to uncover the molecular basis for important traits.

P-2040

Transposon Transcription and Movement During Maize Tissue Culture. S. M. KAEPPLER, A. Smith, and Y. Rhee. University of Wisconsin, Madison, WI 53706. Email: smkaeppl@wisc.edu

Stress induced by the tissue culture environment results in somatically and meiotically heritable variation among cells within a culture, and among regenerated plants and their progeny. Multiple genetic mechanisms underly the observed variation, including derepression of transposable elements. In a microarray study, we have discovered a novel transposon (TCup) in the hAT family of transposons that is highly upregulated in tissue cultures which have undergone many generations of subcultures. This element is sensitive to derepression in newly initiated cultures that have been treated with the demethylation inhibitor 5-azacytidine. We have also discovered a mini-hairpin element which caused a mutation in the c2 gene of a maize line in tissue culture. This is a unique type of element that has not previously been reported in maize. Our observations are consistent with the hypothesis that chromatin reorganization in tissue culture results in reactivation of specific classes of transposons. Our data suggest that certain elements are more sensitive to derepression in culture. This observation indicates that an interplay between overall chromatin state and transcription potential of classes of transposons determines which elements influence somaclonal variation.

P-2041

Development of Efficient In Vitro Systems For Peanut (*Arachis hypogaea* L.) Micropropagation and Seed Production. B. BEY[1], M. Egnin[1], J. Scoffield[1], A. S. Williams[2], D. Mortley[1], L. S. Crawford[1], and M. Quain[1]. [1]Plant Biotechnology and Genomics Research Lab (NASA), Tuskegee University, Tuskegee, AL 36088 and [2]Department of Plant and Soil Sciences, AA&MU, Normal, AL. Email: megnin@tuskegee.edu

In vitro technologies have allowed the introduction of desirable genes into a variety of crop species such as peanut (*Arachis hypogaea* L.). Protocols for peanut regeneration from explants have been effectively used in transformation projects. However, in vitro multiplication and seed production, key in germplasm maintenance and transgene expression screening prior to greenhouse studies, have been a daunting task. We sought to develop a reliable system to clonally propagate peanut plants and produce in vitro peanut pods with viable seeds. In this study, embryo axes from peanut cultivars ('Georgia Red' and 'New Mexico' select) were excised, and germinated on MS basal media (MSO). Seedlings and nodal cuttings were cultured on a modified Hoagland medium supplemented with calcium nitrate until in vitro flowering then transferred to modified Hoagland with 2 mM calcium chloride for in vitro pod production. Two percent charcoal media was used to overlay the Hoagland medium, additional culture vessels were wrapped with aluminum foil to the level of the media to reduce light entry into the e root production zone and cultured for 4 months at 26°C under a 14:10 hour photoperiod. Eighty percent of plantlets on the modified Hoagland nutrient solution developed nodule-like structures on the roots, and flowered in 8 weeks post initiation followed by pegging 3–4 weeks later. For micropropagation, nodal cuttings (2–3 cm long) from the main stem of in vitro plants were used as multiple explant sources to establish micropropagated plantlets on MSO, MSO with 5mg/L GA3 (MMGA3), and MMGA3 supplemented with arginine, ascorbic acid and putrescine (MMADV). Seventy-five to eighty-five percent of the nodal explants successfully developed into healthy plantlets on MSO and MMGA3. Six to eight weeks of culture, micropropagated clones developed well formed shoots, healthy foliage, roots, and were vigorous facilitating further multiplication process. The explants were established in soil in greenhouse for normal seed production. Our results demonstrate the potential for a seed crop clonal propagation and seed production systems to maintain desirable peanut plantlets without the loss of valuable plant material. Work supported by NASA and USDA.

P-2042

Regulating Plant Tissue Growth by Mineral Nutrition. R. P. NIEDZ and T. J. Evens. USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: rniedz@ushrl.ars.usda.gov

The mineral nutrient requirements of nonembryogenic citrus callus were characterized by breaking the MS salts into the following five factors—NH_4NO_3, KNO_3, Ca-Mg-Cl-Mn-SO_4-PO_4, metals, and Fe-EDTA. A D-optimal response surface experimental design where each factor was varied over a range of concentrations was constructed. Callus was grown on each treatment combination, fresh/dry weights and friability were measured, and each measured response analyzed by ANOVA. Callus growth ranged from 31%–135% MS levels and the resulting polynomial model had an R^2 of 0.98 and a predictive R^2 of 0.92. The model was validated by generating predictions of salt combinations not included in the original design but within the original experimental design space. The responses of callus grown on these new salt combinations were then compared to the predicted values. The implications of this approach in defining the appropriate types and concentrations of mineral nutrients for In Vitro responses, including the importance of mineral nutrition, the limitations of traditional methods of defining mineral nutrient formulations, and what it means to "optimize" In Vitro responses will be discussed.

P-2043

Transgenic Plants of Gladiolus Containing the Coat Protein and Replicase Genes of Cucumber Mosaic Virus. K. KAMO, P. Ueng[1], J. Aebig, H. T. Hsu, M. A. Guaragna, and R. Jordan. Floral and Nursery Plants Research Unit, US National Arboretum, Beltsville, MD 20705 and [1]Molecular Plant Pathology, USDA, Beltsville, MD 20705. Email: kamok@ba.ars.usda.gov

Viruses are a major problem for flower bulb crops because the viruses are maintained each year in the infected bulbs used for propagating the crop. The two economically important viruses that infect gladiolus are *Cucumber mosaic virus* (CMV) and *Bean yellow mosaic virus*. Commercially important cultivars of gladiolus resistant to these viruses are unavailable for breeding. Transgenic gladiolus plants were developed using biolistics that contained either the CMV coat protein serotype I, coat protein serotype II, replicase, coat protein serotypes I and II combined, or coat protein serotype II and replicase genes combined. Cucumber mosaic virus is transmitted by aphids making it difficult to challenge the transgenic plants so a method to challenge plants using the hand-held gene gun was developed. All gladiolus plant lines were initially challenged using 2 μg of cucumber mosaic virus isolated from gladiolus when preparing the cartridges to be used in the hand-held gun. Non-transgenic gladiolus plants (100%) will become infected when challenged with only 2 μg cucumber mosaic virus. Several lines containing either the coat protein serotype II or replicase genes were found to be resistant.

P-2044

RNA Interference (RNAi) to Control the Soybean Cyst Nematode (*Heterodera glycines* Ichinohe). WILLIAM R. DALL'ACQUA, Ryan Steeves, Timothy C. Todd, and Harold N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. Email: wdacqua@ksu.edu

The soybean cyst nematode, *Heterodera glycines*, is an important pest in soybean production throughout the United States and around the world with annual yield losses amounting of billions of dollars worldwide. Efforts to control soybean cyst nematode have traditionally been based on conventional plant breeding and crop rotation. However, with the discovery of RNA interference (RNAi) technology, new methods of control are now being possible. In this study, we utilize RNAi as a method for resistance against soybean cyst nematode by expressing double stranded RNA (dsRNA), homologous to essential mRNA transcripts found in *Heterodera glycines*, in roots. Candidate genes that caused sterility and/or embryo lethality when knocked out in *Ceanorhabditis elegans* were selected from WormBase (www.wormbase.org) and their analogs were identified in the nematode EST's (Expressed Sequence Tags) (www.nematode.net) genetic data base using the NemaBlast search tool. High similarity sequences thus found here were further selected for primer design. Sets of primers were designed to amplify the 3'-end portion of each of the candidate genes from genomic DNA and/or a cDNA library from *Heterodera glycines*. The PCR products were sequenced and used to design sense-antisense RNAi constructs. The sense-antisense constructs were sub-cloned into expression vectors for transformation into soybean. Transformation of soybean callus via particle bombardment was done with each of the three sense-antisense RNAi constructs. Fertile transgenic plants from a few of these constructs have already been regenerated. Molecular analysis and bioassays of these are discussed. Preliminary data showed that RNAi control mechanism affected nematode fitness and resulted in a reduction of cysts, juveniles and number of eggs per cyst.

P-2045

Genebanking of Vegetatively-propagated Crops—Cryopreservation of Forty-four *Mentha* Accessions. E. Staats[1], L. Towill[1], J. Laufmann[2], B. Reed[3], and D. ELLIS[1]. [1]National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, CO 80521; [2]T.E.A.M.S Planning Enterprise, USDA-FS, Fort Collins, CO 80526; and [3]National Clonal Germplasm Repository, USDA-ARS, Corvallis, OR 97333. Email: elvis@ars.usda.gov

Globally, the preservation of plant genetic resources is accomplished mainly by storage of seed. Yet many valuable crop genetic resources are vegetatively-propagated and preservation of these resources is usually done in field plantings susceptible to countless biotic and abiotic risks. Preserving this material for long-term conservation in genebanks as cryopreserved shoot tips is one option to reduce risks of losing this valued vegetatively-propagated germplasm. A difficulty in cryopreservation of genetic resource collections is the large number of genotypes involved. *Mentha* offers a good example of what could potentially be expected from a cryopreservation program of a given genus. Forty-six accessions from >20 *Mentha* species were included in this study. Forty-four accessions (96%) were successfully stored in liquid nitrogen (minimum 40% viability and 60 viable shoot tips/accession in storage) at the National Center for Genetic Resources Preservation. All 46 accessions were tested with vitrification without cold acclimation and 65% were successfully stored. The remaining 35% were tested with cold acclimation resulting in an additional 20% stored. Of the remaining genotypes all but 2 (11%) responded to encapsulation/dehydration. Two accessions (4%) did not respond favorably to any of the cryopreservation methods tested. These results highlight the fact that diverse genotypes can be adapted to cryopreservation yet flexibility in the methods used is critical due to differential genotypic response.

P-2046

Embryo Rescue and Meristem Culture Techniques Used in the Development of Oat/Maize Addition Lines. M. W. GALATOWITSCH*, P. A. Huettl*, M. S. Jacobs*, R. L. Phillips* and H. W. Rines.** *Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108 and **USDA-ARS, St. Paul, MN 55108. Email: galat002@umn.edu

Oat/maize addition lines (OMAs) are generated by crossing oat (*Avena sativa*) and corn (*Zea mays*) and promoting embryo growth and development on a simple culture medium in the absence of normal endosperm development. This process has resulted in the generation of oat plants retaining one or more corn chromosomes. Factors important in promoting embryo and subsequent plantlet development include: oat and corn genotypes involved in crosses, composition and timing of hormone application to pollinated plants and adequate air exchange of culture vessels. OMA plants generated through embryo rescue exhibit a range of variation in fertility and morphology. Self-fertile addition lines have been recovered for each of the ten individual corn chromosomes and these materials used in corn genomics and mapping studies. However, the influence of corn and oat genotype effects has hampered efforts to recover complete sets of fertile individual chromosome additions for the elite corn inbreds B73 and Mo17. OMA plants can be maintained in vitro via meristem culture. This technique facilitates the recovery of lines that have relatively poor vigor or fertility and provides an alternative source for generating material for DNA analysis. Meristems proliferate under low light or darkness on a MS medium with 3 mg/l BA + 0.5 mg/l 2,4-D. Sucrose or maltose are utilized as a carbon source. Meristems have been isolated from plants at a variety of stages in development. Terminal and lateral meristem explants were taken from plants grown under short-day conditions and lateral and immature floral meristems from long-day grown plants. The immature floral meristems cultured in vitro revert to the vegetative state. Ongoing experiments are centered on developing addition lines with chromosomes from corn inbreds B73 and Mo17.

P-2047

Enhancement of Somatic Embryogenesis and Plant Regeneration in Japanese Larch (*Larix leptolepis*). Y. W. KIM, H. K. Moon, and S. Y. Park. Biotechnology Division, Korea Forest Research Institute, Suwon, 441–350, REPUBLIC OF KOREA. Email: dragonkim@foa.go.kr

Different kinds of nitrogen sources, abscisic acid (ABA) or gellan gum concentration, osmoticum were evaluated for their effects on the promotion of somatic embryo maturation in Japanese larch (*Larix leptolepis*). Different concentrations of L-glutamine or casein hydrolysate (CH) in the medium were also compared. The highest number of matured embryos was obtained from 1/2Litvay (LM) medium supplemented with 1.71 mM L-glutamine and 250mg/L CH (473.9/g.FW embryogenic tissue). In contrast, the lowest number was matured when 1,000 mg/L CH alone (25.9/g.FW) added to the medium. As for the effects of ABA and gellan gum concentration, the highest number (224/g.FW) of cotyledonary somatic embryos was achieved on a medium containing 60 μM ABA and 0.6 % gellan gum. Based on the results, it was suggested that somatic embryo production was greatly influenced by concentration of ABA rather than that of gellan gum. As for the effects of different osmotica on maturation media, the highest number of cotyledonary somatic embryos was shown to be produced in the medium containing 0.2 M maltose (551.3/g.FW). However, it turned out that the addition of 0.3 M sucrose was most detrimental to somatic embryo maturation (66.6/g.FW). The best occurrence of cotyledons (83.0%), hypocotyls (85.5%), radicle (56.0%), and plant regenernation (35.5%) was obtained from the matured embryos induced from a maturation medium with 60 μM ABA and 0.8% gellan gum.

P-2048

Somaclonal Variation and Stability of the GUS Transgene in Somatic Embryogenic-derived Populations of Transgenic Celery. GUO-QING SONG and K. C. Sink. Plant Transformation Center, Department of Horticulture, Michigan State University, East Lansing, MI 48824. Email: songg@msu.edu

Transgene stability and somaclonal variation (SCV) are both important considerations in sexual or asexual propagation of transformed plants. Thus, instability of the transgene GUS and monitoring of SCV were investigated in callus and plantlet populations obtained by somatic embryogenesis (SE) of two celery lines. The two lines, from transformation with EHA105:pBISN1, both had normal plant phenotypes. GL18 expressed the ß-glucuronidase (GUS) gene (*gus*A) while GL4 had a silenced *gus*A. SE using petiole explants of GL18, GL4, and non-transformed cv. XP166 produced SE-I populations. Phenotypically abnormal and normal plantlets were each observed about 50% of the time in all three SE-I populations. The phenotypic abnormalities were mostly: 1) fused embryos and plantlets, 2) plantlets with multiple roots, 3) plantlets with secondary embryos, and 4) individual plantlets with reduced apical dominance and/or an altered leaf morphology. To determine stability/instability of SCV among SE-I regenerants, SE-II populations, each with 72 plantlets, were produced from 12 individual GL18 SE-I plants that all had abnormal phenotypes. Of these, 83% of the SE-II plantlets from GL18–5 maintained the SCV phenotype similar to the parent GL18–5. Conversely, the other 11 individual SE-I plants of GL18 yielded about 6% abnormal phenotypes like their parents, and 94% normal phenotypes. No loss of *gus*A expression was detected in any of the 1,217 SE-I of GL18 and 1,632 SE-II of the 12 GL18 SE-I derivatives tested. This analysis included embryogenic calluses, somatic embryos at various developmental stages, and morphologically normal and abnormal regenerants. The silenced *gus*A in GL4 remained inactive in the same tissues in all 1,676 SE-I tested. The results indicate: 1) physiological disorders may be responsible for the majority of phenotypic variations, 11 out of 12 GL18 SE-I plants, and 2) the GUS transgene remained consistently active or inactive in GL18 and the GL4, respectively, via plant propagation through somatic embryogenesis.

P-2049

Genetic Transformation and the Expression of the tCUP Promoter in *Prunus domestica*. L. TIAN[1], S. Sibbald[1], X. Wang[1,2] and S. Kohalmi[2]. [1]Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, Ontario, CANADA N5V 4T3 and [2]Department of Biology, University of Western Ontario, London, Ontario, CANADA N6A 5B7. Email: tianl@agr.gc.ca

Important aspects of genetic transformation via *Agrobacterium* were studied for *Prunus domestica* (European plum). Hygromycin resistance was found to be an effective marker for selection of transgenic tissues. Selection of appropriate *Agrobacterium* strains appeared to be important for successful transformation. Immature and mature seeds were evaluated and both can be used as the explants for transformation. L-cystein which was found to promote transformation in some other species did not exhibit a positive effect in plum. Several other aspects related to transformation were also evaluated. Based on the studies, a reliable transformation technology was developed for plum and a large number of transgenic lines containing various DNA constructs were generated. tCUP promoter, a strong and constitutive promoter isolated from tobacco was transferred to plum. High levels of promoter expression, as measured by GUS reporter gene expression, were detected in major organs and tissues of the transgenic plants. tCUP promoter could be a useful gene expression regulatory sequence for transgene expression in plum and other *Prunus* species for different applications.

P-2050

Agrobacterium tumefaciens-mediated Transformation and Efficient Production of Transgenic Potato (*S. tuberosum* L. ssp. Andigena) Plants. A. K. BANERJEE[1], S. Prat[2], and D. J. Hannapel[1]. [1]Department of Horticulture and Interdepartmental Plant Physiology Major, Iowa State University, Ames, IA 50011 and [2]Department of Plant Molecular Genetics, National Center of Biotechnology, Consejo Superior de Investigaciones Cientificas, Cantoblanco Campus University of Madrid, Madrid, SPAIN. Email: anjan@iastate.edu

Potato is the fourth most important crop of the world and is a valuable model system for studying signaling processes. An efficient transformation protocol is a major pre-requisite for rapid genetic analyses. We have developed rapid production of transgenic potato shoots within just 4 weeks from the time of initial inoculation of leaf explants by *Agrobacterium tumefaciens* for *Solanum tuberosum* subspecies andigena. Vigorous stock plants, the precise, uniform wounding of the midrib on the leaf explant, and the composition of the regeneration media play key roles in the development of this fastest shoot regeneration protocol. Leaf explants developed callus in 7–8 days on basal medium supplemented with benzyl-aminopurine (0.1 mg/l) and napthalene acetic acid (5 mg/l). Shoot induction was achieved on basal medium supplemented with a combination of zeatin riboside (2.2 mg/l), napthalene acetic acid (0.02 mg/l), and gibberellic acid (0.15 mg/l) after 28 days of incubation. Induction of roots from putative transformed shoots was established in hormone-free basal medium supplemented with kanamycin (75 mg/l). Shoots developed normal healthy roots within 5 days of incubation and 91 % of the selected shoots rooted on kanamycin. RT-PCR analysis with gene specific primers, of all rooted shoots out of 20 selected from five different lines exhibited expression of the full-length *StBEL5* transgene driven by the CaMV 35S promoter. The protocol described here is simple, highly efficient, and produces transgenic shoots in just 4 weeks (sometime in just 23 days) after inoculation with *Agrobacterium*. Moreover, the present method also describes the most rapid shoot production protocol for potato reported to date.

P-2051

Gene Transfer into Mature Seeds of some Gramineous Species via Electroporation. T. HAGIO[1], A. Sunaga[1], S. K. Oh[2,3], M. Takeda[4], K. Kakeda[5], S. Kamachi[1], K. Konagaya[1], S. Ando[1], M. Tsuda[1], J. Mochizuki[1] and Y. Tabei[1]. [1]NIAS:Tsukuba Ibaraki 305–8602 JAPAN; [2]NARO; [3]NYARI-KOREA; [4]Kagawa U.; and [5]Mie U. Email: hagio94@nias.affrc.go.jp

In an early work, we described the new system that can directly deliver foreign genes into mature seeds of wheat using electroporation after the vacuum treatment. (Patent Application No. PCT/JP03/08937). To further characterize and improve the system, we investigated the GUS gene expression profiles of mature seeds of some gramineous species changing the parameters of gene transfer conditions. Mature seeds of wheat, barley, maize, indica rice and sorghum were tested. Among the tested species, some varieties of barley showed high level of GUS gene expression and their response was reproducible. These varieties will be useful to establish the model system for the determination of physical parameters. The optimal electrical conditions for DNA delivery into barley was estimated to be around 50 V/cm, 50ms pulse duration and 50 times of pulses. After electroporation the seeds were incubated in water supplemented with 0.5% PVP for two days, and they were transferred to GUS assay buffer. Addition of cellulase into the electroporation buffer increased the GUS gene expression. In wheat, presence of *npt*II gene was confirmed by Southern analysis in T_0 and T_1 generations. In T_2 lines of wheat, some plantlets showed geneticine-resistance and the presence of *npt*II gene was confirmed by PCR analysis. We will report the details in our poster. We are also ready for patent licensing negotiations.

P-2052

Tangerine Blush: Engineering Soybean to Produce ß-carotene in Seed Cotyledons. B. JOYCE, P. LaFayette, and W. Parrott. The Center for Applied Genetic Technologies, Crop and Soil Science, Athens, GA 30606. Email: nightone@uga.edu

Consumers associate quality of food with color and flavor. Without certain carotenoids egg yolks, butter, and even shrimp would be white instead of their expected color. Hence, carotenoids must be incorporated into animal diets to obtain products that meet consumer expectations. Such carotenoids can be one of the most expensive feed ingredients. Soybean meal is a chief source of animal feed in the US, but is devoid of carotenoids. If soybean feed already contained carotenoids farming industries would have a more economical choice for animal feed. The goal of the project was to genetically engineer soybean to produce carotene, the precursor for other agriculturally important carotenoids. Soybean embryos were transformed using microprojectile bombardment with a plasmid containing the phytoene synthase (*crtB*) from *Erwinia uredovora* gene for phytoene, placed behind a cotyledon specific promoter and with hygromycin resistance as the selectable marker. Endogenous soybean enzymes then change the phytoene into β-Carotene. Three engineered lines were obtained. One line of the three turned orange denoting the successful expression of carotene in the seed tissues. Engineering with additional genes for carotenoid synthesis should allow for production of other carotenoids, such as canthaxanthin.

P-2053

Evaluation of Inducible Cre/*lox* and FLP/*FRT* Recombination Systems for Marker Gene Deletion in Rice. ABHILASHA KHATTRI and Vibha Srivastava. University of Arkansas, 115 Plant Science, Fayetteville, AR 72701. Email: akhattr@uark.edu

Transgenic plants contain marker genes that are unnecessary after the identification of transformed line. Removing marker gene is important for acceptability of transgenic crops. One of the methods for marker gene removal is use of site-specific recombination system like Cre/*lox* and FLP/*FRT*. To evaluate which of these systems is most efficient in removing marker gene from transgenic rice, we generated heat-shock promoter controlled FLP and Cre DNA constructs and transferred them into rice. The activity of these recombinases was studied in callus, T_0 plants and T_1 seedlings. The data clearly demonstrated that heat-inducible Cre/*lox* is highly efficient, whereas heat-induced FLP/*FRT* is totally ineffective in removing marker gene from rice plants.

P-2054

Agrobacterium-mediated Transformation of Tropical Root Crop (*Dioscorea rotundata*) Vital for Food Security in Sub-saharan African. M. D. QUAIN[1], M. Egnin[2], J. Scoffield[2], B. Bey[2], C. Bonsi[2], and E. Acheampong[1]. [1]Tissue culture Laboratory, Department of Botany, University of Ghana, Legon, Accra, GHANA and [2]Plant Biotechnology and Genomics Research Lab, Tuskegee University, Tuskegee, Alabama. Email: megnin@tuskegee.edu

Root and tuber crops are key dietary components of daily staples in Sub-Saharan Africa. Yam (*Dioscorea rotundata*) in particular is of economic and social importance and is believed to have evolved in West Africa. Several viral and other pathogenic problems adversely affect the shelf life and nutritional value of yam. Yam has 7g/100g dry weight of protein, less than 1g/100g dry wt fat, 80g/100g dry wt carbohydrate, and 3g/100g dry weight dietary fiber. There has been no report of existing program, using biotechnology techniques to improve the nutritional quality of yam. In an effort to enhance yam quality and quantity, this project sought to develop an efficient genetic transformation system utilizing *Agrobacterium tumefaciens strains* C58 and EHA101, both harboring the binary vector pIG121-Hm containing *nptII*, *uidA*-intron and *hpt* genes. Kanamycin concentrations (0–150 mg/l) were screened for treatment that allows explants to maintain their embryogenic potential and regenerate shoots, ensuring the elimination of escapes during the selection. Leaf explants of yam were cocultivated for 5 days on callus production media supplemented with 2,4-D and BAP, then transferred to medium containing 2,4-D, BAP, Kanamycin and Carbenicillin for selection of regenerants. Following cocultivation, explants were subjected to GUS histochemical assay. Transformation frequencies attained using *A. tumefaciens* strain EHA101 were higher (30–50%) than C58 (less than 15%). These successful transient transformation results could be optimized and adapted in engineering of crop qualities for enhanced protein content, and longer shelf life. UNU/INRA, AgSSIP-World Bank and Tuskegee University funded.

P-2055

High-Efficiency *Agrobacterium*-Mediated Transformation of Pear (*Pyrus communis* L.) Leaf Segments and Regeneration of Transgenic Plants. Q. SUN[1,2], W. Wei[1], R. W. Hammond[1], R. E. Davis[1] and Y. Zhao[1]. [1]Molecular Plant Pathology Laboratory, USDA-Agriculture Research Service, Beltsville, MD 20705 and [2]Shandong Institute of Pomology, Taian, P. R. CHINA, 271000. Email: zhaoy@ba.ars.usda.gov

Pear (*Pyrus communis* L.) is a nutrient-dense fruit with strong consumer demand and high commercial value. However, most cultivated pear varieties are often susceptible to diseases caused by fungi, bacteria, and viruses. Since pear is highly heterozygotic and has a long juvenile period, conventional breeding for disease resistance is difficult to achieve. With the recent advances in molecular biology, artificially engineered resistance has become a new approach to plant disease control. Such genetic engineering requires transformation of parent tissues to introduce foreign genes and subsequent regeneration of transgenic plants with desired characters. Major factors that influence transformation and regeneration of pear cultivars were examined and optimal conditions were established for efficient transformation and regeneration from leaves of a popular pear cultivar, 'Old Home'. High transformation efficiency was achieved due to an improved induction stage following initial *Agrobacterium* infection. In the induction stage, *Agrobacterium* cells and parent leaf segments were co-cultivated on a liquid induction medium, which yielded a five-fold increase of transformation frequency over conventional co-cultivation on a solid medium. Transgenic shoots were regenerated from transformed cells *via* an indirect regeneration pathway, which involves a callus proliferation/shoot primordium induction phase using a combination of thidiazuron (TDZ) and naphthalene acetic acid (NAA) as growth regulators, and a shoot elongation phase using a combination of 6-benzylaminopurine (BA) and indole 3-butyric acid (IBA). With the new protocol, independent transgenic pear lines carrying three foreign genes, *i.e.* a β-glucuronidase reporter gene, an antimicrobial peptide gene, and an anti-apoptotic gene, were regenerated. The transgenic pear plants are being analyzed for foreign gene expression and for their potential disease resistance.

P-2056

Hairy Root Cultures induced by *A. rhizogenes* as a Valuable Source of known and Unknown Alkamides in Three Species of *Echinacea*. F.R. ROMERO[1,2], L. Wu[2,3], K. Delate[1], E. Wurtele[2, 3], and D. J. Hannapel[1,2]. [1]Department of Horticulture, Iowa State University, Ames, IA 50011; [2]Interdepartmental Plant Physiology Major, Iowa State University, Ames, IA 50011; [3]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011. Email: djh@iastate.edu

Plant secondary metabolites are useful as potential drugs, nutraceuticals and food additives. Because of difficulties in biosynthesis and *in planta* variation in the levels of bioactive compounds, a uniform consistent production system such as tissue culture is invaluable for isolating select bioactive compounds. *Echinacea* is one of the best-selling medicinal plants in the US, with reported uses as antibiotic, inmuno-stimulant, and anti-inflammatory. It was historically harvested from wild populations, but its demand has increased so substantially that commercial production has become a viable option. The objective of this project was to establish stable hairy root cultures (mediated by *Agrobacterium* spp.) in *Echinacea*, which can be used as an enriched source of dietary supplements. Hairy roots were induced in three species of *Echinacea* (*E. angustifolia* DC, *E. purpurea* (L) Moench, and *E. pallida* (Nutt.) Nutt.) using *Agrobacterium rhizogenes* A4 and *A. tumefaciens* containing only the *rol* ABC genes. Both *Agrobacterium* strains affected root growth and production of secondary metabolites in transformed material. Hairy root cultures induced by wild type *A. rhizogenes* grew faster than hairy roots induced by *A. tumefaciens* containing only the *rol ABC* genes. Transformed roots exhibited modified metabolic profiles of alkamides and produced unknown secondary metabolites. Our results establish the potential of hairy root cultures as a bioreactor system to produce natural products with therapeutic bioactivity.

P-2057

Functional Role of Potato Virus X Movement Protein Domains in the Virion RNA Translational Activation. M. A. Arkhipenko, A. A. MUKHAMEDZHANOVA, S. V. Kozlovsky, O. V. Karpova, N. P. Rodionova, and J. G. Atabekov. Lomonosov State University, Moscow 119899, RUSSIA. Email: usinskmax@mail.ru

Potato virus X (PVX) is a helical positive-strand RNA plant virus. Recently, we have reported that a selective binding of PVX-coded movement protein (TGBp1) to one end of a coat protein (CP) helix converted PVX virion RNA from non-translatable into a translatable form. Binding of TGBp1 to CP subunits located at one extremity of PVX particles induced a linear destabilization of the CP helix and TGBp1-dependent ribosome-triggered PVX disassembly representing a novel phenomenon. To localize theTGBp1 domain binding to terminal CP subunits of PVX particle, a series of TGBp1 protein N- and C-terminal deletion mutants were constructed (including deletion in superfamily 1 NTPase/helicase motif of TGBp1). And the mutant with substitution in the conservative tripeptide GKS (/>Walker box) were produced. PVX TGBp1 mutants were used in in vitro translation system to analyze their translation activating ability. TGBp1 mutant proteins were tested for their ability to form complexes with PVX; the PVX-binding ability was examined by Far-Western Blotting and Immunoelectron microscopy. Only the substitution mutation was capable to convert nontranslatable PVX virion RNA into a fully translatable form as well as a full-length TGB1 but not other deletion mutants. These data mainly were in agreement with result of protein-protein interactions analyses by Far-Western Blotting. In vitro TGBp1 phosphorilation is not enable to activate translation of PVX virion RNA.

P-2058

In Vitro Assembly of Triple Complexes from Potato Virus X RNA, Coat Protein and the 25-kDa Movement Protein. N. A. NIKITIN, O. V. Karpova, O. V. Zayakina, M. A. Arkhipenko, N. P. Rodionova, and J. G. Atabekov. Lomonosov State University, Moscow, RUSSIA, 119899. Email: aleolay@hotmail.ru

Two models have been proposed for nature of the infectious potexvirus transport form that moves from cell to cell: (i) filamentous virions are involved in cell-to-cell movement of potato virus X (PVX); (ii) non-virion ribonucleoprotein particles (RNP) complexes consisting of RNA, coat protein (CP) and movement protein (TGBp1) moved. However, that exact structure of these RNPs has not been yet studied. Here we characterized the products assembled in vitro from PVX RNA, CP and TGBp1 by electron and atomic force microscopy. The complexes appeared as single-tailed particles (STPs) with helical head-like structure comprised of CP subunits located at the 5'-proximal region of PVX RNA; the TGBp1 was bound to the terminal CP molecules of the "head". No particular non-virion RNP complexes were observed. STPs could be assembled from RNA and CP in the absence of TGBp1. STPs without TGBp1 were not translatable; however, they were rendered translatable by binding of TGBp1. Also CP phosphorilation result in translation activation of STPs. We suggested that RNA-mediated assembly of STPs proceeds in the following way. Firstly, nontranslatable single-tailed CP-RNA particles are produced due to the 5'-terminal region of RNA encapsidation. Secondly, the TGBp1 molecules bind to the end of a polar "head", resulting in conversion of the STPs into a translatable form. The single-tailed particles or/and with PVX virions may represent the "transport form" of viral infection.

P-2059

Polyamine Level Modulation Affect to Tobacco BY-2 Cell Growth. F. Serrano, A. Piqueras[1], and J. L. CASAS. Unidad de Biotecnología Vegetal. Instituto Universitario de Investigación CIBIO. Universidad de Alicante, Carretera de San Vicente del Raspeig, s/n. E-03690 San Vicente del Raspeig. Alicante, SPAIN and [1]Departamento de Mejora Vegetal, Campus Universitario de Espinardo, P.O. Box 164, E-30100 Espinardo, Murcia, SPAIN. Email: jl.casas@ua.es

Free polyamine (PA) levels of tobacco BY-2 cell were modulated by means of the application of selected inhibitors of polyamine biosynthesis, and their consequences on cell growth and viability evaluated subsequently. Methylglyoxal (bis-guanylhydrazone) (MGBG), an inhibitor of AdoMet decarboxylase, or aminoguanidine (AG), an inhibitor of polyamine oxidase was independently supplied to a BY-2 cell suspension at 0.1, 1.0 and 5.0 mM. After fifteen days of growth in the presence of the inhibitor, the cell viability and growth rate were evaluated and correlated with endogenous polyamine content. AG at 1.0 and 5.0 mM caused a dose-dependent reduction in cell viability, with a concomitant reduction in cell growth rate that was 0.18 g fresh weight day^{-1} and 0.05 g fresh weight day^{-1}, respectively, far from the 0.46 g fresh weight day^{-1} exhibited by untreated control cells.

Treatment	Concentration (mM)	Cell viability (%)	Cell growth rate (g fw day^{-1})	Polyamine/ diamine ratio	Total PAs (nmol g fw^{-1}
Control	—	100	0.46	1.56	208.6
AG	0.1	100	0.46	0.74	340.9
	1	64.8	0.18	0.76	451.9
	5	29.7	0.05	0.59	483.0
MGBG	0.1	91.9	0.30	0.51	313.4
	1	100	0.27	0.36	424.0
	5	72.9	0.28	0.41	708.2

MGBG was less harmful than AG because only at the highest concentration tested caused a significant reduction of cell viability, much less pronounced than that caused by AG. PA analysis revealed the presence of putrescine (diamine), spermidine and spermine (polyamines) in tobacco BY-2 cells. In view of relationships observed between PA pattern and cellular viability or growth rate, the following can be deduced:

a) The strong effect of 5 mM AG should neither be addressed to a lower polyamine/diamine ratio, nor to an accumulation of polyamines within cells. We hupothesise that some product from PA oxidation (1,3-diaminopropane or 4-aminobutyric acid), could play a pivotal role in maintaining cellular homeostasis and growth.
b) Polyamine/diamine ratio, though apparently a good indicator of situations of active growth, should always be given along with other parameters such as total PA content to be fully informative of the physiological status of a plant tissue or cell.

[1]Triphenyltetrazolium chloride reduction test.

P-2060

Potential Mechanisms for Contaminations of Green Onions with Hepatitis A. DAVID D. CHANCELLOR, Shachi Tyagi, Virginia M Dato, Sara Bacvinskas, Michael B Chancellor, Fernando de Miguel. Department of Urology and Department of Behavioral and Community Health Services, University of Pittsburgh Graduate School of Public Health. Pittsburgh, PA. Email: ddato@aol.com

Introduction: The largest Hepatitis A outbreak occurred in Pennsylvania in November 2003, with over 600 people infected with the deadly virus. The source of the outbreak was traced backed to green onions. Two biomarkers were used to determine ways that hepatitis virus can contaminate green onions. **Methods:** Fluorescent microspheres and Hepatitis A attenuated virus vaccine (HAV) were placed on the outside and soil of pot grown green onion to determine if hepatitis virus can contaminate the growing green onions for up to 60 days. In addition, green onions were grown in a self contained hydroponic system. Fluorescent microspheres and HAV were placed in the circulating water and the plant was analyzed after 1, 2, 7 and 21 days. Reverse transcription-polymerase chain reaction (RT-PCR) was used to identify HAV RNA in the RNA isolated from the green onions. **Results:** Microspheres were seen on the outside and inside of the pot-grown onion for up to 60 days, even after the outermost 3 layers were carefully peeled off. RT-PCR revealed Hepatitis A RNA in well-washed green onions. In the hydroponic grown onion, microspheres were seen in the bottom, middle and tip of the onion after only 1 day. There were greater fluorescent illuminiscence in the middle and base vs. the tip of the plant in day 1 and 2 ($p < 0.001$) but microspheres distribution was similar throughout the onion at day 7 and 21. RT-PCR demonstrated HAV RNA in the onion body in hydroponically grown onion after one week. **Conclusions:** Both biomarkers support that HAV can contaminate the inside of the growing onion and be taken up intracellularly through the roots. Once inside, the particles are impossible to remove with cleaning.

P-2061

High Expression Levels of Erythropoietin(EPO) in Plants Cells Using TMV and PVX-Based Viral Vectors. C. LACORTE [1,2], J. Shrestha[1], H. Beenen[1], D. Lohuis[1], R. Goldbach[1] and M. Prins[1]. [1]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD, Wageningen, THE NETHERLANDS and [2]EMBRAPA-Recursos Genéticos e Biotecnologia, Brasília, DF, BRAZIL. Email: Cristiano.Lacorte@wur.nl

Expression of heterologous proteins in plants has become an attractive alternative for expression systems based on animal, yeast or bacteria cell culture. A number of proteins of pharmaceutical and industrial significance have been produced successfully in plants, where the low cost of production, easy scaled-up, and low risk of contamination with animal pathogens are the main advantages. In this report, we tested the expression of human Erythropoietin (EPO) in plant cells using viral vectors. EPO is a hormone acting as a growth factor for erythrocytes and has has important therapetical application for anemia resulting from chemotherapy, AIDS or chronical renal failure. A synthetic EPO gene, with a codon usage optimized for plant expression was cloned into a Gateway™ entry vector and transferred by LR reaction to a destination binary vector pK7GW and to TMV and PVX-based viral vectors. Expression vectors containing the EPO gene were transferred to *Agrobacterium tumefaciens* and infiltrated in *N. benthamiana* leaves in vitro and *in planta*. Extracts from inoculated tissues were analyzed after 5 days and the EPO protein was detected by Western blot using specific polyclonal antibodies. Expression from TMV vector produced high amounts of recombinant EPO, but also induced a strong necrotic reaction after 5–10 dpi at the inoculated leaves and in systemically infected tissues. These results indicate that high levels of EPO can be obtained in plant cells which thus represent a novel alternative for recombinant EPO production.

P-2062

Polyamines and Ethylene as Stress Indicators During Root Development in Micropropagated Rosa Plantlets Acclimatized to Ex Vitro Conditions. A. PIQUERAS, S. Hussein, M. D. Serna[1], and J. L. Casas[1]. CIBIO, Plant Biotechnology Unit. Universidad de Alicante, Campus de San Vicente PO Box 99, 3080, Alicante, SPAIN and [1]Dept. Plant Breeding (CEBAS) CSIC. PO BOX 164, 30100 Espinardo. Murcia SPAIN. Email: piqueras@cebas.csic.es.

The development of a functional root system is an essential requisite for the successful acclimatization of micropropagated plantlets to ex vitro conditions. We have used the roots of micropropagated rosa plantlets at several stages during the acclimatization process (0, 2, 7, 15 and 30 days) to study changes in the different polyamine fractions as well as ethylene production. Both polyamines and ethylene have been involved in the response of plants to environmental stress and could be usefull parameters to improve the quality control of the acclimatization process. The level of total polyamines in roots showed a significant decrease with the progression of acclimatization to ex vitro conditions. For the different polyamine fractions studied, the conjugated fraction resulted to be clearly reduced particularly between 15 and 30 days of acclimatization. The free polyamine fraction experimented fluctuations until the last week of the process when a clear increase could be observed. The only fraction with a continued increase was the bound one. The qualitative analysis of free polyamines during the first three weeks only allowed the detection of putrescine and spermidine while spermine and cadaverine could only be measured at day 30. The pattern of conjugated polyamines during acclimatization showed the continuous presence of putrescine and spermidine with the discontinuous absence of spermine. The pattern of bound polyamines only allowed the detection of putrescine, espermidine and a progressive and significant increase in cadaverine as the main polyamine. The ethylene produced by the roots of micropropagated rosa plantlets during acclimatization showed a progressive decrease with time. However, at day 2 a marked increase in the production of ethylene was observed followed of a continuous reduction until day 30.

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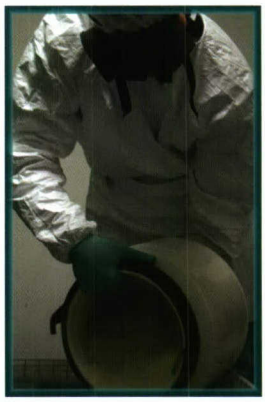

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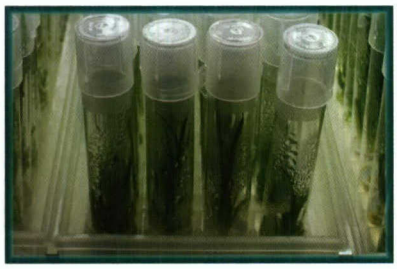

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