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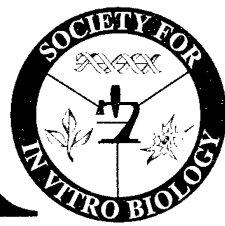

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Portland, Oregon
May 31 - June 4, 2003

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The 2003 Congress on In Vitro Biology's program includes symposia, workshops, contributed paper and poster sessions, continuing education programs, exhibits, and other events that reflect state-of-the-art in vitro biology and biotechnology. A program has been developed that will provide you with the latest research in the diverse areas of vertebrate, cellular toxicology, invertebrate, and plant cell and tissue culture. Whether your interest is reproductive health, insect hormones, artificial chromosomes, regulatory affairs, habitat restoration, micropropagation, or tropical plant transformation, there is something for you at the Congress. The Congress is also an opportunity to meet and share science with colleagues from around the world in a comfortable, intimate setting.

INVITRO

VOLUME 39 NUMBER ABSTRACT
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The Future of Serum: A Roundtable Discussion ... iii-A
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Interactive Poster Session: In Vitro Tools and Techniques ... v-A
Interactive Poster Session: Insect Midgut Stem Cell Lines and Insect Cell Lines for Biocontrol ... vi-A
Interactive Poster Session: Stress Biology ... vi-A
Plenary Session: "ARTistic Use of Nonhuman Primates: IVF to Cloning and Beyond" ... vii-A

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2003 Congress on In Vitro Biology

Time	Saturday May 31	Location	Sunday June 1	Location	Monday June 2
Morning 6:00 am - 8:00 am			7:00 am - 8:00 am Plant Program Committee Meeting	Overton Room	7:00 am - 8:00 am Publication Committee Meeting
			CABI/IAPTC&B/SIVB Meeting	Pettygrove Room	Student Affairs Breakfast
			Education Committee Meeting	Glisan Room	
8:00 am-10:00 am	8:00 am - 12:00 pm SIVB Board of Directors Meeting	Crown Zellerbach	Cryopreservation	Washington/Clark	Biopharmaceutical Manufacturing: Address the Needs of Staffing, Facilities, and Processes
					Biotechnology of Grapevine Improvement
					Growth Factors: Part A
					Monocot Transformation: Contributed Paper Session
10:00 am - 10:30 am			Coffee Break	Grand Ballroom	Coffee Break
10:30 am-12:30 pm			Creating Connections Between Scientists and Educators: Bringing Biotech to the Classroom	Timberline	Epigenetic Toxicants, Altered Intra-cellular Signalling and Modulated Gap Junctional Intercellular Communication
			The Future of Serum: A Roundtable Discussion	Multnomah	Forest Biotechnology: Should, Could, and Wood
			High Throughput Methods	Washington/Clark	Growth Factors: Part B
			In Vitro Tools Contributed Paper Session	Clackamas	
12:30 pm-2:30 pm	12:30 pm - 1:30 pm 2003 Program Committee Meeting	Weyerhaeuser	12:30 pm - 1:30 pm Lunch and Posters		12:30 pm - 1:30 pm Lunch and Posters
			Plant Editorial Board Meeting	Pettygrove	
			1:30 pm - 2:30 pm Interactive Poster Sessions: In Vitro Tools and Techniques	Grand Ballroom	1:30 pm - 2:30 pm Interactive Poster Sessions: Biotechnology
			Insect Cell Lines for Biocontrol	Grand Ballroom	In Vitro Tools/Evaluation of Toxicity
			Stress Biology	Grand Ballroom	
Afternoon 2:30 pm - 5:00 pm	3:00pm - 6:00 pm Poster Set up	Grand Ballroom	3:00 pm - 4:30 pm Plenary Session ARTistic Use of Nonhuman Primates: IVF to Cloning and Beyond	Multnomah/Clackamas	Dicot Transformation Contributed Paper Session
					Growth Regulators
					In Vitro Quantitation of Angiogenesis: A BDBiosciences Workshop
Evening 5:00 pm - 11:00 pm	5:00 pm - 6:00 pm History Society Meeting	Grand Ballroom	5:00 pm - 9:00 pm Plenary Reception and Dinner	Pittock Mansion and the World Forestry Center	6:00 pm - 9:00 pm Plant Business Meeting and Social
	6:00 pm - 7:00 pm Student Reception/Poster Session	Presidential Suite			Vertebrate/Toxicology Business Meeting and Social
	7:00 pm - 9:00 pm Opening Reception	Grand Ballroom			Invertebrate Business Meeting and Social
	City of Roses Silent Auction Kickoff	Grand Ballroom			

Saturday: Registration 7:00 am - 7:00 pm; 11:00 am - 5:00 pm The Scotts Compay Tour

Sunday: Registration 7:00 am - 6:00 pm

Monday: Registration 7:00 am - 6:00 pm

Program at a Glance

Location	Time	Tuesday June 3	Location	Wednesday June 4	Location
Overton Room Pettygrove Room	Morning 6:00 am - 8:00 am	6:00 am - 7:30 am Fun Run/Walk 7:00 am - 8:00 am Development Committee Meeting Membership Committee Meeting	Grand Ballroom Foyer Pettygrove Room Overton Room	Long Range Planning Committee Meeting	Pettygrove Room
Multnomah Washington/Clark Timberline Clackamas	8:00 am-10:00 am	Bioreactors and Biopharming Countering Chemical and Biological Threats: Current Research	Washington/Clark Multnomah	Biomass Conversion for Fuels Biotechnology Contributed Paper Session	Washington/Clark Multnomah
Grand Ballroom	10:00 am - 10:30 am	**Coffee Break**	**Grand Ballroom**	**Coffee Break**	**Mt. St. Helen's Foyer**
Multnomah Washington/Clark Timberline	10:30 am-12:30 pm	Advances in Cancer Modeling and Treatment Disease and Pest: Pathways to Resistance Plant Tissue Culture and Micropropagation Contributed Paper Session	Multnomah Washington/Clark Clackamas	Transgenes Blowing in the Wind?	Washington/Clark
Grand Ballroom Grand Ballroom	12:30 pm-2:30 pm	12:30 pm - 1:30 pm Lunch and Posters 2004 Program Committee Meeting 1:30 pm - 3:00 pm Cell Models and Cellular Differentiation Contributed Paper Session	 Pettygrove Room Clackamas	12:30 pm - 1:30 pm Lunch and Posters	
Clackamas Washington/Clark Timberline	Afternoon 2:30 pm - 5:00 pm	2:00 pm - 3:00 pm Poster Breakdown and Removal 2:00 pm City of Roses Silent Auction Final Bidding 3:00 pm - 5:00 pm Delivery of Genes to Mammalian Cells with Baculoviruses Predictive Toxicology Roundtable Transgenic Cereals	Grand Ballroom Grand BallroomFoyer Multnomah Timberline Washington/Clark	2:30 pm - 4:30 pm Gene Silencing: Use for High Throughput Gene Validation and/or Functional Genomics New and Developing Technologies for Micropropagation	Washington/Clark Multnomah
Clackamas/Multnomah Washington Off Property	Evening 5:00 pm - 11:00 pm	5:00 pm - 6:00 pm SIVB Business Meeting 7:00 pm - 8:00 pm Reception Announcement of City of Roses Silent Auction Winners 8:00 pm - 10:00 pm Closing Banquet	Multnomah Grand Ballroom Foyer Grand Ballroom Foyer Grand Ballroom	**Thursday June 5 Special Event** 10:00 am - 4:00 pm Greyhorse Winery Tour	McMinnville, OR

Registration 7:00 am - 5:30 pm (Tuesday)

Registration 7:00 am - 5:00 pm (Wednesday)

Schedule of Functions

TIME	TYPE OF FUNCTION	ROOM
FRIDAY, MAY 30		
5:00 pm – 8:00 pm	SIVB Board of Directors Meeting	Crown Zellerbach
SATURDAY, MAY 31		
7:00 am – 7:00 pm	**Registration**	Grand Ballroom Foyer
8:00 am – 12:00 pm	SIVB Board of Directors Meeting	Crown Zellerbach
12:30 pm – 1:30 pm	2003 Program Planning Committee Meeting	Weyerhaeuser
11:00 am – 5:00 pm	The Scotts Company Tour	The Scotts Company
3:00 pm – 6:00 pm	Poster Set-up	Grand Ballroom
5:00 pm – 6:00 pm	History Society Meeting	Presidential Suite
6:00 pm – 7:00 pm	Student Reception/Poster Session	Grand Ballroom
7:00 pm – 9:00 pm	Opening Reception	Grand Ballroom
7:00 pm – 9:00 pm	City of Roses Silent Auction Kickoff	Grand Ballroom
SUNDAY, JUNE 1		
7:00 am – 6:00 pm	Registration	Grand Ballroom Foyer
7:00 am – 8:00 am	SIVB/CABI/IAPTC&B Business Meeting	Pettygrove Room
7:00 am – 8:00 am	Education Committee Meeting	Glisan Room
7:00 am – 8:00 am	Plant Program Committee Meeting	Overton Room
10:00 am – 3:00 pm	**Exhibits and Posters**	Grand Ballroom
10:00 am – 10:30 am	Coffee Break	Grand Ballroom
12:30 pm – 1:30 pm	In Vitro – Plant Editorial Board Meeting	Pettygrove Room
12:30 pm – 1:30 pm	Lunch	Grand Ballroom
5:00 pm – 9:00 pm	Plenary Reception	Pittock Mansion and the World Forestry Center
MONDAY, JUNE 2		
7:00 am – 6:00 pm	Registration	Grand Ballroom Foyer
7:00 am – 8:00 am	Student Affairs Breakfast	Pettygrove Room
7:00 am – 8:00 am	Publications Committee Meeting	Overton Room
10:00 am – 3:00 pm	**Exhibits and Posters**	Grand Ballroom
10:00 am – 10:30 am	Coffee Break	Grand Ballroom
12:30 pm – 1:30 pm	Lunch	Grand Ballroom
6:00 pm – 9:00 pm	Plant Business Meeting and Social	Clackamas/Multomah
6:00 pm – 9:00 pm	Vertebrate/Toxicology Business Meeting and Social	Washington
6:00 pm – 9:00 pm	Invertebrate Business Meeting and Social	OFF PROPERTY
TUESDAY, JUNE 3		
6:00 am – 7:30 am	Fun Run/Walk	Grand Ballroom Foyer
7:00 am – 5:30 pm	Registration	Grand Ballroom Foyer
7:00 am – 8:00 am	Development Committee Meeting	Pettygrove Room
7:00 am – 8:00 am	Membership Committee Meeting	Overton Room
10:00 am – 2:00 pm	**Exhibits and Posters**	Grand Ballroom
10:00 am – 10:30 am	Coffee Break	Grand Ballroom
12:30 pm – 1:30 pm	2004 Program Planning Committee Meeting	Pettygrove Room
12:30 pm – 1:30 pm	Lunch	Grand Ballroom
2:00 pm – 3:00 pm	Poster Breakdown and Removal	Grand Ballroom
2:00 pm	City of Roses Silent Auction Final Bidding	Grand Ballroom
5:00 pm – 6:00 pm	SIVB Business Meeting	Multnomah
7:00 pm – 8:00 pm	Reception & Announcement of City of Roses Silent Auction Winners	Grand Ballroom Foyer
8:00 pm – 10:00 pm	Closing Banquet	Grand Ballroom
WEDNESDAY, JUNE 4		
7:00 am – 5:00 pm	Registration	Grand Ballroom Foyer
7:00 am – 8:00 am	Long-Range Planning Committee Meeting	Pettygrove Room
10:00 am – 10:30 am	Coffee Break	Mount St. Helen's Foyer
THURSDAY, JUNE 5		
10:00 am – 4:00 pm	Greyhorse Winery Tour	McMinville, OR

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, MAY 31

7:00 am – 7:00 pm	Registration	Grand Ballroom Foyer
8:00 am – 1:00 pm	**SIVB BOARD OF DIRECTORS MEETING**	Crown Zellerbach
7:00 pm – 9:00 pm	**2003 CONGRESS OPENING RECEPTION**	Grand Ballroom
7:00 pm – 9:00 pm	**CITY OF ROSES SILENT AUCTION KICKOFF**	Grand Ballroom

Saturday, May 31
All Poster Authors will be present
7:30 pm – 8:30 pm
(See list of posters on pages 32-A to 59-A)

SUNDAY, JUNE 1

7:00 pm – 6:00 pm | Registration | Grand Ballroom Foyer

CRYOPRESERVATION

Conveners: Barbara M. Reed, USDA-ARS
Valerie C. Pence, Cincinnati Zoo and Botanical Garden
Lia H. Campbell, Organ Recovery Systems, Inc.

8:00 am – 10:00 am | Joint Symposium (See Abstracts page 6-A) | Washington/Clark

Techniques for storing living materials in liquid nitrogen have become important tools in the areas of agriculture, medicine, and conservation, and in vitro systems are often particularly suited for long-term storage at freezing temperatures. Research into cryoprotective and recovery mechanisms, while dealing with specific taxa, can provide guidance for the application of cryopreservation to a variety of systems. This session will provide an update on recent work on oxidative stress and desiccation tolerance in cryobiological systems as well as important principles needed for successful cryopreservation.

8:00 Introduction (B. Reed, V. Pence, and L. Campbell)
8:15 J-1 The Role of Small Amphiphilic Solutes in Desiccation Tolerance
Ann E. Oliver, *University of California – Davis*
8:45 J-2 Cryopreservation Principles for Mammalian Tissues: The Basis of Success or Failure
Michael J. Taylor, *Organ Recovery Systems, Inc.*
9:15 J-3 A Free Radical View of Cryopreservation
David H. Bremner and Erica E. Benson, *University of Abertay Dundee*

10:00 am – 10:30 am | Coffee Break | Grand Ballroom

10:00 am – 3:00 pm | Exhibits and Posters | Grand Ballroom

CREATING CONNECTIONS BETWEEN SCIENTISTS AND EDUCATORS: BRINGING BIOTECH TO THE CLASSROOM

Convener: Carol M. Stiff, Kitchen Culture Kits, Inc.

10:30 am – 12:30 pm | Education Symposium (See Abstracts page 2-A) | Timberline

Incredible advances are being made in the fields of plant, vertebrate, and invertebrate in vitro biology. It is our duty as scientists to insure that instructional resources, conceptual background information, and hands-on laboratory experiences can be incorporated into classrooms. This serves to both educate the public and foster development of our future scientists and educators. The session will discuss the various ways this has been accomplished and will provide web resources for continued networking.

Sunday, June 1

10:30		Introduction (C. Stiff)
10:45	E-1	Connecting with Teachers Through Hands-on Workshops, Online Courses and Listservs, and User-friendly Kits ***Carol M. Stiff,*** *Kitchen Culture Kits, Inc.*
11:10	E-2	LIGASE Loaners: Bringing Scientific Expertise to the High School Classroom ***Patricia E. Bossert****, Northport/East Northport School District*
11:35	E-3	Creating a Viable Biotechnology Program in High School ***Judi Heitz,*** *San Diego High School*
12:00	E-4	Bio-Link: Promoting Biotechnology Education in Community Colleges ***Beth Pitonzo,*** *Mount Hood Community College*

THE FUTURE OF SERUM: A ROUNDTABLE DISCUSSION

Convener: William J. Smith, U.S. Army Medical Research Institute of Chemical Defense

10:30 am – 12:30 pm   Animal Symposium   Multnomah

Serum has been a critical supplement to cell culture media for many years. Developments in serum collection and preparation have refined the quality of the product, but questions persist as to the standardization and purity of serum in critical cell culture studies. Many efforts have been made to produce serum-free or defined media. A number of successes have resulted for a limited span of cell types. The panel, consisting of representatives of three major cell culture media and reagent suppliers, will help us understand the problems and benefits associated with the use of serum in culture systems. They will also give us insights into what the future holds in the formulation of media and the selection of supplements.

Panelists: ***David Jayme****, Invitrogen Corporation*
Bill B. Barnett, *HyClone Laboratories*
Gary Shipley, *Cascade Biologics, Inc.*

HIGH THROUGHPUT METHODS

Conveners: Allan R. Wenck, Syngenta
Ebrahim Firoozabady, Del Monte Fresh Produce

10:30 am – 12:30 pm   Plant Symposium   Washington/Clark
(See Abstracts page 7-A)

We have entered an era where high quality sequence is being made available to researchers throughout the world. The rice, Arabidopsis and soon other sequences will be known. As methods improve, more and more information will flow through the scientific databases. How do we deal with this sequence information? How can science make biological sense out of the genetic code and identify important areas of interest? How are we to identify and validate genes with potential impact on such things as yield, stress tolerance and pest resistance? Computer programs are in place to help sift out potentially important sequences from the billions of base pairs into thousands or tens of thousands of genes. This subset of genes needs to be expressed in a high throughput manner within biological systems generating thousands or tens of thousands of events for analysis. Both molecular and other characterization data must be collected in a high throughput manner in order to validate the predicted functions of these chosen sequences. This session will provide examples of how we can identify potential genes of interest, transform them in a high throughput manner, and characterize them in an equally high throughput manner.

Sunday, June 1

10:30		Introduction (A. Wenck and E. Firoozabady)
10:45	P-1	High-throughput Methods for Determining Transgene Copy Number and Expression in Plants ***Wen-Jin Yu,*** *Syngenta Biotechnology, Inc.*
11:15	P-2	A Maize "Whiskers" Transformation System ***W. Paul Bullock,*** *Garst Seed Company*
11:45	P-3	High Throughput Plant Gene Function Analysis ***Keith Davis,*** *Paradigm Genetics, Inc.*

IN VITRO TOOLS

Moderators: Bernadette Lourdes Plair, Cincinnati Zoo and Botanical Garden
Maureen M. M. Fitch, USDA/ARS

10:30 am – 12:30 pm Plant Contributed Paper Session (See Abstracts pages 19-A to 20-A) Clackamas

10:30	P-1000	Development of an In Vitro Tuberization System for Sweet Potato Micro-storage Root Formation ***Marceline Egnin****, Tuskegee University, Latrice Carwford, and Anne Sama*
10:45	P-1001	Cryopreservation of Shoot Tips of the Endangered *Asimina tetramera* by Encapsulation-vitrification ***Bernadette Lourdes Plair,*** *Cincinnati Zoo and Botanical Garden, and V. C. Pence*
11:00	P-1002	In Vitro Collecting and Establishment of Tissue Culture Lines of Three Endangered Florida Pawpaws ***Valerie C. Pence,*** *Cincinnati Zoo and Botanical Garden, and S. M. Charls*
11:15	P-1003	Control of Bacterial Contamination in Large Scale Papaya Micropropagation ***Maureen M. M. Fitch,*** *USDA/ARS, T. Leong, N. Saito, G. Yamamoto, A. Dela Cruz, A. Yeh, S. White, S. Maeda, S. Ferreira, and P. Moore*
11:30	P-1004	Factors Affecting In Vitro Establishment of Cocoyam (*Xanthosoma sagittifolium* L Schott) ***Anne Eyango Sama,*** *Tuskegee University, M. Egnin, and S. Zok*
11:45	P-1005	Somaclonal Variation Detection and Chimerism in Somatic Embryo-derived Cocoa ***Carlos Marcelino Rodriguez Lopez,*** *Reading University, M. J. Wilkinson, and A. C. Wetten*
12:00	P-1006	Encapsulation of Orchid-Phalaenopsis Shoot Tips for Storage and Exchange of Germplasm ***W. T. P. S. K. Senarath,*** *Chonbuk National University, Kui Jae Lee, and S. Rehman*
12:15	P-1007	A Novel Disposable Film Culture Vessel for Photoautotrophic Micropropagation of *Epidendrum* Orchid ***Giang Thi Thanh Dam,*** *Kagawa University, H. Watanabe, M. Ujike, Y. Kume, and M. Tanaka*

Sunday, June 1

Sunday, June 1
Non-interactive Even Poster Authors will be present
1:30 pm – 2:30 pm
(See list of posters on pages 32-A to 59-A)
Interactive Poster Authors listed below will be present during their sessions

IN VITRO TOOLS AND TECHNIQUES

Moderator: John W. Harbell, Institute for In Vitro Sciences, Inc.

1:30 pm – 2:30 pm Joint Interactive Vertebrate/Toxicology Poster Session — Grand Ballroom
(See list of posters on pages 54-A to 55-A and 57-A)

VT-2000 An Animal Origin Free Trypsin Alternative to Harvest Cells
***Lori L. Nestler**, Invitrogen Corporation, E. K. Evege, J. A. McLaughlin, D. G. Munroe, T. C. Tan, K. E. Wagner, and B. Stiles*

VT-2001 A Filter for Trapping Metastatic Breast Cancer Cells
***Jessica L. Moore,** Washington State University, P. Elias, C. Davitt, A. Bandyopadhyay, S. Bose, S. Kalita, and H. Hosick*

VT-2002 Induction of a Zone of Cell Death in Multi-well Plates by Refeeding
***John W. Harbell,** Institute for In Vitro Sciences, Inc., H. Raabe, G. Moyer, G. Mun, and M. Clear*

VT-2003 Comparative Cytotoxcity of Three In Vitro Cell Viability Assays
***Ann M. Wright,** CiBA Vision/Novartis Company, and Mary Mowery-McKee*

VT-2004 Mycoplasma Testing Experience of an Academic Support Facility
***Margaret L. Smith,** Riggs Consulting, Lovella Cacho, and Phan Tu*

VT-2005 Use of a Self-assembling Pore for the Indtroduction of Impermeant Molecules through Mammalian Cell Membranes
***Lia H. Campbell,** Organ Recovery Systems, K. Sarver, K. Ratcliff, M. J. Taylor, J. Walsh, and K. G. M. Brockbank*

VT-2006 Enhanced Effectiveness of Non-viral Gene Transfer Using Electroporation
***Richard Heller,** University of South Florida, Richard Gilbert, Kathleen Merkler, and Loree Heller*

VT-2007 Confocal Imaging of Epidermal Growth Factor Peptide Binding Along the Stem Cell Compartment of Mammalian Colonic Crypt
***Bertrand A. Kaeffer,** Institut National Recherche, Lissia Pardini, and Alain Trubuil*

VT-2015 Protein Kinase C Disrupts the Formation of VA Gene Transcription Initiation Complex
***Calvin B. James**, Ohio University*

Sunday, June 1

INSECT MIDGUT STEM CELL LINES AND INSECT CELL LINES FOR BIOCONTROL

Moderator: Guy Smagghe, Ghent University

1:30 pm – 2:30 pm Interactive Invertebrate Poster Session — Grand Ballroom
(See list of posters on page 34-A)

I-2001 Effect of Bt Proteins on the Viability of Selected Insect Cell Lines
Cynthia L. Goodman, *USDA/ARS/BCIRL, H. Nabli, J. Baum, T. Malvar, B. Isaac, Y.-J. Lee, A. H. McIntosh, and S. J. Phipps*

I-2002 Insulin-like Peptides Stimulate Midgut Stem Cell Proliferation of Lepidopteran Larvae *In Vitro*
Shintaro Goto, *Kobe University, Marcia J. Loeb, and Makio Takeda*

I-2003 Effects of Insect Hormone Actions, 20E and JH, on Midgut Stem Cells of Lepidoptera
Guy Smagghe, *Ghent University, W. Vanhassel, C. Moeremans, K. Elsen, and M. Loeb*

I-2004 Who Controls Midgut Stem Cell Differentiation – the Stem Cell or the Environment?
Raziel S. Hakim, *Howard University, M. Loeb, and J. Young*

STRESS BIOLOGY

Moderator: Lynn Dahleen, USDA/ARS

1:30 pm – 2:30 pm Interactive Plant Poster Session — Grand Ballroom
(See list of posters on pages 36-A to 37-A)

P-2000 Transformation of Barley with Two Antifungal Genes
Lynn S. Dahleen, *USDA/ARS, and M. Manoharan*

P-2001 Regeneration and Genetic Transformation of Durum Wheat
M. Manoharan, *USDA/ARS, L. S. Dahleen, and P. B. Jauhar*

P-2002 Effects of UV-B on the Development of In Vitro Propagated African Violet Chimera (*S. ionantha*)
Wai-Yei Leung, *Champlain Regional College Saint Lambert, P. Castillo-Ruiz, E. Belanger, and S. Taylor*

P-2003 Overexpression Antioxidant Gene in Tomato Increases Tolerance to Heat and Chilling Stress
Yueju Wang, *Oregon State University, Michael Wisniewski, Lailiang Cheng, Richard Meilan, Minggang Cui, and Leslie Fuchigami*

P-2004 Expression of the *Arabidopsis* CBF1 Gene in Poplar Confers Elevated Freezing Tolerance
Yongjian Chang, *Oregon State University, Jeffery S. Skinner, and Tony H.H. Chen*

Sunday, June 1

PLENARY SESSION

Convener: William J. Smith, US Army Medical Research Institute of Chemical Defense

3:00 pm – 4:30 pm | Plenary Session | Multnomah/Clackamas

ARTistic Use of Nonhuman Primates: IVF to Cloning and Beyond

(See abstract page 1-A)

3:00 Introduction: William J. Smith, 2003 Congress Program Chair

Opening Remarks: Sandra L. Schneider, Research and Clinical Systems and President, Society for In Vitro Biology

PS-1 Plenary Speaker: **Richard Stouffer**, Head of the Reproductive Sciences Division, Oregon Regional Primate Research Center

5:00 pm – 9:00 pm **PLENARY RECEPTION AND DINNER** Pittock Mansion and World Forestry Center

MONDAY, JUNE 2

7:00 am – 6:00 pm Registration Grand Ballroom Foyer

GROWTH FACTORS IN GROWTH, REGENERATION AND DIFFERENTIATION OF INVERTEBRATE CELLS

Conveners: Marcia J. Loeb, USDA
Raziel S. Hakim, Howard University

8:00 am – 10:00 am Animal Symposium Timberline
(See Abstracts pages 3-A to 4-A)

Growth factors play a large role in regulating the growth, development, and differentiation of mammalian tissues. It is only recently that the roles of peptidic growth factors in the growth, development, and differentiation of invertebrates have been revealed. There are a very few known factors that are specific to development in insects and molluscs; most of the invertebrate organisms that have been studied in this respect are controlled by the same factors that regulate vertebrates. Their mechanisms of action for those invertebrates, such as Caenorhabditis elegans, that have been extensively characterized, are similar as well. Therefore, invertebrate tissues may serve as models for vertebrate organisms. In addition, invertebrate pest control agents whose mechanisms involve manipulation of growth factors will have to be studied in depth in order to avoid doing harm to the general environment where all animals have to live in harmony with each other.

8:00 Introduction (R. Hakim)

8:15 I-1 Co-culture of *Trypanosoma musculi* with Spleen-derived Adherent Fibroblasts and Macrophages Ensures Survival In Vitro
Winston A. Anderson, *Howard University*

8:45 I-2 Lymnaea EGF, Roles in Neurite Outgrowth In Vitro and In Vivo
Andrew G. M. Bulloch, *University of Calgary*

9:15 I-3 Growth-blocking Peptide Family and Its Multiple Effects on Insect and Vertebrate Cells
Yoichi Hayakawa*, Hokkaido University*

9:45 Discussion

10:00 – 10:30 Invertebrate Session Coffee Break Grand Ballroom

10:30 I-4 Regeneration of Walking Legs in the Fiddler Crab, *Uca pugilator*: The Role of Growth Factors
Penny M. Hopkins, *PhD, University of Oklahoma*

11:00 I-5 Regulation of the Fate of Stem Cells from the Midgut of the Caterpillar, *Heliothis virescens*
Marcia Loeb, *USDA*

11:30 I-6 Modulation of Growth Factor Signaling During *C. elegans* Vulva Development
Nadeem Moghal*, California Institute of Technology*

Monday, June 2

BIOPHARMACEUTICAL MANUFACTURING: ADDRESSING THE NEEDS FOR STAFFING, FACILITIES, AND PROCESSES DEVELOPMENT

Moderators: Dennis A. Laska, Eli Lilly and Company
William J. Smith, U.S. Army Medical Research Institute of Chemical Defense

8:00 am – 10:00 am — Animal Roundtable Session — Mutlnomah
(See Abstracts page 17-A)

Current bottlenecks and future needs for state of the art biopharmaceutical manufacturing facilities coupled with shortages of highly trained technical and professional staff threaten to slow or even impede development and commercialization of greatly needed bio-molecules, vaccines, and therapeutics. This roundtable session will address in depth the current situation, pose scenarios for remediation, and project future trends in professional and technical training, process improvement and efficiency, as well as quality initiatives.

8:00 Introduction (D. Laska)

8:15 W-1 Academic Institutions Response to the Staffing Needs of the Biopharmaceutical Industry
Kamal A. Rashid, *Biotechnology and Genomics Research Center*

8:45 W-2 Industrial Mammalian and Microbial Cell Culture System for Biopharmaceutical Manufacture of Therapeutics
Sarad Parekh, *Dow AgroSciences, LLC*

9:15 W-3 TBA
Julia Cino, *New Brunswick Scientific Co., Inc.*

BIOTECHNOLOGY OF GRAPEVINE IMPROVEMENT

Convener: Cecilia Zapata, Yoder Brothers, Inc.

8:00 am – 10:00 am — Plant Symposium — Washington/Clark
(See Abstracts pages 7-A to 8-A)

Techniques for genetic modification in grapevine (Vitis spp.) using somatic embryogenesis (SE) include transformation and in vitro selection. All successful examples of genetic transformation in grapevine have utilized embryogenic cells as targets for gene insertion and somatic embryos for recovery of transformed plants. Transgenics have been used to solve problems with disease and stress resistance as well as manipulation of qualitative traits. In vitro selection has also been used as an alternate method to select for disease resistance in grapevine. In the area of grape genomics, incredible advances have been made in the recent past, where an estimate of 44,928 grape (Vitis vinifera) ESTs have been produced. These code for approximately 18,500 grape genes, representing 2/3 of the grape genome. This symposium will present a talk on the uses of somatic embryogenesis and transformation for grapevine improvement. The session will include another presentation on in vitro selection to enhance disease resistance. It also includes a presentation on the grape genome project focusing on gene discovery.

8:00 Introduction (C. Zapata)

8:15 P-4 Applications of Somatic Embryogenesis and Transformation in Grapevine Improvement
Dennis J. Gray, *University of Florida*

8:45 P-5 Gene Discovery in Grapes: The Grape Genome Project
Effie Ablett, *Southern Cross University*

Monday, June 2

9:15 P-6 In Vitro Selection to Enhance Disease Resistance in Grapevine
Jayasankar Subramanian, *University of Guelph*

MONOCOT TRANSFORMATION

Moderators: Jane Vishnevetsky, The Volcani Center
Diaa F. Al-Abed, The University of Toledo

8:00 am – 10:00 am Plant Contributed Paper Session Clackamas
(See Abstracts pages 21-A to 22-A)

8:00 P-1008 Transformation and Regeneration of Wetland Monocot *Juncus accuminatus* Using Different Binary Vectors
Li Chen, *Salem International University, R. Nandakumar, P. K. Lai, and S. M. D. Rogers*

8:15 P-1009 An Efficient System for Biolistic Transformation and Plant Regeneration of Pearl Millet Using Spikelets Shaved from Immature Inflorescences
Jason James Goldman, *University of Georgia, W. W. Hanna, G. Fleming, and P. Ozias-Akins*

8:30 P-1010 Alleviation of Wheat Allergenicity Using the Thioredoxin System
Hyun-Kyung Kim, *University of California-Berkeley, M.-J. Cho, H. R. Jung, Y.-B. Kim, S. Morigasaki, J. H. Wong, P. G. Lemaux, and B. B. Buchanan*

8:45 P-1011 Transgenic *Spirodela*: A Unique, Low-risk, Plant Biotechnology System
Avihai Perl, *Volcani Center, Marvin Edelman, Ron Vunsh, Jihong Li, Uri Hanania, Moshe Flaishman, and Jane Vishnevetsky*

9:00 P-1012 Expression of a Synthetic Avidin Gene in Maize for Control of Corn Rootworm (*Diabrotica* spp.) and Other Insect Pests
Serena B. McCoy, *Kansas State University, Mitsuhiro Ueda, Karl J. Kramer, Subbaratnam Muthukrishnan, and Harold N. Trick*

9:15 P-1013 Shoot Meristem: An Ideal Explant for *Zea mays* (L.) Transformation
Diaa F. Al-Abed, *The University of Toledo, R. V. Sairam, and S. L. Goldman*

9:30 P-1014 Enhanced Fungal Tolerance in Transformed Banana (*Musa* spp. AAA cv. 'Grand Nain') Plants Regenerated Through Somatic Embryogenesis
Jane Vishnevetsky, *The Volcani Center, Y. Cohen, M. A. Flaishman, and A. Perl*

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

10:00 am – 3:00 pm Exhibits and Posters Grand Ballroom

Monday, June 2

EPIGENETIC TOXICANTS, ALTERED INTRA-CELLULAR SIGNALING AND MODULATED GAP JUNCTIONAL INTERCELLULAR COMMUNICATION

Convener: Alda Vidrich, University of Virginia Health System

10:30 am – 12:30 pm | Animal Symposium | Multnomah

(See Abstracts page 15-A)

Many chemicals capable of inducing multiple health risks do so without causing genetic mutations. The mechanisms whereby these chemicals cause disease apart from mutagenesis include cytotoxicity (either necrosis or apoptosis) and epigenetic toxicity. Via epigenetic toxic mechanisms chemicals can alter gene expression at the level of transcription, translation or post-translational events in a stem cell, a precursor cell or a terminally differentiated cell. This session will explore the role of intercellular signaling and communication in the cellular response to epigenetic toxicants as well as the mode by which epigenetic toxicants can alter the channels of intercellular communication.

10:30 Introduction (A. Vidrich)
10:45 VT-1 Gap Junctions, Homeostasis, and Epigenetic Toxicology
Randall J. Ruch, *Medical College of Ohio*
11:15 VT-2 Integrated Signaling Effects of Epigenetic Toxicants
Brad Upham*, Michigan State University*
11:45 VT-3 Stem Cells, Cell-cell Communication and Epigenetic Toxicants: Risk Assessment Implications
James E. Trosko, *Michigan State University*

FOREST BIOTECHNOLOGY: SHOULD, COULD, AND WOOD

Conveners: Todd J. Jones, Weyerhaeuser Technology Center
Maud A. Hinchee, ArborGen

10:30 am – 12:30 pm | Plant Symposium | Washington/Clark

(See Abstracts pages 8-A to 9-A)

Researchers now have the tools to apply genetic engineering and biotechnology for the improvement of commercially important forest tree species. We are currently on the threshold of major advancements in our understanding of gene function in trees. The Poplar Genome Sequencing Project is the first full-scale sequencing of a forest tree species and it will provide the fundamental basis for functional tree genomics. This project, which is nearing completion, will add thousands of tree genes to the molecular toolbox and provide new insights into what genes contribute to commercially valuable tree phenotypes. For some biochemical pathways, we already know which genes encode many of the important enzymes. One such pathway is the lignin biosynthetic pathway, and efforts to modify the quantity and quality of lignin have already begun. Deployment of transgenic trees will face potential challenges on the regulatory and public acceptance fronts. Trees present unique concerns, such as large scale and widespread pollen dispersal, along with perennial persistence in the environment. For instance, certain plantation tree species are grown in their native habitat, and in these cases, the potential for gene flow of engineered traits from modified trees into native stands is a real possibility. Several approaches have been taken to mitigate gene flow. This session will present speakers who will address the potential and challenges associated applying biotechnology to tree improvement.

10:30 Introduction (T. Jones and M. Hinchee)
10:45 P-7 Gene Flow Control in Trees: Technology Development in Transgenic Poplars
Steven H. Strauss*, Oregon State* University

11:15 P-8 The *Populus* Genome: Development of the Information Resource
Gerald A. Tuskan, *Oak Ridge National Lab*

11:45 P-9 Genetic Engineering of Wood Formation in Forest Trees
Vincent L. Chiang, *North Carolina State University*

Monday, June 2
Non-interactive Odd Poster Authors will be present
1:30 pm – 2:30 pm
(See list of posters on pages 32-A to 59-A)
Interactive Poster Authors listed below will be present during their sessions

BIOTECHNOLOGY

Moderator: Nancy A. Reichert, Mississippi State University

1:30 pm – 2:30 pm — Interactive Plant Poster Session — Grand Ballroom
(See list of posters on pages 37-A to 38-A)

P-2005 Kanamycin Resistant Alfalfa Has a Point Mutation in the 16S Plastid rRNA
Pierluigi Barone, *University of Perugia, Italy, D. Rosellini, P. LaFayette, F. Veronesi, and W. A. Parrott*

P-2006 An Approach for Fiber Improvement in Kenaf Using a Gibberellin Oxidase Gene
Margaret M. Young, *Mississippi State University, and N. A. Reichert*

P-2007 A Poplar Promoter Functions in Potato and is Induced by Wounding and Fungal Infection
Dmytro P. Yevtushenko, *University of Victoria, Rafael Romero, William W. Kay, and Santosh Misra*

P-2008 Screening *Arabidopsis thaliana* and *Chlamydomonas reinhardtii* for Their Phenotypic Response to 2,4,6-trinitrotoluene (TNT)
Nrupali Patel, *University of Tennessee at Knoxville, and C. Neal Stewart, Jr.*

P-2009 DNA Fingerprinting of Orchardgrass (*Dactylis glomerata* L.) cv. Persist for Plant Variety Protection
Judith K. McDaniel, *University of Tennessee, and B. V. Conger*

P-2009A Reactivation of a Disabled Virus Vector by the Utilization of an Inducible Recombination System in Tobacco Plants
Arianne Tremblay, *Federal Government of Canada, C. Beauchemin, V. Bougie, A. Séguin, and J.-F. Laliberté*

Monday, June 2

IN VITRO TOOLS/EVALUATION OF TOXICITY

Moderator: Bobbie L. Thompson, HyClone Laboratories

1:30 pm – 2:30 pm Joint Interactive Vertebrate/Toxicology Poster Session — Grand Ballroom
(See list of posters on pages 56-A to 57-A)

VT-2008 Design and Phase Ia Results of a Validation Study to Evaluate In Vitro Cytotoxicity Assays for Predicting Rodent and Human Acute Systemic Toxicity
Michael W. Paris, *NIEHS, J. A. Strickland, W. S. Stokes, S. Casati, A. P. Worth, H. Raabe, C. Cao, R. Clothier, J. Harbell, R. Curren, J. Haseman, R. R. Tice, M. L.* Wenk, M. K. Vallant, G. Mun, M. Clear, G. O. Moyer, J. Madren-Whalley, C. Krishna, M. Owen, and N. Bourne

VT-2009 The EpiOcular Prediction Model: In Vivo Versus In Vitro Draize Scores for Consumer Products
George L. DeGeorge, *MB Research Laboratories, M. Klausner, M. Osborn, K. Bellavance, B. Breyfogle, J. Kubilus, and D. R. Cerven*

VT-2010 Enhanced Phototoxicity Assay in Reconstituted Skin (EPARS)
George L. DeGeorge, *MB Research Laboratories, A. C. Gilotti, T. L. Ripper, T. L. Fox L. Wagner, L. Lewis, S. H. Young, C. A. Kirk, M. K. Reeder, and B. Jones*

VT-2011 Serum Alternatives to the Use of Fetal Bovine Serum in Cell Culture
Bobbie L. Thompson, *HyClone Laboratories, B. Fujimoto, and B. Barnett*

VT-2012 Toxic Effects of Organic and Inorganic Forms of Selenium in Murine Erytholeukemia and Human Prostate Cancer Cells
Shyamal K. Majumdar, *Lafayette College, S. A. Satuh, J. H. Tchaicha-Pavlic, and E. A. Lucisano*

VT-2013 Metalloprotease Inhibitors, Non-microbial Chemically Modified Tetracyclines and Ilomastat, Block Anthrax, Lethal Factor Activity In Vitro
Salih Silay Kocer*, State University of New York at Stony Brook, and Sanford Simon*

VT-2014 The Effects of Glutamine and Ammonia Concentrations on CHO Cells in Serum-free Media
Paula N. Decaria, *HyClone Laboratories, Inc., J. Camire, and B. Barnett*

DICOT TRANSFORMATION

Moderator: Y. Judy Zhu, Hawaii Agriculture Research Center
Zhiwu Li, Kansas State University

3:00 pm – 5:00 pm Plant Contributed Paper Session — Clackamas
(See Abstracts pages 22-A to 24-A)

3:00 P-1015 Heterogeneity in Terpenoid Gene Expression in Transformed Roots of *Artemisia annua* L. Grown in Bioreactors
Pamela J. Weathers,*Worcester Polytechnic Institute, F. F. Souret, Y. J. Kim, B. E. Wyslouzil, and K. K. Wobbe*

3:15 P-1016 Utilization of RNA Interference to Confer Resistance to the Soybean Cyst Nematode, *Heterodera glycines*
Ryan Matthew Steeves, *Kansas State University, Timothy C. Todd, and Harold N. Trick*

3:30	P-1017	Genetic Transformation and Plant Regeneration in *Stevia rebaudiana* Using Microprojectile Bombardment ***Kaye M. Knowles,*** *Fort Valley State University, Seema Dhir, M. Singh, and Sarwan Dhir*
3:45	P-1018	Shoot Formation of *Agrobacterium* Co-cultivated Tissues of Teak ***Sri N. Widiyanto,*** *Institut Teknologi Bandung, H. Rahmania, and S. Suhandono*
4:00	P-1019	Towards a Mucosal Vaccine Against HIV ***Nobuyuki Matoba,*** *Arizona State University, M. Bomsel, C. J. Arntzen, and T. S. Mor*
4:15	P-1020	Metabolic Engineering Phytoalexins from the *Vitaceae* Improves Antifungal Activity in Tropical Plants ***Y. Judy Zhu,*** *Hawaii Agriculture Research Center, C. S. Tang, and P. H. Moore*
4:30	P-1021	A Novel Protocol for Regeneration of Soybean from Mature and Immature Cotyledon; Suitable for Genetic Transformation ***Gregory Franklin,*** *University of Toledo, E. Davis, S. Ismail, L. Carpenter, A. Hampton, B. Smith, S. Ibeji, J. McDougall, B. Sadia, M. Parani, S. L. Goldman, and R. V. Sairam*

GROWTH REGULATORS

Convener: David D. Songstad, Monsanto Company

3:00 pm – 5:00 pm — Plant Symposium — Washington/Clark

(See Abstracts page 9-A)

The influence of growth regulators on plant tissue culture and biotechnology is profound. The routine manipulation of cells in vitro was possible only through the use of specific plant growth regulators to promote either undifferentiated cell division or the formation of plants via organogenesis or embryogenesis. In this symposium, the speakers will cover three plant growth regulators, abscisic acid, gibberellins and ethylene, as they relate to in vitro culture and regenerated plants.

3:00		Introduction (D. Songstad)
3:15	P-10	Persistent Abscisic Acid Analogs ***Sue R. Abrams,*** *Plant Biotechnology Institute at National Research Council of Canada*
3:45	P-11	Gibberellins, Their Effects on and Roles in Growth and Differentiation of *In Vitro* Cultured Plant Tissues, Organs, and Somatic Embryos ***Richard Persons Pharis,*** *University of Calgary*
4:15	P-12	Ethylene Based Opportunities in Horticultural Biotechnology ***Franzine D. Smith,*** The Scotts Company

IN VITRO QUANTITATION OF ANGIOGENESIS: A BD BIOSCIENCES WORKSHOP

Convener: Steve Ilsely, BD Biosciences Discovery Labware

3:00 pm – 5:00 pm — Animal Workshop — Timberline

Angiogenesis, the formation of new blood vessels, is essential for normal growth and homeostasis. Certain disease states can be exacerbated by the loss of tight control of angiogenesis, which results in

either excessive or insufficient blood vessel formation. The modulation of angiogenesis as a therapeutic strategy is a rapidly expanding field for the drug discovery and research scientist. Several *in vitro* assays have been developed to identify potential therapeutic molecules and to understand the mechanisms of angiogenesis. However, because most of these assays are cumbersome, laborious, poorly quantitative, and lack standardization, rapid progress in screening for effective therapeutic agents has been hampered. BD Biosciences Discovery Labware has developed a portfolio of products designed to provide the scientist easy, quick, robust, reproducible, standardized and readily available assay platforms for quantifying the effects of angiogenesis modulating compounds. These assay products recapitulate one or more of the neoangiogenic processes such as endothelial cell migration, invasion, and differentiation into vessels. The use of these products for the *in vitro* quantitation of angiogenesis will be presented.

3:00 Introduction (S. Ilsley)

Panelists: ***Stephen Ilsley,*** *BD Biosciences Discovery Labware*
Min Wu, *BD Biosciences Discovery Labware*
James Maliakal, *BD Biosciences Discovery Labware*

TUESDAY, JUNE 3

7:00 am – 5:30 pm	Registration	Grand Ballroom Foyer

BIOREACTORS AND BIOPHARMING

Conveners: Marceline Egnin, Tuskeegee University
Mary Ann Lila, University of Illinois

8:00 am – 10:00 am	Plant Symposium (See Abstracts pages 10-A and 28-A)	Washington/Clark

High value plant-derived pharmaceutical proteins promise to reduce human and animal suffering, and can be manufactured using a delivery and production system that is safe, cost-efficient, and convenient. Plants can be engineered to produce drugs for disease prevention or therapy, nutraceutical or health-protective compounds, or agents that may combat terrorism's threats. This session will overview the commercial outlook for molecular farming approaches, the use of plants as bioreactors for production of uniform, scaled-up quantities of active compounds, and the prospects for capitalizing on plants to produce agents that can intervene in the event of biological or chemical warfare.

8:00		Introduction (M. Egnin and M. Lila)
8:15	P-13	Molecular Farming: Current Products and Future Prospects ***Michael E. Horn,*** *ProdiGene*
8:45	P-14	Improving Nutraceuticals Through Tissue Culture ***Alison M. R. Ferrie****, Plant Biotechnology Institute*
9:15	P-1036	Chemical Warfare Countermeasures: Expression of Human Acetylcholinesterase in Plants ***Samuel Patrick Fletcher,*** *Arizona State University*
9:35	P-1037	Plant-based Production of a Subunit Mucosal Vaccine for Pneumonic Plague ***Bonnie Jean Woffenden****, Virginia Tech*

COUNTERING CHEMICAL AND BIOLOGICAL THREATS: CURRENT RESEARCH

Convener: Elizabeth J. Roemer, State University of New York – Stony Brook

8:00 am – 10:00 am	Animal Symposium (See Abstracts pages 15-A to 16-A)	Multnomah

Continuing concern about the threat of chemical and biological agents both on the battlefield and as tools of terror has lead to increased research in these arenas. Multiple agents ranging from organisms such as anthrax and small pox; potent toxins including botulinum toxin and aflotoxin, and chemicals such as ricin, sulfur mustard, and phosgene are all considered to pose potential dangers. Research is underway in a variety of venues, both civilian and military, to develop better understanding of the mechanism of action of these and others. Today's session will present three talks on current, ongoing projects in the area of chemical and biological threats.

Tuesday, June 3

8:00 Introduction (E. Roemer)

8:15 VT-4 Ex Vivo Utilization of Dendritic Cells to Identify Vaccine Targets for Control of Infectious Biothreat Agents
Kamal U. Saikh, *US Army Medical Research Institute of Infectious Diseases (USAMRIID)*

8:45 VT-5 Evaluating Potential Inhibitors of Anthrax Lethal Factor Protein
Sanford R. Simon, *State University of New York – Stony Brook*

9:15 VT-6 Morphological Expression of Mustard Gas-induced Lesions In Vivo and In Vitro
John P. Petrali, *US Army Medical Research Institute of Chemical Defense (USAMRICD)*

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

10:00 am – 2:00 pm Exhibits and Posters Grand Ballroom

ADVANCES IN CANCER MODELING AND TREATMENT

Conveners: Richard Heller, University of South Florida
Paul J. Price, GIBCO Invitrogen

10:30 am – 12:30 pm Animal Symposium Multnomah
(See Abstracts page 16-A)

The first decade of this century may very well be the turning point in our diagnosis and treatment of cancer. Cancer models are allowing us to understand what changes are occurring when a normal cell becomes a cancer cell and how different approaches may signal out the cancer cell for destruction. It is the understanding of unique or up-regulated pathways in the cancer cell that is allowing for targeted therapy. The knowledge of up-regulation of specific genes yielding up-regulated proteins and peptides is allowing for the priming of the cytotoxic "T" to seek and destroy the tumor cells. Cancer immunotherapy is predicted to become a significant adjunct to other areas of cancer treatment. The three speakers will cover these new and exciting approaches to cancer treatment.

10:30 Introduction (R. Heller and P. Price)

10:45 VT-7 STI571: A Tyrosine Kinase Inhibitor for the Treatment of CML - Validating the Promise of Molecularly Targeted Therapy
Michael W. N. Deininger, *Oregon Health and Science University*

11:15 VT-8 Dendritic Cell Immunotherapy of Cancer
Reiner Laus*, Dendreon Corporation*

11:45 VT-9 Identification of Casual Genetic Alterations in Human Breast Cancer Using New Model Cell Lines and Xenografts
Stephen P. Ethier*, University of Michigan Cancer Center*

DISEASE AND PEST: PATHWAYS TO RESISTANCE

Convener: Heidi F. Kaeppler, University of Wisconsin

10:30 am – 12:30 pm Plant Symposium Washington/Clark
(See Abstracts pages 10-A to 11-A)

Pathogens and insects are the major causes of crop quality and yield losses worldwide. Breeding efforts aimed at enhancing resistance to diseases and pests have been successful in many cases. Continued

research aimed at improving resistance is needed, however, because current levels of resistance are inadequate, the resistance is of a nondurable form and/or sources of adequate resistance are unavailable within the crop species germplasm. Expression of simple antifungal proteins in transgenic plants has resulted in mixed effects on resistance, and has led to investigation of more complex transgenic strategies for enhancing resistance. Detailed characterization of plant defense genes and regulatory cascades should result in improved design of genetic resistance strategies, both for breeding and transgenic approaches toward enhancing resistance. The speakers in this symposium will discuss findings from investigations of plant defense products and pathways, and how results can be used for enhancing plant resistance to pathogens and pests.

10:30 Introduction (H. Kaeppler)

10:45 P-15 The XA21 Receptor Kinase Mediated Defense Response in Rice
Pamela C. Ronald, *University of California – Davis*

11:15 P-16 Lipid Signaling in Plant Defense
Jyoti Shah, *Kansas State University*

11:45 P-17 The Interface Between Bacterial Pathogens and Plants: Virulence Functions and Resistance Responses
David Mackey, *The Ohio State University*

PLANT TISSUE CULTURE AND MICROPROPAGATION

Moderator: Baochun Li, University of Kentucky
M. Manoharan, USDA/ARS

10:30 am – 12:30 pm Plant Contributed Paper Session Clackamas
(See Abstracts pages 24-A to 26-A and 29-A)

10:30 P-1022 Shoot Organogenesis in *Nicotiana* Species: Shoot Production per Responsive Leaf Explant Increases Exponentially with Explant Organogenic Potential
Baochun Li, *University of Kentucky, W. (Q. W.) Huwang, and T. Bass*

10:45 P-1023 Micropropagation of *Cordyceps sinensis* (Berk) Sacc., a High Value Medicinal Fungus Wildly Growing in Himalayan Region
Narendra Kumar, *Government of India – Ministry of Defence, and P. S. Negi*

11:00 P-1024 Micropropagation Saves the Endangered *Musa* Germplasm in the FSM
Hattie Andrew, *Micronesia Plant Propagation Research Center, P. C. Josekutty, N. H. Nena, R. A. George, T. N. Kilafwasru, and S. S. Cornelius*

11:15 P-1025 Micropropagation and Field Performance Evaluation of Eight Micronesian Bananas
Puthiyaparambil Chacko Josekutty, *Micronesia Plant Propagation Research Center*

11:30 P-1026 *In Vitro* Initiation of *Artocarpus heterophyllys* Lam. (Jak Fruit) – Effect of the Explant Type and the Season of Explant Collection
W. T. P. S. K. Senarath, *Chonbuk National University, K. A. H. K. Kasturiarachchi, and Kui Jae Lee*

11:45 P-1027 Shoot Tip Culture: A Powerful Model System for In Vitro Flowering and Transformation Studies in Maize (*Zea mays* L.)
Swati Bhargava, *G. B. Pant University of Agriculture and Technology, Alok Shukla, and R. C. Pant*

Tuesday, June 3

12:00 P-1028 Micropropagation of *Papuacalia versteegii*, an Important Endemic Plant to Mount Jaya
Erly Marwani, *Institute of Technology Bandung, and W. Sarosa*

12:15 P-1029 Somatic Embryogenesis from Ovules of Kinnow (*Citrus nobilis* X *C. deliciosa*) for Elimination of ICRSV
Gita Rani, *Guru Nanak Dev University, B. Singh, S. Sharma, A. A. Zaidi, V. Hallan, A. Nagpal, and G. S. Virk*

12:30 P-1040 A Comparative Account of the Studies on Embryogenesis and Organogenesis in Various Cultivars of Cotton (*Gossypium spp.*)
Tanveer Khan, *G. B. Pant University of Agriculture and Technology, and R. C. Pant*

CELL MODELS AND CELLULAR DIFFERENTIATION

Moderator: Kim C. O'Connor, Tulane University

1:30 pm – 3:00 pm Joint Vertebrate/Toxicology Contributed Paper Session — Clackamas
(See Abstracts pages 30-A to 31-A)

1:30 VT-1000 Differentiation Kinetics of In Vitro 3T3-L1 Preadipocyte Cultures
Kim C. O'Connor, *Tulane University, H. Song, K. D. Papadopoulos, and D. A. Jansen*

1:45 VT-1001 Characterization of I-11.15, an Immortalized Murine Splenic Macrophage Cell Line
Aicha Delafoulhouse, *California State University at Fullerton, C. Calumpong, and D. B. Drath*

2:00 VT-1002 Improving the Embryonic Stem Cell Test (EST) by Establishing Molecular Endpoints of Tissue-specific Development
Roland Buesen, *Federal Institute for Risk Assessment, A. Seiler, A. Visan, B. Slawik, E. Genschow, and H. Spielmann*

2:15 VT-1003 Growth and avb3 Expression In OPC1 Cells Grown on Thermanox® Plastic and Collagen Substrates
Jessica Lee Moore, *Washington State University, C. Davitt and H. Hosick*

2:30 VT-1004 The Extracellular Matrix Laminin, Fibronectin, and Collagen IV in Green Sea Turtle Gonadal Cell Cultures
Anggraini Barlian, *Institut Teknologi Bandung, S. Sudarwati, L. A. Sutasurya, and H. Hayashi*

Tuesday, June 3
All Poster Authors will be present
1:30 pm – 2:00 pm
(See list of posters on pages 32-A to 59-A)

2:00 pm – 3:00 pm Poster Breakdown and Removal — Grand Ballroom

2:00 pm City of Roses Silent Auction Final Bidding — Grand Ballroom Foyer

Tuesday, June 3

DELIVERY OF GENES TO MAMMALIAN CELLS WITH BACULOVIRUSES

Convener: Guido F. Caputo, Natural Resources Canada

3:00 pm – 5:00 pm | Animal Symposium | Multnomah
(See Abstracts pages 4-A to 5-A)

3:00 Introduction (G. Caputo)
3:15 I-7 Isolation and Characterization of Transgenic Insect Cell Lines with Humanized Glycoprotein Processing Pathways
Donald L. Jarvis, *University of Wyoming*
3:45 I-8 Recombinant Baculoviruses as Mammalian Cell Gene-delivery Vectors
Tom A. Kost, *GlaxoSmithKline*
4:15 I-9 Enhancing the Sensitivity of Rainbow Trout Cells in Culture to the Toxicity of Metals
Vivian Rashida Dayeh, *University of Waterloo*

PREDICTIVE TOXICOLOGY

Moderator: John W. Harbell, Institute for In Vitro Science, Inc.

3:00 pm – 5:00 pm | Animal Roundtable Session | Timberline
(See Abstracts pages 17-A to 18-A)

Traditionally, application of in vitro methods to drug discovery or toxicology often focused on an understanding of the mechanisms underlying the changes in target tissues or organs occurring in vivo. Increasingly though, in vitro methods are being applied prospectively to predict the action of a chemical on the target tissue or whole organism. There are several forces driving the increased emphasis on in vitro cell and tissue based models and accompanying test procedures. One primary force has been the need to support the high through put screens to address the chemical libraries developed from combinatorial chemistry. The large number of potential active compounds and the small quantity of each chemical have precluded most in vivo assessments. Concerns about extrapolation across species have also increased the focus on human cell systems, especially in organ-specific and metabolism studies. In vivo, absorption, distribution, metabolism and excretion (ADME) are measured to determine the effective dose and duration of exposure to the parent compound and any active metabolites. To predict the potential action of the chemical in vitro, the ADME factors must be addressed along with the action on the target tissue(s). This symposium will focus on the in vitro approaches to assessing intestinal absorption, hepatocyte metabolism (phase 1 bioactivation and phase 2 inactivation) as well as basal and organ-specific toxicity.

3:00 Introduction (J. Harbell)
3:15 W-4 The Caco-2 Assay: An In Vitro Models for Prediction of Intestinal Permeability
Dennis A. Laska, *Eli Lilly and Company*
3:45 W-5 Hepatocyte Systems for Predicting First-pass Metabolism and Bioavailability of Chemicals In Vitro
Edward L. LeCluyse, *University of North Carolina*
4:15 W-6 Model Systems for Cytotoxicity Screening and Predicting Target Organ Effects
Charles A. Tyson, *SRI International*

Tuesday, June 3

TRANSGENIC CEREALS

Conveners: Mark C. Jordan, Agriculture & Agri-Food Canada
Harold N. Trick, Kansas State University

3:00 pm – 5:00 pm | Plant Symposium (See Abstracts pages 18-A and 28-A) | Washington/Clark

The present large-scale commercial production of transgenic maize is largely dependent on advances in transformation technology leading to high throughput transformation systems. The procedures and practices for selection of an appropriate transformation system in maize will ultimately be applied to other cereals such as wheat. The next bottleneck in cereals is the ability to isolate genes for important traits due to the large and complex genome. In spite of this, wheat is now coming into its own as a tool for functional genomics. Several international programs in wheat genomics have generated a wealth of sequence information and expression data. Transgenic wheat is poised to play a major role in the assignment of function to these sequences and the potential to develop gene tagging strategies in wheat will be discussed. After successful transformation and gene identification strategies are in place for wheat, there are still barriers to commercial production. There is no commercial production of transgenic wheat in the world today; however, there are products in advanced field trials that could be ready for release in 2004. Given potential impacts on international export markets as well as other issues, there has been pressure exerted on governmental agencies to prevent the release of transgenic wheat. Herbicide tolerant transgenic wheat has been bred and tested in the Pacific Northwest and we will hear from a wheat breeder who has worked with the product and can discuss the risks and benefits.

3:00 Introduction (M. Jordan and H. Trick)

3:15 P-17A Gene Identification in Wheat
***Camille M. Steber**, USDA-ARS, Washington State University*

3:45 P-1038 Statistical Analysis of Frequency and Quality of Transgenic Maize Production from Three Transformation Methods: *Agrobacterium*, Gunpowder Gun, Electric Gun
***David D. Songstad,** Monsanto Company*

4:15 P-18 Risk Assessment of RoundUp® Ready Wheat Production in the Pacific Northwest
***Kimberlee K. Kidwell,** Washington State University*

5:00 pm – 6:00 pm | **SIVB Business Meeting** (All Members are Urged to Attend) | Multnomah

7:00 pm – 8:00 pm | **Reception** | Grand Ballroom Foyer

7:00 pm – 8:00 pm | **Announcement of City of Roses Silent Auction Winners** | Grand Ballroom Foyer

8:00 pm – 10:00 pm | **Closing Banquet** | Grand Ballroom

Seating is Limited. Admittance to Banquet by Advance Ticket Holders Only.

WEDNESDAY, JUNE 4

7:00 am – 5:00 pm | Registration | Grand Ballroom Foyer

BIOMASS CONVERSION FOR FUELS

Conveners: Michael E. Horn, Prodigene
Elizabeth E. Hood, Plant Biotechnologist

8:00 am – 10:00 am | Plant Symposium | Washington/Clark

(See Abstracts pages 11-A to 12-A)

Fossilized hydrocarbon-based energy sources, such as coal, petroleum and natural gas, provide a limited, non-renewable resource pool. Because of the worlds increasing population and increasing dependence on energy sources for electricity and heating, transportation fuels, and manufacturing processes, energy consumption is rising at an accelerating rate. Renewable resources, such as those derived from plants, make economic and environmental sense, and we should investigate and implement models for moving to these new energy sources. The goal is to derive 10% of our liquid fuels from renewable plant biomass by 2020, a 10-fold increase over todays production levels. This session will present current research in several areas affecting our ability to produce fuels from lignocellulosic materials and the impact those issues have on the economics of the process.

8:00 Introduction (M. Horn and E. Hood)

8:15 P-19 Transgenic Plant-produced Cellulases for Biomass Conversion
Elizabeth E. Hood, *Plant Biotechnologist*

8:45 P-20 The Impact of Feedstock Composition on Biomass Conversion Process Economics
Steven R. Thomas, *National Renewable Energy Laboratory*

9:15 P-21 Economics and Opportunities for Improvements for Biological Conversion of Cellulosic Biomass to Ethanol
Charles E. Wyman, *Dartmouth College*

BIOTECHNOLOGY

Moderator: Anton S. Callaway, North Carolina State University
Ryan Matthew Steeves, Kansas State University

8:00 am – 10:00 am | Plant Contributed Paper Session | Multnomah

(See Abstracts pages 26-A to 27-A)

8:00 P-1030 Interactions Between Post-transcriptional and Transcriptional Silencing Pathways in *Arabidopsis thaliana*
Anton S Callaway, *North Carolina State University, William F. Thompson, George C. Allen, Lindsay D. Jones, and Dolores, A. Sowinski*

8:15 P-1031 A New Glyphosate Tolerance Strategy in Transgenic Crops
Michael W. Lassner, *Verdia Inc., Daniel L. Siehl, Rebecca Gorton, Sean Bertain, Hyeon-Je Cho, Donglong Liu, James Wong, Nick Duck, and Linda A. Castle*

Wednesday, June 4

8:30	P-1032	Profiling of Differentially Expressed Genes in Yam (*Dioscorea rotundata*) During Post-harvest Storage ***Sali Kone-Coulibaly,*** *Tuskegee University, M. Egnin, and G. He*
8:45	P-1033	Chromatin Structure of T-DNA Integration Sites in *Arabidopsis* ***Kirk E. Francis,*** *North Carolina State University, and S. L. Spiker*
9:00	P-1034	SSR Markers as a Suitable Tool for Checking Recombination Events During Somatic Embryogenesis from Floral Explants in Grapes ***Lucia Martinelli,*** *Instituo Agrario Provinciale, M. S. Grando, J. Zambanini, V. Poletti, E. Maffettone, and R. Marconi*
9:15	P-1035	Transgenic "Sweet Rice" Expressing a Thermostable Amylopullulanase in Seeds Leads to Starch Autohydrolysis and Production of Nutritionally-improved High-protein Flour ***Su-May Yu,*** *Academia Sinica, C.-M. Chiang, F.-S. Yeh, and J.-F. Shaw*

10:00 am – 10:30 am   Coffee Break   Mount St. Helen's Foyer

TRANSGENES BLOWING IN THE WIND?

Convener: Wayne Parrott, University of Georgia

10:30 am – 12:30 pm   Plant Symposium   Washington/Clark
(See Abstracts pages 12-A to 13-A)

Pollen has been blowing in the wind ever since it first appeared on earth, facilitating gene flow among plants. With the advent of transgenic crops, gene flow is being viewed increasingly not as a natural phenomenon, but as something that must be avoided. Hence, gene flow and its control is playing a larger role in the regulatory approval of transgenic crops. This session will present a talk on measuring gene flow and determining its impact, another on the legal aspects of gene flow, and specific study of gene flow from transgenic grasses.

10:30		Introduction (W. Parrot)
10:45	P-22	Transgenic Turfgrasses and Issues with Gene Flow ***Eric K. Nelson,*** *The Scotts Company*
11:15	P-23	Gene Flow from Transgenic Crops to Wild and Weedy Relatives: When is It a Problem? ***Allison A. Snow,*** *Ohio State University*
11:45	P-24	Liability Issues Related to Transgene Flow in Agriculture ***Drew L. Kershen****, University of Oklahoma College of Law*

GENE SILENCING: USE FOR HIGH THROUGHPUT GENE VALIDATION AND/OR FUNCTIONAL GENOMICS

Conveners: Peggy J. Ozias-Akins, University of Georgia
Dwight T. Tomes, Pioneer Hi-Bred International, Inc.
Theodore M. Klein, Pioneer/DuPont Crop Genetics

2:30 pm – 4:30 pm   Plant Symposium   Washington/Clark
(See Abstracts page 13-A)

Wednesday, June 4

Science magazine's "Breakthrough of the Year: New Roles for RNAs" (2002. Science 298:2296) highlights a rapidly emerging realization of the important and varied roles for small RNAs. Independent research in plants and animals has converged to reveal related mechanisms for phenomena variously known as RNA interference, quelling, co-suppression, or post-transcriptional gene silencing. The outcome, RNA-mediated gene silencing, is similar for all of these phenomena, and involves double-stranded RNA that is recognized by the host cell machinery as aberrant and is subsequently cleaved into smaller molecules. These smaller molecules continue to propagate degradation in a sequence-dependent manner. RNA silencing in the form of co-suppression was first recognized in plants, and the silencing of endogenous genes by homologous transgenes remains a useful tool for exploring gene function. More recently, RNA silencing also has been determined to play a role in plant development and defense. The use of RNA silencing as a tool for functional genomics and its participation in natural biological processes will be addressed in this symposium.

2:30		Introduction (P. Ozias-Akins, D. Tomes, and T. Klein)
2:45	P-25	Inhibition of Plant Gene Expression Technologies for Fun and Profit ***Gregory P. Pogue***, *Large Scale Biology Corporation*
3:15	P-26	Use of Transiently Expressed RNAi to Dissect Signaling Pathways in Plants ***Tuan-Hua David Ho***, *Washington University*
3:45	P-27	Plant MicroRNA's and Their Targets ***James Carrington,*** *Oregon State University*

NEW AND DEVELOPING TECHNOLOGIES FOR MICROPROPAGATION

Convener: Bruce A. Stermer, PhD

2:30 pm – 4:30 pm — Plant Symposium — Multnomah

(See Abstracts page 14-A and 28-A)

Plant micropropagation by conventional technology is labor intensive and requires considerable inputs of space and materials. However, new technologies are being developed that offer significant improvements over conventional micropropagation. These emerging liquid culture systems can reduce the costs of labor and materials, lessen the time required for production and also result in increased product quality. Today's session will present talks on the application of liquid culture systems for increased efficiency and quality in plant product production.

2:30		Introduction (B. Stermer)
2:40	P-28	Somatic Embryo Development in Liquid Medium for Large-scale Propagation of Conifer Trees ***Pramod K. Gupta,*** *Weyerhaeuser Company*
3:10	P-29	Liquid Systems for Micropropagation, Storage, Shipping, and Acclimatization ***Jeffrey W. Adelberg***, *Clemson University*
3:40	P-1039	Gibberellin Synthesis Inhibitors Improve Conifer Embryogenic Tissue Initiation ***Gerald S. Pullman,*** *Institute of Paper Science and Technology*
4:10	P-30	Alternative Media for the Micropropagation of Plants ***Kenneth C. Torres,*** *Phytotechnology Laboratories, L. Williams, N. Philman, and M. Kane*

Education and Invertebrate Posters

SATURDAY, MAY 31	SUNDAY, JUNE 1	MONDAY, JUNE 2	TUESDAY, JUNE 3
7:00 pm – 9:00 pm	10:00 am – 3:00 pm	10:00 am – 3:00 pm	10:00 am – 2:00 pm

Posters mounted Saturday, May 31, 3:00 pm – 6:00 pm.
Posters must be removed from Grand Ballroom by 3:00pm, June 3.
Authors will be present at their posters the following days and times:

SATURDAY, MAY 31	SUNDAY, JUNE 1	MONDAY, JUNE 2	TUESDAY, JUNE 3
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
7:30 pm – 8:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:00 pm

EDUCATION POSTERS

STUDENT POSTERS

E-2000 The Effect of Trace Metals on Bioluminescent Dinoflagellates
***Peter Douglas Clark**, St. Andrew's Episcopal School*

E-2001 Identification of Drug Resistant Loci in Breast Cancer Cells by RAPD-PCR Fingerprinting
***Raymond Mailhot**, The Altamont School, Kamleshwar P. Singh, Satya Narayan, and Deodutta Roy*

E-2002 Some Effects of Plant Growth Regulators on Mammalian Cell
***Kendra C. Cawley,** Portland Community College, R. Aleman, M. Blackledge, C. Campbell, J. Cohen, B. Lindsey, M. Kondapalli, J. Marsh-Haffner, N. Myint, and K. Taylor*

EDUCATOR POSTERS

E-2003 Plant Tissue Culture and Plant Conservation: In Vitro Methods for Introducing Concepts to the Classroom
***Bernadette Lourdes Plair,** Cincinnati Zoo and Botanical Garden, V. C. Pence, and S. M. Charls*

E-2004 Investigating the Use of Oil Degradation Microbes in the Secondary Science Classroom
***Carol Harrison,** Booker T. Washington High School and Tuskegee University*

INVERTEBRATE POSTER

SILENT ABSTRACT

I-2005 Ecological Monitoring of the Ai River (Ural, Russia) Under the Influence of Oil Spills
***Anton Ilyich Vagapov,** Municipal Ecological Lyceum of Chelyabinsk*

Plant Posters

SATURDAY, MAY 31	SUNDAY, JUNE 1	MONDAY, JUNE 2	TUESDAY, JUNE 3
7:00 pm – 9:00 pm	10:00 am – 3:00 pm	10:00 am – 3:00 pm	10:00 am – 2:00 pm

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All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
7:30 pm – 8:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:00 pm

BIOTECHNOLOGY

P-2010 Positive Correlation Occurs Between Cytosine Methylation and Adventitious Shoot Induction in Petunia
Prakash P. Kumar, *National University of Singapore, A. P. Prakash, A. Kush, and P. Lakshmanan*

P-2011 Plant Transformation Center
Kenneth C. Sink, *Michigan State University*

P-2012 Aluminum Tolerance in Alfalfa with the Citrate Synthase Gene
Pierluigi Barone, *University of Georgia, D. Rosellini, J. H. Bouton, P. L. LaFayette, M. Sledge, F. Veronesi, and W. A. Parrott*

P-2013 A Non-antibiotic Marker for the Selection of Transformed Plants
Peter LaFayette, *University of Georgia, P. M. Kane, and W. A. Parrott*

P-2014 Co-ordinate Expression of β and δ Zeins in Transgenic Tobacco
Jennifer Randall, *New Mexico State University, Dennis Sutton, Soumitra Ghroshroy, Suman Bagga, and John D. Kemp*

P-2015 Characterizing the Plastid Ribosomal Region Among 25 Diverse Angiosperms: An Early Step Towards Universal Plastid Transformation Vectors for Plant Expression
James R. Wright, *Madigan Army Medical Center, and P. M. McNutt*

P-2016 DNA Methylation and Transgene Silencing in Wheat Transformed with the Wheat Streak Mosaic Virus Coat Protein Gene
Zhiwu Li, *University of Idaho, J. Hanson, R. S. Zemetra, and P. H. Berger*

P-2017 RAPD Analysis Concerning Genetic Variability of *Phytophtora infestans* in Potato
Constantin Botez, *University of Agricultural Sciences and Veterinary Medicine, D. Pamfil, M. Ardelean, R. Sestra, Elena Tãma, Katalin Kovacs, and J. Ekart*

P-2018 Expression of GFP Reporter Gene in *Arundo donax* Following Microprojectile Bombardment
M. Singh, *Fort Valley State University, K. Knowles and S. K. Dhir*

Plant Posters

P-2070 Establishing Cryopreservation Methods for Conserving European Plant Germplasm
Erica E. Benson, *University of Abertay Dundee, B. Panis, J. Geuns, R. Swennen, K. Harding, D. Bremmer, P. Lynch A. Hargreaves, P. Bonner, S. Dussert, N. Chabrillange, C. Damiano, C. Forni, S. Beninati, L. Alessandro, P. Bruno, H. M. Schumacher, E. Heine-Dobbernack, H. Takagi, L. Maggioni, and F. Engelmann*

IN VITRO TOOLS

P-2019 Cryopreserved Storage of Hops (*Humulus* L.) Germplasm
Barbara M. Reed, *USDA/ARS, Carolyn Paynter, Jeanine DeNoma, Jeff D'Achino, and Nese Okut*

P-2020 Elimination of Grapevine Virus A (GVA) by Cryopreservation of *In Vitro*-grown Shoot Tips of *Vitis vinifera* L.
Qiaochun Wang, *The Hebrew University of Jerusalem, M. Mawassi, P. Li, R. Gafny, I. Sela, and E. Tanne*

P-2021 *In Vitro* Selection of Somaclones Through Phosphate Starvation in Maize (*Zea mays* L.)
Anshu Miglani, *G. B. Pant University of Agriculture and Technology, Alok Shukla, and R. C. Pant*

P-2022 The Use of Clontech Coral Reef Proteins as Tools in Plant Transformation
Allan R. Wenck, *Syngenta, Celine Pugieux, Mark Turner, Martha Dunn, Cheryl Stacy, Annalisa Tiozzo, Erik Dunder, Emiel Van Grinsven, Rafiqul Kahn, Marina Sigareva, Wen Chung Wang, Janet Reed, Shanaaz Tayab, Paul Drayton, Duncan Oliver, Gaston Legris, Helen Rushton, Hugh Trafford, Karen Launis, Yin-Fu Chang, Dong-Fang Chen, and Leo Melchers*

P-2023 Growth Response of In Vitro Cultured *Campanula* Plantlets to Trophic Phase, Photosynthetic Photon Fluxes, and Temperature
Kim Gyeong Hee, *Gyeongsang National University, Nam Hee Choi, Mi Young Lim, Young Hoe Kim, and Byoung Ryong Jeong*

P-2024 Monitoring Phytohormone-induced Changes in Cotton Ovule Culture Gene Expression Using Real-time, Reverse-transcription Polymerase Chain Reaction
Barbara A. Triplett, *USDA/ARS, and H. J. Kim*

TRANSFORMATION

P-2025 Accumulation Profiles of Lipid and Phenolic Antioxidant Compounds During a Growth Cycle of Suspended Cells of *Salvia officinalis* L.
Manuel Fernandes-Ferreira, *Universidade Do Minho, Paulo S. C. Braga, Paula C. Santos-Gomes,, Rosa M. Seabra, and Paula B. Andrade*

P-2026 Stable Transformation of *C. annuum* and *C. baccatum* Explants Inoculated with *A. rhizogenes* and *A. tumefaciens*
Luis L. Valera-Montero, *New Mexico State University, and Gregory C. Phillips*

Plant Posters

P-2027 Herbicide Resistant Celery (*Apium graveolens* L.) Plants Produced by *Agrobacterium*-mediated Transformation Using the Bar Gene
Andrey V. Loskutov, *Michigan State University, and K. C. Sink*

P-2028 Transgenic Chrysanthemum (*Dendranthema grandiflora* cv. Subangyuk) with *Ls Like* Gene Expresses Branchlessness Habit
Su Young Lee*, National Horticultural Research Institute, B. H. Han, H. J. Yoo, H. K. Shin, I. G. Mok, E. J. Suh, and Y. P. Lim*

P-2029 Phloem-specific Expression of the β-glucuronidase Reporter Gene in Transgenic Strawberry (*Fragaria vesca* and *F. x ananassa*)
Qingzhong Liu, *USDA/ARS, Y. Zhao, and R. E. Davis*

P-2030 *Agrobacterium*-mediated Transformation and Plant Regeneration from Leaf Segments of Sweet Cherry Dwarf Rootstock 'Gisela 6' (*Prunus cerasus X P. canescens*)
Qingzhong Liu, *USDA/ARS, Y. Zhao, R. W. Hammond, H. Zhao, and R. E. Davis*

P-2031 Transformation of Rice with Bacterial Artificial Chromosomes (BAC) DNA
Bao H. Phan, *University of Georgia, C. Topp, C. Zhong, U. Akoh, J. Jiang, R. K. Dawe, and W. A. Parrott*

P-2032 *Agrobacterium*-mediated Genetic Transformation of Pigeon Pea with Hemagglutinin Nuraminidase (HN) Gene of *Peste des Petits Ruminants Virus* as a Source of Edible Vaccine
Valluri Venkata Satyavathi, *Indian Institute of Science, V. Prasad, K. J. M. Valli, K. Abha, M. S. Shaila, and G. Lakshmi Sita*

P-2033 *Vaccinium angustifolium* Cell Cultures: An Alternative Method of Studying the Anti-cancer Potential of Wild Blueberries
Tristan F. Burns Kraft, *University of Illinois, Chris Knight, Barbara Schmidt, Randy Rogers, David Seigler, and Mary Ann Lila*

P-2034 Differential Accumulation of Flavonoids in Cell Cultures of *Vitis vinifera*: Implications for Pharmacological Research
Sinee Pauline Kopsombut, *University of Illinois, K. A. Marley, R. A. Larson, and M. A. Lila*

P-2035 Transformation of Carnations with Flavonoid Biosynthesis Related Genes
Byung Joon Ahn, *Dankook University, K. H. Hwang, B. H. Min, and H. Y. Joung*

P-2036 Development of a Robust Tissue Culture System: Ideal for *Agrobacterium* Mediated Transformation in Sorghum
Bushra Sadia, *University of Toledo, W. Alaiwi, J. MacDougall. K. Meeker, B. Smith, S. Ibeji, G. Franklin, M. Parani, S. L. Goldman, and R. V. Sairam*

P-2037 Biolistic Transformation and Expression of Fertile Soybean Transgenics for Chitinase and Glucanase
Jung-Hoon Lee, *Kansas State University, William Schapaugh, Subbaratnam Muthukrishnan, and Harold N. Trick*

P-2038 Genetic Transformation of Chilli (*Capsicum annuum* L. var Pusa Jwala and California Wonder) via *Agrobacterium tumefaciens* with Coat Protein of Pepper Vine Binding Virus (PVBV)
K. J. Maragatha Vally, *Texas A&M University, and G. Lakshmi Sita*

P-2039 Using Leaf Disk for Guayule Transformation
Niu Dong, *USDA/ARS, and Katrina Cornish*

Plant Posters

P-2040 Increased Transformation and Rooting Efficiencies in Canola (*Brassica napus* L.) Using *Agrobacterium* Mediated Transformation
Vinitha Cardoza, *University of Tennessee, and C. Neal Stewart, Jr.*

P-2041 Utilization of Glufosinate Selection and Cysteine in *Agrobacterium*-mediated Cotyledonary Node Transformation in Twelve Soybean Cultivars
Margie Margarita Mercado Paz, *Iowa State University, Z.-B. Guo, Z.-Y. Zhang, A. K. Banerjee, and K. Wang*

P-2042 Use of a Site Directed Recombination Strategy for Selectable Marker Removal in *Glycine max* (Soybean)
Joanne L. Ekena, *Monsanto Company, Michael Petersen, and Larry Gilbertson*

P-2043 Selection of Transgenic Papaya Seedlings Using Kanamycin and DMSO
Thomas W. Zimmerman, *University of the Virgin Islands, and Nina St. Brice*

P-2044 High-frequency Transformation of Undeveloped Plastids in Tobacco Suspension Cells
Camri L. Langbecker, *Monsanto, Guang-Ning Ye, Peter T. J. Hajdukiewicz, Charles W. Xu, Charles L. Armstrong, and Jeffrey M. Staub*

P-2045 Somatic Embyrogenesis and Transformation in Alfalfa Using Gene Gun
Kaye Knowles, *Fort Valley State University, Seema Dhir, and S. K. Dhir*

PLANT TISSUE CULTURE AND MICROPROPAGATION

P-2046 Field Performance and Evaluation of Micropropagated FHIA Hybrid Banana Plants in the Marshall Islands
Dilip Nandwani, *College of the Marshall Islands, Arwan Soson, and Diane Myazoe*

P-2047 Development of Efficient Plant Regeneration Systems for *Agrobacterium*-mediated Transformation of Sour Cherry (*Prunus cerasus* L.)
Guo-qing Song, *Michigan State University, and K. C. Sink*

P-2048 Dispersal and Filtration of Embryogenic Callus Increases the Frequency of Embryo Maturation and Conversion for Hybrid Tea Roses
Kathryn K. Kamo, *UDSA, B. Jones, J. Castillon, and F. Smith*

P-2049 Effect of Growth Regulators on Callus Induction and Somatic Embryo Formation from Scutella Cultures of Durum Wheat
Valluri Venkata Satyavathi, *North Dakota State University, and Prem P. Jauhar*

P-2050 Induction of Somatic Embryogenesis in O'Henry Cultivar of Peach (*Prunus persica)*
Nirmal Joshee, *Fort Valley State University, A. K. Yadav, J. Subramanyan, and F. A. Hammerschlag*

Plant Posters

P-2051 Effect of Stage II Duration on Rooting and Survival of Sea Oats (*Uniola paniculata* L.) Genotypes
Carmen Valero-Aracama, *University of Florida, M. E. Kane, S. B. Wilson, and N. L. Philman*

P-2052 Micropropagation of *Lavendula angustifolia* L. for Production of Essential Oils
Ana M. S. Vicente, *Universidade Do Minho, Paulo S. C. Braga, Maria José Vilaça-Silva, Gilda Ramos, Cecilia Araújo, and Manuel Fernandes-Ferreira*

P-2053 Germplasm Preservation of Fruit, Small Fruit, and Grape Cultures in Kazakhstan
Irina Y. Kovalchuk, *Kazakh Research Institute of Horticulture and Viticulture, and S. Kushnarenko*

P-2054 In Vitro Regeneration of the Himalayan Medicinal Plant *Lilium nepalense* (D. Don) via Shoot Organogenesis
Prakash Raj Malla, *Tribhuvan University, and Sajani Malla*

P-2055 Rooting in Cultures of Two Endangered Florida Pawpaws, *Asimina tetramera and Deeringothamnus Rugelii*
Susan M. Charls, *Cincinnati Zoo and Botanical Garden, J. R. Clark, and V. C. Pence*

P-2056 Regeneration of *Aloe arborescence* via Somatic Organogenesis from Young Inflorescences
M. Velcheva, *Volcani Center, Z. Faltin, A. Vardi, Y. Eshdat, and A. Perl*

P-2057 Induction of Somatic Embryogenesis in Ovaries of *Vitis vinifera* L.: Effect of the Developmental Stage and Growth Regulators
Hélia Guerra Cardoso, *Instituto de Ciência Aplicada e Tecnologia, A. Peixe, and M. S. Pais*

P-2058 High Frequency Somatic Embryogenesis from Leaf and Floral Explants of 'Chancellor' Grape
Richard Mulwa, *University of Illinois, M. A. Norton, and R. M. Skirvin*

P-2059 *In Vitro* Propagation and Phytochemical Production of Kava (*Piper methysticum*)
Hideka Kobayashi, *University of Illinois, M. C. Gawienowski, M. A. Lila, and D. P. Briskin*

P-2060 In Vitro Shoot Induction of *Pinus maximartinezii*
Elizabeth Cardenas, *Universidad Atónoma de Nuevo Leõn, M. C. Ojeda, and T. E. Torres*

P-2061 Approaches to Increase Embryogenic Culture Initiation and Cell Line capture in Loblolly Pine
John Joseph Clark, *Arborgen, M. R. Becwar, M. K. Chowdhury, N. S. Nehra, M. R. Rutter, M. J. Cook, J. M Victor, T. J. Stout, A. M. Perry, P. J. Wade, and M. A. Hinchee*

P-2062 Plant Regeneration of *Arundo donax* L. Through Somatic Embryogenesis
M. Singh, *Fort Valley State University, D. Moore, K. Knowles, and S. K. Dhir*

Plant Posters

P-2063 Development of Thin Cell Layer Culture System for Rapid and Direct Regeneration of Sugarcane and Other Monocot Species
Prakash Lakshmanan, *David North Plant Research Centre, R. J. Geijskes, A. R. Elliot, L. F. Wang, M. G. McKeon, R. S. Swain, Z. Borg, N. Berding, C. P. L. Grof, and G. R. Smith*

P-2064 Somatic Embryogenesis from Immature Embryos of *Phaseolus aureus*
Magfrat Muminova, *Institute of G&PEB, M. Nasretdinova, S. Djataev, and A. Abdukarimov*

P-2072 Cryopreservation Studies in *Carica papaya* – Effect of Some Cryoprotectants on Regrowth and Somatic Embryogenesis in Sunrise Solo Papaya
Sadanand A. Dhekney, *University of Florida, R. E. Litz, N. Joshee, and A. K. Yadav*

PLANT SILENT ABSTRACTS

P-2066 Somatic Embryogenesis from Callus Cultures of Teak (*Tectona grandis* L.f.) Derived from Leaf Explants
Luluk Yunaini, *Institut Teknologi Bandung, and S. N. Widiyanto*

P-2067 Alternative Media for In Vitro Propagation of Fe'i Bananas
Flordeliza Briones Javier, *College of Micronesia – FSM*

P-2068 Saffron Micropropagation by Somatic Embryogenesis in a Temporary Immersion System
Silvia Blázquez Martin, *Instituto de Desarrollo Regional, Abel Piqueras, and José Antonio Fernández*

P-2069 Characterization of a Putative Rab-related Small GTP-binding Protein, *LeRab6* in Tomato (*Lycopersicon esculentum, Miller)*
Hyder Ali S. Khoja, *INP/ENSAT, J. Leclercq, A. Latché, J. C. Pech, and M. Bouzayen*

P-2071 Cellular Location of Oxidative Stress and Antioxidant Enzymatic Response in Hyperhydrated Carnation Plantlets
Abel Piqueras, *CEBAS (CSIC), S. Saher, E. Hellin, and E. Olmos*

Vertebrate / Toxicology Posters

SATURDAY, MAY 31	SUNDAY, JUNE 1	MONDAY, JUNE 2	TUESDAY, JUNE 3
7:00 pm – 9:00 pm	10:00 am – 3:00 pm	10:00 am – 3:00 pm	10:00 am – 2:00 pm

Posters mounted Saturday, May 31, 3:00 pm – 6:00 pm.
Posters must be removed from Grand Ballroom by 3:00pm, June 3.
Authors will be present at their posters the following days and times:

SATURDAY, MAY 31	SUNDAY, JUNE 1	MONDAY, JUNE 2	TUESDAY, JUNE 3
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
7:30 pm – 8:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:30 pm	1:30 pm – 2:00 pm

VERTEBRATE/TOXICOLOGY SILENT ABSTRACTS

VT-2016 A Non-animal Alternative Carcinogenicity Assay Using Fertilized Avian Eggs: The In Ovo Carcinogenicity Assay (IOCA)
George L. DeGeorge, *MB Research Laboratories, D. R. Cerven, M.J. Iatropoulos, G. M. Williams, C. Perrone, and H. Enzmann*

VT-2017 Development of an Alternative Test for Photoirritants: The In Ovo Phototoxicity Assay (IOPA)
George L. DeGeorge, *MB Research Laboratories, D. R. Cerven, T. L. Ripper, T. L. Fox, and S. H. Young*

VT-2018 In Vitro Models of Full-thickness Human Skin (EpiDerm-FT) and Airway Epithelium (EpiAirway-FT) for Toxicology and Drug Development Applications
Patrick J. Hayden, *MatTek Corporation, M. Klausner, J. Kubilus, B. Burnham, and G. R. Jackson*

Plenary Session

PS-1

ARTistic Use of Nonhuman Primates: IVF to Cloning and Beyond. R. L. STOUFFER and D. P. Wolf. Division of Reproductive Sciences, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR 97006. E-mail: stouffri@ohsu.edu

Twenty-five years ago, the use of assisted reproductive technologies (ART) became ensconced in human society and medicine with the birth of the first test-tube baby by in vitro fertilization (IVF). The availability of "spare" human eggs and embryos following IVF procedures in infertility clinics also facilitated further technological advances, including the isolation of human embryonic stem (ES) cells, and generated controversy on reproductive versus therapeutic cloning. The application of ART to nonhuman primate models occurred more slowly over the last 10-15 years, but offers great promise for performance of basic primate biology, preservation of endangered primate species, generation of valuable animals for disease-related research, and performance of preclinical trials to evaluate efficacy and safety of gene- or ES-based therapies. Development of the nonhuman primate model resulted in many achievements using rhesus monkeys (*Macaca mulatta*), including the routine production of infants from fresh and cryopreserved-thawed embryos derived by IVF or intracytoplasmic sperm injection (ICSI). In addition, births have been achieved following splitting of early embryos or transfer of the nucleus from embryonic cells into the oocyte. The ability of cultured fertilized eggs to develop to the blastocyst stage is facilitating studies on early embryogenesis; macaque ES cells have been isolated, characterized and are currently used to study the processes controlling cell differentiation into diverse lineages, including neurons and pancreatic β cells. Efforts are also ongoing to use gametes from genetically characterized monkeys or to genetically modify embryos as a means to generate novel disease models. The lecture will provide an overview of the advances and challenges associated with the nonhuman primate ART program, as well as the opportunities to facilitate animal husbandry and clinical research. Supported by HD28484, HD18185, A142709, RR12804 and RR00163.

E-1

Connecting with Teachers Through Hands-on Workshops, Online Courses and Listservs, and User-friendly Kits. C. M. STIFF. Kitchen Culture Kits, Inc., Lufkin, TX 75901. E-mail: kck@turbonet.com

Creating connections between scientists and educators is critical if we are to bring scientific discoveries to the classroom. Knowledge of these new discoveries aids students and their communities in understanding and appreciating the latest science innovations. Creating these connections can be difficult due to the typical educators' limited time and funds. Through use of hands-on workshops, online courses, listservs and user-friendly kits, these connections can be implemented. Details of some of these ongoing activities will be discussed.

E-2

LIGASE Loaners: Bringing Scientific Expertise to the High School Classroom. P. E. BOSSERT. Northport High School, Northport, NY 11768. E-mail: pbossert@optonline.net

The ***Long Island Group Advancing Science Education*** (LIGASE) is funded by a 1.9 million dollar grant from the Howard Hughes Medical Institute to Stony Brook University. David Bynum, Associate Professor of Biochemistry directs this grant that enables Stony Brook to work with school districts, teachers, teachers-in-training and students to integrate current science concepts into K-12 education. The Northport/East Northport School District was a pioneer partner in a LIGASE initiative that enables school districts to borrow a thermal cycler and the equipment necessary to do polymerase chain reaction (PCR) in the classroom. The loan of equipment is supported by teacher workshops designed to teach both the theory and practice of PCR. Students at Northport High School now routinely do PCR with equipment that the district has purchased and some have used the technology to do research projects that received recognition as Finalist and Semifinalist in national science competitions. A new initiative (pending grant) will 'lend a scientist' to the high school classroom after teachers attend seminars and hands on workshops given by university faculty and graduate students.

E-3

Creating a Viable Biotechnology Program in High School. JUDY HEITZ. Chula Vista High School, 820 4th Ave., Chula Vista, CA 91911. E-mail: jmheitz@earthlink.net

Chula Vista High School is an underperforming, predominantly Hispanic school, located five miles from the Mexican border. Using state and local funds, we have created a three year biotech program aimed at those students who are not traditionally successful in math and science. The first two years are academic, liberally sprinkled with job shadows and speakers from industry. The third year is either an industry internship or college attendance via a 2 + 2 program with local community colleges. This talk will provide the basics of how the program was started and how it was institutionalized into the school academic programs.

E-4

Bio-link: Promoting Biotechnology Education in Community Colleges. B. J. PITONZO and E. A. Johnson. Mt. Hood Community College, Division of Science, Gresham, OR 97030. E-mail: pitonzob@mhcc.edu and City College of San Francisco, Advanced Technological Education for Biotechnology National Center, San Francisco, CA 94103. E-mail: ejohnson@biolink.ucsf.edu

Bio-Link is an Advanced Technological Education (ATE) Center for the nation's biotechnology industry and is funded primarily by the National Science Foundation to promote and improve technological education. The ATE program focuses on using the resources of community and technical colleges that can prepare technicians for today's high-performance workplace. Bio-Link, headquartered at City College of San Francisco, has Regional Centers in the states of New Hampshire, Texas, Wisconsin, Washington, California and North Carolina. Bio-Link is also affiliated with the Northern California Biotechnology Center at City College of San Francisco. The national and regional Centers all work closely with the biotechnology industry to help define curriculum and training that will best meet industry's needs. They also work with secondary schools and four-year institutions to help create career paths for students who are interested in the cutting edge field of biotechnology. The Center concentrates on professional development for instructors, curriculum improvement, and the sharing of information and materials with biotechnology programs across the country. There is an extensive curriculum and instructional materials clearinghouse and many other services for business, educators, and technicians on the website at www.bio-link.org. Bio-Link is currently in the fifth year of a six-year commitment to NSF and is planning for sustainability as a Biotechnology National Resource Center for many years in the future.

Invertebrate Symposia

I-1

Co-culture of *Trypanosoma musculi* with Spleen-derived Adherent Fibroblasts and Macrophages Ensures Survival In Vitro. W. A. ANDERSON, A. Gugssa, and C. M. Lee. Department of Biology, E. E. Just Hall, Howard University, Washington, DC 20059. E-mail: wanderson@howard.edu

The search for methods of culturing trypanosomes has occupied the efforts of many researchers. *Trypanosoma musculi* cultivated in medium containing serum alone transformed into rounded, immotile cells, incapable of division and infectivity. However, *T. musculi* co-cultured with adherent splenic fibroblasts or peritoneal macrophages survived and grew indefinitely so long as cellular contact was retained. Immature forms of *T. musculi* made contact with adherent cells by flagellar or lateral surfaces for an indefinite period of time. Eventually, trypanosomes transformed to trypomastigotes, severed contact with adherent cells, divided and eventually died, possibly by apoptosis. Cellular contact, therefore, seemed to be essential for trypanosomal survival and growth. Transmission and scanning electron microscopy confirmed intimate membrane-to-membrane contact between trypanosomes and adherent cells. However, neither membrane fusion nor junctions were apparent. Immunocytochemical studies demonstrated intense fibroblast growth factor (FGF) and a complex cytoskeletal system of F-actin underlying the plasmalemma of adherent fibroblasts. Among the cytoskeletal and membrane glycoproteins, fibronectin, I-CAM, laminin, occludin, vinculin and desmin were the most prominent markers. Intimate connection between trypanosomes and adherent cells suggested transfer of anti-apoptotic and growth factors that maintained survival of trypanosomes. *T. musculi* adhered firmly to splenic macrophages that demonstrated intense nitric oxide synthase (I-NOS). *T. musculi* parasites associated and proliferated in the presence of non-LPS-activated macrophages, but not in the presence of activated macrophages. Activated macrophages appeared to secrete several cytokine species (detected by Western Blotting) including TNFa, INFa, IL-6 and IL-10 that may be related to death of *T. musculi* cells in the medium. Our results suggest that adherent spleen-derived fibroblasts and macrophages produced a growth and/or anti-apoptotic factor (s) that enhanced the survival of immature forms of trypanosomes in co-cultures.

I-2

Lymnaea EGF, Roles in Neurite Outgrowth In Vitro and In Vivo. A. G. M. BULLOCH, W. Wildering, and P. Hermann. University of Calgary, Calgary, Alberta, T2N 4N1, CANADA. E-mail: bulloch@ucalgary.ca

Our research is directed at the cellular and molecular responses of the CNS to injury. We have shown that EGF from the freshwater snail, Lymnaea (L-EFG) mRNA is expressed throughout embryonic development and in the juvenile CNS. Although not detectable in the normal, adult CNS, its expression is upregulated after injury. These observations led us to test the neurotrophic properties of purified L-EGF both on isolated neurons and in the organ cultured CNS. When assayed on different adult neurons in vitro, L-EGF evoked neurite outgrowth in some, but not all, cell types. This outgrowth could be blocked by application of a specific EGF inhibitor. We then tested whether L-EGF could promote axonal regeneration of the same specific neurons in the organ cultured CNS. Unlike our in vitro data, all neurons types responded to L-EGF in vitro. Taken together, our data suggests that L-EGF exerts both direct and indirect actions on molluscan neurons.

I-3

Growth-blocking Peptide Family and Its Multiple Effects on Insect and Vertebrate Cells. YOICHI HAYAKAWA. Institute of Low Temperature Science, Hokkaido University, Sapporo, 060-0819 JAPAN. E-mail: hayakawa@lowtem.hokudai.ac.jp

Growth-blocking peptide (GBP), is a 25-amino acid cell growth factor found in Lepidopteran insects: it stimulates thymidine incorporation into human keratinocytes at a level comparable with that of human EGF and also induces thymidine incorporation into Sf-9 insect cells to higher levels than does human EGF. Further, GBP possesses other biological activities, which include stimulation of immune cells (spreading of plasmatocytes), regulating larval growth and causingtemporary paralysis when injected into larvae. It is unknown, however, how GBP exerts such diverse biological activities. We recently synthesized deletion and point mutation analogs of GBP to investigate the relationship between the structure of GBP and its mitogenicand plasmatocyte spreading activity. Deletion of the N-terminal residue, Glu(1), eliminated all plasmatocyte spreading activity but did not reduce mitogenic activity. In contrast, deletion of Phe(23) along with remainder of the C-terminus destroyed all mitogenic activity but only slightly reduced plasmatocyte spreading activity. Further, GBP analogs replaced Asp(16) with either a Glu, Leu, or Asn residue had no mitogenic activity but retained about 50% of their plasmatocyte spreading activity. Based on these results, it is reasonable to conclude that multifunctional properties of this peptide may be mediated by different types of a GBP receptor.

I-4

Regeneration of Walking Legs in the Fiddler Crab, *Uca pugilator*: The Role of Growth Factors. P. M. HOPKINS. Department of Zoology, University of Oklahoma, Norman, OK 73019. E-mail: phopkins@ou.edu

This paper will summarize recent work on the histological, physiological and molecular aspects of limb regeneration in the fiddler crab *Uca pugilator*. Information will be presented on mitotic activity in the blastema during the first days of blastemal organization. The localization of vertebrate growth factor immunoreactivity (FGF 2 and FGF 4) in the regenerating blastema will be described. The first part of this paper will review recent histological findings concerning the physical events that accompany autotomy of limbs with the presentation of a model for the events of early blastema regeneration. The second part of the paper will review our recent findings on the identification and characterization of the Uca ecdysteroid receptor (UpEcR, and its potential dimer partner, the retinoid-X-receptor, UpRXR). Using Uca-specific antibody probes raised in our lab, we have been able to identify specific cells in the early blastema that express receptor proteins. The regenerating limb of the fiddler crab is responsive in vitro to both steroids and retinoids and mRNA for steroid and retinoid receptors are expressed in the regenerating limb buds during all stages of regeneration. The DNA and deduced amino acid sequences of the ecdysteroid receptor is very similar to the sequences of insect EcRs, while the retinoid receptor is similar to insect protein (ultraspiracle) in the DNA-binding domain, but closer to vertebrate RXRs in the ligand binding domain. The identification of isoforms of both receptor partners will be discussed.

I-5

Regulation of the Fate of Stem Cells from the Midgut of the Caterpillar, *Heliothis virescens*. M. J. LOEB[1], R. S. Hakim[2], and G. Smagghe[3]. [1]Insect Biocontrol Laboratory, U. S. Department of Agriculture, Beltsville, MD 20705; [2]Department of Anatomy, Howard University, Washington, DC 20059; and [3]Free University of Brussels, Brussels, BELGIUM. E-mail: loebm@ba.ars.usda.gov

Stem Cells derived from midguts of several species of Lepidoptera can be induced to multiply and to differentiate *in vitro* when incubated with growth factors. Four different peptide factors that induce differentiation of mature larval midgut cells have been isolated from conditioned medium in which *Manduca sexta* midgut cells have been grown and from the blood of *Lymantria dispar*. A protein isolated from pupal fat tissue is necessary to maintain stem cell mitosis in the presence of low titers of a steroid, the insect molting hormone, 20-hydroxyecdysone; the crude fat body extract will disrupt midgut issue and kill *Spodoptera littoralis* larvae *in vivo*. Mammalian growth factors epidermal growth factor, all trans retinoic acid and platelet-derived growth factor also induce stem cell differentiation, albeit to mature midgut cells characteristic of pupal and adult midgut tissue, as well as other epidermal structures. We previously showed that conditioned medium from cultures grown from pre-pupal or pupal midgut induced larva-derived midgut stem cells to differentiate to pre-pupal and pupal midgut types. Thus, the stem cells of Lepidopteran midgut are multipotent to epidermal fates. It has been shown that the interaction of factors and their receptors determine fate selection in stem cells from bone marrow and epidermis. We will show that calcium titers in the cells and external medium, as well as the receptor, integrin, have roles in fate regulation in insect midgut stem cells.

I-6

Modulation of Growth Factor Signaling During *C. elegans* Vulva Development. N. MOGHAL and P. W. Sternberg. Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125. E-mail: nmoghal@caltech.edu

Growth factors are widely used in development to promote cell survival, proliferation, and differentiation. We use the nematode *C. elegans* to study mechanisms of regulating growth factor signaling during the specification of cell fates. *C. elegans* has one ErbB/EGFR family member, LET-23, whose activation by the LIN-3, EGF-like growth factor, drives the induction of vulval fates. This process involves signaling from a conserved RAS-MAP kinase cassette. We have used molecular genetic approaches to elucidate mechanisms of regulating this pathway. We find that the output of this pathway is subject to multiple levels of regulation extending from the plasma membrane to the nucleus. Moreover, we have identified a novel pathway for regulating vulva development that involves signaling from muscles and neurons. This new pathway may exist to ensure that correct developmental decisions are made even under changing environmental conditions.

I-7

Isolation and Characterization of Transgenic Insect Cell Lines with Humanized Glycoprotein Processing Pathways. DONALD L. JARVIS, Jason R. Hollister, and Jared J. Aumiller. Department of Molecular Biology, University of Wyoming, Laramie, WY 82071. E-mail: dljarvis@uwyo.edu

The baculovirus-insect cell expression system is widely used to produce recombinant proteins, including glycoproteins, for various biomedical applications. However, one limitation of this system is that it cannot produce recombinant mammalian glycoproteins containing terminally sialylated glycans. A fundamental basis for this limitation is that insect cells lack some of the functions needed to produce sialylated glycoprotein glycans. This can be a serious problem, as the presence or absence of terminal sialic acids can strongly influence the behavior of recombinant glycoproteins, particularly in vivo. During the past several years, our group has undertaken efforts to address this problem and to gain a better understanding of protein glycosylation pathways in insect cells. One of our approaches has been to incorporate genes encoding mammalian glycan processing enzymes into the insect cell lines commonly used as hosts for baculovirus expression vectors. Towards this end, we have constructed expression plasmids and developed methods for stable transformation of lepidopteran insect cells. We then used these tools to produce transgenic insect cell lines and extensively characterized their biological and biochemical properties. As a result of these efforts, we have produced stable, transgenic insect cell lines that constitutively express mammalian genes encoding various functions required for glycoprotein sialylation. That is, we have isolated transgenic insect cell lines with humanized glycoprotein processing pathways. This presentation will focus on the creation and functional properties of these cell lines.

I-8

Recombinant Baculoviruses as Mammalian Cell Gene-delivery Vectors. TOM KOST. Gene Expression Protein Biochemistry, GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709. E-mail:Tom.A.Kost@gsk.com

The baculovirus expression system has been used extensively for the production of recombinant proteins in insect cells. Recently, recombinant baculoviruses vectors, engineered to contain mammalian cell-active promoter elements, have been used successfully for transient and stable gene delivery in a broad range of primary and established mammalian cells. Many commonly used cell lines including CHO, HEK 293, COS and HeLa cells are efficiently transduced. In contrast to other commonly used viral vectors, baculoviruses have the unique property of replicating in insect cells while being incapable of initiating a replication cycle and producing infectious virus in mammalian cells. The viruses can be readily manipulated, accommodate large insertions of DNA, initiate little to no microscopically observable cytopathic effect, are readily scalable and have a good biosafety profile. We have used baculovirus vectors containing a CMV-IE1 promoter cassette for a variety of gene delivery applications. Recombinant viruses have been developed to deliver hepatitis B virus into hepatocyte cell lines and to extend the lifespan of human hepatocyes by delivery of SV40 TAg. Vectors have also been developed for establishing assays for measuring estrogen receptor α and β and ATP-sensitive potassium (K_{ATP}) ion channel activity. Functional expression of the K_{ATP} ion channel requires the delivery of the sulfonylurea receptor (SUR) and an inward rectifier potassium channel (K_{IR}), demonstrating the utility of these vectors for studying multi-subunit protein complexes.

I-9

Enhancing the Sensitivity of Rainbow Trout Cells in Culture to the Toxicity of Metals. V. R. DAYEH and N. C. Bols. Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1. E-mail: vrdayeh@sciborg.uwaterloo.ca

A gill cell line, RTgill-W1, from rainbow trout was used to investigate the toxic actions of 5 metals: Cu, Zn, Cd, Fe and Ni. Cellular viability was measured using three fluorometric assays. These were alamar Blue for metabolic activity, CFDA-AM for membrane integrity and neutral red for lysosomal activity. The toxicity of these metals was affected by exposure medium. Little or no toxicity occurred in fish cell cultures exposed to the metals in basal L-15 medium or in L-15 medium with a fetal bovine serum (FBS) supplement. However, exposure in the minimal medium L-15/ex, which contains the salts, galactose and sodium pyruvate of L-15, resulted in cytotoxicity. In this exposure medium, the rank order of toxicity from least to most toxic was Ni < Fe < Cd < Zn < Cu. Sensitivity to copper was enhanced by modifying L-15/ex in two different ways. Firstly, toxicity was increased upon removal of pyruvate from the L-15/ex medium. Secondly, a further increase in sensitivity was seen upon treatment with DL-Buthionine-[S,R]-sulfoximine (BSO), which is an inhibitor of gamma-glutamyl cysteine synthetase and decreases glutathione levels. The increased sensitivity depended on the timing of the BSO addition to cell cultures. Cytotoxicity was not enhanced by concurrent exposure of cells to BSO and copper. On the other hand, sensitivity was increased dramatically when the cells were treated with BSO for 24 h followed by the removal of BSO and by the addition of copper. This enhancement of sensitivity opens up the possibility of using fish cells as a bioassay tool to detect toxic metals in industrial effluent.

J-1

The Role of Small Amphiphilic Solutes in Desiccation Tolerance. A. E. OLIVER[1] and D. K. Hincha[2]. [1]Section of Molecular and Cellular Biology, University of California, Davis, CA 95616 and [2]Max-Planck Institute for Molecular Plant Physiology, D-14424 Potsdam, Germany. E-mail: aeoliver@ucdavis.edu

Anhydrobiosis, or life without water, involves different processes depending on the specific organism or tissue involved, but one common mechanism is the accumulation of disaccharides. Desiccation-tolerant plants and animals accumulate sucrose or trehalose during the drying phase, which helps to maintain the membranes in a liquid crystalline state and forms a protective glassy matrix. Damage can continue in the presence of the disaccharides, however, and includes the possibility of adventitious enzymatic reactions. Together with free-radical mediated damage, these reactions can cause lipid hydrolysis and loss of membrane integrity. Organisms capable of surviving the severe stresses of the low-water environment must have evolved methods of minimizing this type of damage as well. Small amphiphilic solutes may participate in this type of protection. One example is arbutin, which is found in very high concentrations in the resurrection plant *Myrothamnus flabellifolia*. Arbutin can inhibit phospholipase A_2 activity at low water contents and is also an effective antioxidant. The lipid composition of the membrane determines the exact effect of arbutin under stressful conditions. In the presence of nonbilayer-forming lipids, arbutin can serve a protective function during desiccation or freeze/thaw-induced stresses, whereas it is disruptive to membranes composed entirely of phosphotidylcholine. Other antioxidant amphiphiles, such as certain naturally occurring flavonols, might have similar lipid composition-dependent effects. These compounds, therefore, are likely to be localized near target membranes where they could provide the greatest benefit at the least liability to the organism.

J-2

Cryopreservation Principles for Mammalian Tissues: The Basis of Success or Failure. M. J. TAYLOR. Organ Recovery Systems, Charleston, SC 29403. E-mail: mtaylor@organ-recovery.com

Modern day medicine has entered the post-genomic era when decoding of the human genome is expected to provide the basis for mapping a route that will define the genetic basis and provide the keys to the eventual cure of human diseases. This new era has also seen the emergence of new disciplines that involve manipulative engineering to provide remedies to a wide variety of conditions. The new discipline of tissue engineering, in its broadest sense, seeks to apply the principles of biology and engineering to produce substitutes for a variety of human tissues and organs in need of repair, restoration or replacement. Moreover, within these emerging new fields of medicine there is a crucial need for the parallel development of enabling technologies such as biopreservation. It is universally recognized that effective methods of preservation are critically important for ensuring an on-demand supply of the best quality cells, tissues and organs. Biopreservation is also important for the stabilization of natural and engineered tissues to permit transportation and extended shelf life. Tissue engineering is an interdisciplinary field that has largely trivialized the importance of cell and tissue storage. Methods of preservation are crucial for both the source of cells and the final tissue constructs or implantation devices. Tissue preservation technology involves both hypothermic (above freezing) methods for short-term storage, and cryopreservation for long-term banking. Both approaches call for consideration of the cell in relation to its environment and as interventionalists we can control or manipulate that environment to effect an optimized protocol for a given cell or tissue. A considerable degree of understanding of the mechanisms of freezing injury in cells and its prevention has ensued from early discoveries of the need for protective agents. It has long been anticipated that these cryobiological techniques would also provide the means for long-term storage of more complex tissues and possibly organs. However, it is now recognized that organized muticellular tissues are subject to additional mechanisms of cryoinjury and the destructive effect of extracellular ice, in particular, must be minimized by reducing, or eliminating ice crystallization during low temperature storage. Two basic approaches to avoiding ice damage in tissues will be reviewed. The first is to maintain the storage temperature above the freezing point (short-term hypothermic storage), and the second is to restrict the formation or growth of ice at sub-zero temperatures. The relative merits and disadvantages of these fundamentally different approaches to biopreservation of complex tissues will be discussed.

J-3

A Free Radical View Of Cryopreservation. ERICA E BENSON. Conservation and Environmental Chemistry Centre, Plant Conservation Group, University of Abertay Dundee, Bell Street, Dundee, DD1 1HG, Scotland. E-mail: e.e.benson@abertay.ac.uk

Free radical and electron transfer reactions are an important component of metabolism in all aerobic organisms. However, antioxidant protection and the tight metabolic coupling of reactions involving O_2 and activated oxygen species (H_2O_2, $\cdot OH$, O_2^1, $O_2^{\cdot -}$) are essential in order to prevent oxidative stress and the attack of key macromolecules such as proteins, lipids and DNA. At cryogenic temperatures (e.g. $-196°$ C in liquid nitrogen) all metabolic processes are considered to cease. However, it is known from chemical studies (using epr) that some radical reactions may proceed, even at ultra low temperatures. Thus, the study of free radical mediated oxidative stress in cryopreserved cells, tissues and organs may be important. Particularly as chemical reactions may still occur in the absence of antioxidant enzyme protection systems, which will be rate limited at ultra low temperatures. This presentation explores evidence for free radical damage being a component of cryoinjury and considers the implications that this has for storage stability at the physical, chemical, and molecular level. The presentation will include an inter-kingdom comparison of cryopreserved animal, plant and microbial systems. It will also consider the possibility that different storage states (e.g. liquid/vapour phase of LN_2, frozen/vitrified, hydrated/desiccated) may have different behaviours with respect to pro- and antioxidant status.

P-1

High-throughput Methods for Determining Transgene Copy Number and Expression in Plants. W. J. YU, D. J. Ingham*, M. Fielder, J. Huang and L. Ireland. Syngenta Biotechnology, Inc., Research Triangle Park, NC 27709. E-mail: wenjin.yu@syngenta.com

Conventional methods to detect transgene copy number and expression level by Southern blotting and northern blotting, respectively, are both time-consuming and low throughput. We have applied real-time quantitative PCR (qPCR) and real-time quantitative RT-PCR (qRT-PCR) as standard approaches to replace blotting techniques. Combining automated nucleic acid extraction using silica magnetic beads-binding method and qPCR, thousands of assays may be performed daily. As these approaches significantly reduce the time to screen putative transgenic plants and increase the assay accuracy, they help scientists to make quick decisions to select desired events, thus, greatly reducing cost and accelerating progress of commercial transgenic plant production. *Current address: BASF Plant Science, 26 Davis Drive, Research Triangle Park, NC 27709.

P-2

A Maize "Whiskers" Transformation System. P. BULLOCK, D. Dias, K. Cook, S. TeRonde, S. Bagnall, J. Ritland, J. Christensen, D. Spielbauer, and B. Budke. Garst Seed Company, Slater, IA 50244. E-mail: paul.bullock@garstseedco.com

The use of silicon carbide "whiskers" to genetically engineer maize has now become a very routine and high throughput transformation methodology. Recent enhancements of the "whisker" transformation system are largely based upon the use of a more robust and scaled-up "whisker"/cell collision system. More specifically, a commercial (Red Devil™) paint shaker is now being used to batch process large amounts of cultured maize cells with silicon carbide "whiskers". Thus, very substantial numbers (many 1000's of PCR + clones/year) of transgenic maize callus clones can be produced on a regular basis from embryogenic suspensions. PCR analysis of several thousand transgenic callus clones derived from the co-whiskering of multiple genes located either on two separate constructs or in the same construct indicates a co-transformation frequency of approximately 68% and 89%, respectively. Cryo-preservation of maize suspensions (frozen for over 10 years) provides for uninterrupted (non-greenhouse-dependent) year-round target tissue availability and, in a related sense, for the production of fertile transgenic maize plants. An overview of the "whiskers" transformation system will be provided as it relates to the commercial utilization of this technology.

P-3

High Throughput Plant Gene Function Analysis. K. R. DAVIS, D. C. Boyes, A. M. Zayed, S. Kjemtrup, C. Christensen, and J. Woessner. Paradigm Genetics, Inc., 108 T. W. Alexander Drive, Research Triangle Park, NC 27709. E-mail: kdavis@paragen.com

The completion of the Arabidopsis genome sequence provided a major opportunity to develop systems for a comprehensive high throughput functional genomics program in a higher plant. As a model organism for agricultural biotechnology, Arabidopsis provides a facile system that can provide key insights into the way that gene function can affect commercial crop production. To aid in the rapid discovery of gene function, we have established two complementary high-throughput phenotypic analysis platforms for capturing detailed data describing the effects of specific gene modifications on growth and development throughout the entire life of the plant. The early analysis platform characterizes early seedling growth on vertical plates over a period of two weeks. The soil phenomics platform consists of comprehensive measurements of pot-grown plants over a period of approximately two months. The platforms are based on a series of defined growth stages that serve both as developmental landmarks and as triggers for the collection of key morphological data at specific stages of development. When combined with parallel processes for metabolic and gene expression profiling, these platforms can serve as a core technology in the high throughput determination of gene function. We present here analyses of the development of wild-type and selected mutants to illustrate a methodology that can be used to identify and interpret phenotypic differences in plants resulting from genetic variation and/or the impact of environmental stress.

P-4

Applications of Somatic Embryogenesis and Transformation in Grapevine Improvement. D. J. GRAY, Z. Li, M. Van Aman, N. Barnett, A. Raveendran, and K. Kelly. Mid-Florida Research & Education Center, Institute of Food and Agricultural Sciences, University of Florida, 2725 Binion Road, Apopka, FL 32703. E-mail: djg@mail.ifas.ufl.edu

Although somatic embryogenesis (SE) is considered to be genotype dependent in grapevine, it has become common for an increasing number of species and varieties. SE offers the potential of efficient clonal germplasm storage, since embryos can be kept under seed repository conditions for long periods of time. Plants derived from SE appear to originate from single cells; thus, manipulation of embryogenic cell cultures can yield genetically modified non-chimeric plants. Techniques for genetic modification using SE includes transformation and in vitro selection; the latter will be discussed separately in this symposium. With only a few exceptions, all successful examples of genetic transformation in grapevine have utilized embryogenic cells as targets for gene insertion and SE for plant recovery. Use of high performance vectors containing visual marker genes like GUS and GFP, along with selectable markers like BAR and NPTII, have enabled protocols to be refined so that transformation is now routine for a number of grapevine species and cultivars. Resultant improved transformation efficiencies have enabled genes that encode traits of practical interest to be demonstrated. Such genes include those from viruses, like coat protein and replicase genes, to be inserted for virus resistance. Genes for chitinases and other PR-related proteins have been tested for fungal resistance. Lytic peptide genes are being utilized for both fungal and bacterial disease resistance. Herbicide resistance also has been explored via use of the BAR gene. In addition to using transgenics for solving problems with disease and stress resistance, manipulation of qualitative traits, such as induction of seedlessness and ripening have been explored. A number of transgenic field tests are underway. Beyond the obvious requirement that plants express the desired traits conferred upon them by given transgenes, it will be necessary to determine whether resulting grapevines exhibit the particularly strict levels of clonal fidelity required of this crop.

P-5

Gene Discovery in Grapes: The Grape Genome Project. E. M. ABLETT[1], L. Slade Lee[1], Toni Pacey-Miller[1], Dan Waters[1], Guo-Hua Miao[2], Maureen Dolan[2], Michael Hanafey[2], Scott V. Tingey[2], and Robert Henry[1]. [1]Centre for Plant Conservation Genetics, Southern Cross University, P.O. Box 157, Lismore, NSW 2480, Australia and [2]Dupont Genome Sciences, Newark, DE. E-mail: eablett@scu.edu.au

The grape genome project is combining large-scale EST analysis, a 16 times BAC library, and functional analysis of grape genes in Arabidopsis to advance gene discovery in *Vitis vinifera*. From two projects undertaken by Southern Cross University, 44,928 grape (*Vitis vinifera*) ESTs have been produced from a range of tissues and cultivars, and these code for approximately 18,500 grape genes. With the grape genome having a size of 511 Mb, similar to rice, we estimate that the 18,500 genes represent 2/3 of the grape genome. The first project produced almost 5,000 sequences (2,330 distinct gene matches). The list of genes is being made available via the World Wide Web and includes transcripts relating to proteins involved in the whole range of functional categories. The Web-based catalogue of the gene functions revealed from the first project and the method of access to selected gene sequences may be viewed at *http://wwwdev.scu.edu.au/research/cpcg/genomics/*. ESTÕs from both projects have been used in "electronic northern" and microarray analysis to investigate new aspects of gene function from expression data. The abundance of transcripts with predicted cellular roles in leaf, berry and developing buds, were estimated by classifying the EST primary BLAST matches into 80 functional categories. Comparison with "electronic northern" analysis of 33 human tissues suggests plant cells may involve their cellular machinery to a greater extent in specialised activities than is the case in animal cells. Hybridization of mRNA sampled during budburst to arrayed ESTÕs indicate altered levels of expression of large numbers of genes before and during budburst, and profiles of candidate genes with possible roles in this process have been analysed.

P-6

In Vitro Selection to Enhance Disease Resistance in Grapevine. S. JAYASANKAR. Department of Plant Agriculture -Vineland Station, University of Guelph, 4890, Victoria Ave N, PO Box 7000, Vineland Station, Ontario L0R 2E0, Canada. E-mail: jsubrama@uoguelph.ca

Grapevine (*Vitis* spp.) is a subject of a several fungal, bacterial and viral diseases. Resistance breeding through conventional methods is a laborious and time-consuming process with a low success rate. Contemporary approaches such as genetic transformation are quite promising, but the progress is hampered by several restrictions. Any alternate method to incorporate disease resistance in this important crop, with minimal or no damage to its 'clonal integrity', will be highly rewarding. *In vitro* selection is one such alternate method that can help develop disease resistant grapevine. Though *in vitro* selection is not a new approach to develop disease resistance, our understanding of the process at the molecular level has greatly improved in the past decade. Experience in the past six years with grapevine has lead to interesting results such as a) resistance induced by in vitro selection at the cellular level is retained in the plants regenerated from such cells, b) such induced resistance is broad-spectrum, and c) resistance appears to be mediated by constitutive induction of certain PR-proteins belonging to PR-1, PR-3 and PR-5 groups and a lipid transfer protein (LTP). The details of selection process and how it feeds into functional proteomics by tapping into the innate immunity gene cascade will be discussed.

P-7

Gene Flow Control in Trees: Technology Development in Transgenic Poplars. S. H. STRAUSS, A. Brunner, R. Meilan, and V. Busov. Department of Forest Science, Oregon State University, Corvallis, OR 97331-5752. E-mail: Steve.Strauss@orst.edu

For all crops, domestication is incomplete. Wild or feral relatives are present with which they can interbreed in the proximity of cropping areas. This is particularly true for trees, which have undergone very few generations of selection and can spread pollen and/or seeds widely. For some kinds of transgenes, such as those for herbicide resistance and exogenous forms of pest resistance, means to greatly reduce, or possibly prevent, the spread of transgenic organisms or introgressed transgenes are highly desirable. This would avoid the exacerbation of control difficulties in the case of herbicide resistant crops, prevent the spread of genes whose products could impart new non-target organism toxicities in wild populations, or that might threaten the viability of small endangered populations. Because many kinds of transgenic trees will be vegetatively propagated, insertion of strong, redundant male and female sterility transgenes, and/or fitness reduction genes, are far simpler than the complex systems such as "terminator" required to mitigate gene flow in seed propagated crops. We describe our efforts to mitigate gene flow in transgenic poplars using several transgenic mechanisms. These include floral tissue ablation, and RNAi and dominant negative protein suppression of floral homeotic gene function. We also describe activation tagging of a dominant dwarfing gene that affects gibberellin metabolism; testing of Arabidopsis dwarfism genes in poplar; and the possibilities of using the poplar genome sequence to develop novel transgenic approaches to mitigation of transgene dispersal. The key obstacles to developing effective sterility systems for trees are the extremely limited industry or grant support for the needed applied biotechnology studies, especially the production of transgenic trees and their long-term monitoring.

P-8

The *Populus* Genome: Development of the Information Resource. G. A. TUSKAN[1], S. DiFazio[1], S. Wullschleger[1], K. Ritland[2], J. Bohlmann[2], C. Douglas[2], B. Ellis[2], M. Marra[2], J. Chapman[3], P. Richardson[3], and Dan Rokhsar[3]. [1]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, [2]Genome Canada–University of British Columbia, Vancouver, BC, CANADA, and [3]Joint Genome Institute, Walnut Creek, CA. E-mail: tuskanga@ornl.gov

Forest trees are the dominant life form in many ecosystems. They provide structural and functional habitat for two-thirds of the Earth's terrestrial species and contain greater than 90% of all terrestrial biomass. Among biologically derived materials, wood-based products are second only to maize in their monetary contribution to the U.S. economy. Moreover, approximately 25% by volume of all industrial materials are derived from forest-based resources. The genus *Populus*, including cottonwoods, aspens, and their many hybrids, is recognized as the model forest tree species due to its ease of propagation, relatively short reproductive cycle [1-12 yr.], small genome size [550 Mb], extensive known pedigrees, developed genetic resources, and transformability. The poplar genome is being sequenced using a whole-genome shotgun method by the U.S. Department of Energy's (DOE) Joint Genome Institute. To date approximately 3x shotgun coverage of the genome has been generated; primarily as paired end-sequences from random 3 kb insert plasmid libraries constructed from a single heterozygous female genotype, 'Nisqually-1' (http://genome.jgi-psf.org/poplar0/poplar0.home.html). The anticipated 6x sequence database should be completed by time this presentation is being made. The final 6x draft will also be derived from a random shotgun approach though using 3 kb, 8 kb and 40 kb libraries. The basal assembly will be created by JAZZ via aligning end read overlaps among the various libraries, BAC end sequences, and the BAC fingerprint tiling path. Currently, the tiling path, at 1x e^{-4} Sulston score, contains ca. 7000 clones with an average size of 118 kb. It is anticipated that the basal assembly will yield between 2000 and 4000 scaffolds each containing multiple contigs [0.5 to 100 kb each]. The current estimate of nucleotide polymorphisms is 1.5 substitutions per 100 bases, slightly higher than in humans. Based sequence assembly there is also no evidence at this point for genome wide duplication. In addition to the nuclear genome data, the complete chloroplast genome of *Populus* has been sequenced, assembled and annotated. This relatively small genome, 157 kb, contains approximately 118 genes (http://genome-devel.ornl.gov/microbial/poplar/).

P-9

Genetic Engineering of Wood Formation in Forest Trees. V. L. CHIANG, L. Li, Y. Zhou, X. Cheng, J. Sun, J. M. Marita, and J. Ralph. Forest Biotechnology Group, College of Natural Resources, Department of Forestry, North Carolina State University, Raleigh, NC 27695. E-mail: vincent_chiang@ncsu.edu

One of the major uses of forest trees is the production of woodpulp. However, lignin quantity and reactivity are two major barriers to woodpulp production. To verify our contention that these traits are regulated by distinct monolignol biosynthesis genes, encoding 4-coumarate:coenzyme A ligase (4CL) and coniferaldehyde 5-hydroxylase (CAld5H), we used *Agrobacterium* to co-transfer antisense *4CL* and sense *CAld5H* genes into aspen (*Populus tremuloides*). Trees expressing each one and both of the transgenes were produced with high efficiency. Lignin reduction by as much as 40% with 14% cellulose augmentation was achieved in antisense *4CL* plants; S/G (lignin syringyl:guaiacyl constituent ratio) increases as much as 3-fold were observed without lignin quantity change in sense *CAld5H* plants. Consistent with our contention, these effects were independent but additive, with plants expressing both transgenes having up to 52% less lignin, 64% higher S/G ratio and 30% more cellulose. S/G increase also accelerated cell maturation in stem secondary xylem, pointing to a role for syringyl lignin moieties in coordinating xylem secondary wall biosynthesis. The results suggest that this multigene co-transfer system should be broadly useful for plant genetic engineering and functional genomics.

P-10

Persistent Abscisic Acid Analogs. S. R. ABRAMS, L. I. Zaharia, A. J. Cutler, D. C. Taylor, A. Jadhav, and M. K. Walker-Simmons. Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, SK S7N 0W9, Canada; Agriculture Research Service, USDA, Washington State University, Pullman, WA 99164-6420. E-mail: sue.abrams@nrc-cnrc.gc.ca

Abscisic acid (ABA) is involved in numerous developmental processes in seeds from embryogenesis through to germination. For example, ABA prevents precocious germination and regulates synthesis of storage proteins and lipids in developing seeds. In addition, ABA is involved in the development of desiccation tolerance and induction and maintenance of dormancy. We have developed ABA analogs that are more slowly metabolized by plant enzymes than the natural hormone, while conferring ABA-like physiological effects over a longer time [Abrams et al (1997) Plant Physiology 114: 89-97]. The analogs are useful as tools for studying regulation of ABA-inducible genes and as growth regulators for manipulating embryo ontogeny in tissue culture. Examples will be presented showing the effects of ABA and long-lasting analogs on regulation of genes involved in storage protein accumulation and fatty acid synthesis and modification in microspore-derived embryos of Brassica napus, in growth of maize suspension cultured cells and in germination and gene expression in wheat embryos. In microsomes from maize cells, some of the persistent ABA analogs are irreversible inhibitors of ABA 8¢-hydroxylase, a key enzyme in the catabolism of natural ABA [Cutler et al (2000) Biochemistry 39: 13614-13624]. Studies on the metabolism of ABA and analogs in the maize cells will be discussed.

P-11

Gibberellins, Their Effects on and Roles in Growth and Differentiation of *In Vitro* Cultured Plant Tissues, Organs, and Somatic Embryos. R. P. PHARIS[1], R. Zhang[1], A. B. Pereira Netto[2], B. McCown[3], S. Welsh[4], and S. Jackson[4]. [1]Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4, Canada; [2]Department of Botany-SCB, Centro Politecnico, C.P. 19031, 81531-970 Curitiba-PR, Brazil; [3]Department of Horticulture, Univeristy of Wisconsin, Madison, WI 53706; and [4]Plant and Microbial Sciences Dept., University of Canterbury, Christchurch, New Zealand. E-mail: rpharis@ucalgary.ca

The gibberellins (GAs) are a potent group of naturally occurring plant growth hormones that are causal for a wide range of growth and differentiation processes in higher plants. GAs also occur in *in vitro*-cultured plant shoots, organs, cells and somatic and microspore-derived embryos. The *in vitro* growth and development of these tissues can be modified by applied GAs. Changes in concentration of naturally occurring GAs are presumed to influence a wide range of developmental processes under *in vitro* conditions. Using examples from embryo and cell cultures, and explants of vegetative shoots, induced floral apices and pith and cambial region tissues, we will examine the likely roles of GAs in a range of developmental processes under *in vitro* conditions. This will include xylem differentiation, tracheid growth, microfibril angle, shoot elongation, floral apex development and (somatic) embryogenesis. Additionally, the usefulness of GAs of several structural types as "working tools " for *in vitro* cultures will be discussed. Finally, pitfalls posed by *in vitro* culture conditions, especially where high sugar concentrations are utilized, will be discussed relative to effects on the metabolism and catabolism of both endogenous and applied GAs.

P-12

Ethylene Based Opportunities in Horticultural Biotechnology. FRANZINE D. SMITH[1], Jyothi Bolar[1], Jeff Lowe[1], Holly Loucas[2], David Clark[2], and Robert Harriman[1]. [1]The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041 and [2]University of Florida, Environmental Horticulture Department, Gainesville, FL 32611-0670. E-mail: franzine.smith@scotts.com

Ethylene is one of the most intensely studied plant hormones, and affects several aspects of plant growth and development. Many of the ethylene-related processes are highly significant to horticulture. Processes affected by ethylene include seed germination, stem and root elongation, flower initiation, senescence of leaves and flowers, organ abscission and fruit ripening. The ethylene biosynthetic pathway has been very well characterized. Genetic manipulation of key enzymes in the biosynthetic pathway has resulted in significant extension of postharvest storage life of several perishable crops. However, these transgenic flowers/fruits remain capable of sensing and responding to exogenous sources of ethylene. During the past decade, considerable progress has been made in the applications of molecular genetic approaches to better understand the mechanisms of ethylene perception and signal transduction. The Scotts Company is developing ornamental plants with flowers that last longer by using key technologies in the ethylene perception pathway. By slowing the "senescence" in the flowers, we have produced desirable ornamental plants with more bloom and hence more color.

P-13

Molecular Farming: Current Products and Future Prospects. MICHAEL E. HORN. ProdiGene, 101 Gateway Blvd. Suite 100, College Station, TX 77845. E-mail:mhorn@prodigene.com

The molecular farming industry is a relatively new, rather diverse group of research organizations with the aim of producing proteins of economic value in plants or animals. While the path to commercialization would first appear to be a simple one, the reality has been quite the opposite. In regard to a plant-based system, the decisions of which plant species to use, the location of the plantings, the method of confinement, transportation of the raw material, and processing of the final product must all be taken into consideration. The target product(s) must be of high enough value and a large enough market to justify the investment. Initial products such as trypsin have met these challenges and are selling well. Several potential products are currently in clinical trials and many other proteins are in the pipeline. This presentation will give an overview of the molecular farming industry with special focus on current and future products from plant-based systems.

P-14

Improving Nutraceuticals Through Tissue Culture. A. M. R. FERRIE, W. A. Keller, and C. E. Palmer. Plant Biotechnology Institute, National Research Council, Saskatoon, SK, S7N 0W9, Canada. E-mail: Alison.Ferrie@nrc-cnrc.gc.ca

The herb/spice and nutraceutical markets are growing industries, which offers producers new opportunities and markets. At the present time, the world consumption of nutraceuticals is about $15–$20 billion and is projected to grow to $500 billion by 2010. There is very little activity underway to improve the genetics of herbs, spices and other nutraceutical crops. Much of the industry relies on "wild" plants (i.e. not genetically improved/enhanced), therefore, the potential for variability in crop performance and active ingredients is high. This presents significant challenges for the industry, which is striving to achieve national and international market credibility and meet current regulatory standards. Uniform varieties would also be beneficial for use in clinical trials. The industry requires plants that will perform consistently under various environmental conditions and will produce a consistent quality and quantity of desired active ingredients. This research cannot solely rely on traditional plant breeding but must be supported by the development of tissue culture methods for the species of interest. Tissue culture is also valuable for propagation of species endangered through over collection or where seed production is low and dormancy intractable. Haploidy technology can accelerate the breeding of new varieties of these species. The time required to develop a new variety is decreased by three to four years when using this technology. This is beneficial to the producer, as high yielding cultivars with improved characteristics can be available more readily. Having a product that is consistently of high quality is also beneficial to the consumer. Haploidy technology has been developed for a number of species (e.g. *Brassica* sp., wheat, barley) but very little work has been done on the herbs, spices, and nutraceutical species. This presentation will discuss the tissue culture methods that can be used to improve herbs, spices and nutraceuticals.

P-15

The XA21 Receptor Kinase Mediated Defense Response in Rice. PAMELA C. RONALD[1], Chris Dardick[1], LiYa Pi[2], Andy Howden[1], Yi-Ren Chen[1], Todd Richter[1], WenYuan Song[2], M. Gribskov[3], and M. Fromm[4]. [1]Department of Plant Pathology, University of California-Davis, 1 Shields Ave., Davis, CA 95616; [2]Department of Plant Pathology, University of Florida, FL; [3]University of Nebraska, Lincoln, NE; and [4]University of California - San Diego, San Diego, CA. E-mail: pcronald@ucdavis.edu

Perception of extracellular signals by cell surface receptors is of central importance to eukaryotic development and immunity. Many of these receptors possess intrinsic protein kinase activity in their cytoplasmic domains (RKs) and regulate transcription of target genes through phosphorylation events. The rice Xa21 gene, encodes an RK with leucine rich repeats (LRRs) in the extracellular domain and is a key recognition and signaling determinant in the innate immune response, a pathogen defense pathway widely conserved between plants and animals. Yeast two hybrid (Y2H) screens identified at least several XA21 binding proteins (Xbs). These include Xb10, encoding a putative transcriptional regulator; and Xb15 encoding a PP2c phosphatase-like protein. Preliminary characterization of these genes suggest that they play a key role in transducing the XA21-mediated defense response. The availability of a draft sequence of the rice genome provides an opportunity for genome wide analysis of the protein kinase gene super family. We estimate that rice contains ca. 1600 protein kinases that can be classified into ~300 subfamilies. We are using the Y2H and TAP tagging to obtain protein interaction data for 275 protein kinases. This information, together the analysis of selected knockout mutants, will facilitate the interpretation of phosphorylation-mediated signaling pathways in cereals.

P-16

Lipid Signaling in Plant Defense. J. SHAH, A. Nandi, V. Pegadaraju, and R. Welti. Division of Biology and the Molecular, Cellular and Developmental Biology Program, Kansas State University, Manhattan, KS 66506. E-mail: Shah@ksu.edu

Lipids are important structural components of biological membranes. In addition, lipid derived second messengers participate in signal transduction mechanisms influencing plant growth, development and response to abiotic and biotic stresses. Polyunsaturated fatty acids (PUFA) released from membrane lipids are precursors for the synthesis of oxidized lipids (oxylipins), some of which are potent signal molecules. For example, jasmonic acid (JA), which is synthesized from linolenic acid (18:3) by the octadecanoid pathway, participates in plant development and stress responses. In addition, a parallel hexadecanoid pathway in *Arabidopsis thaliana* synthesizes other oxylipins from hexadecatrienoic acid (16:3). Mutations in predicted lipases encoded by the Arabidopsis *EDS1* and *PAD4* genes alter plant defense response to pathogens. In addition, our studies have shown that *PAD4* is also involved in plant defense to the phloem-feeding green peach aphid. Likewise, alterations in the activity of fatty acid/lipid biosynthesis enzymes also affect plant defense to pathogens and insects. Loss of a stearoyl-ACP desaturase activity encoded by the Arabidopsis *SSI2* (*FAB2*) gene confers enhanced resistance to the bacterial pathogen *Pseudomonas syringae*, the oomycete pathogen *Peronospora parasitica* and green peach aphid. In contrast, resistance to the necrotrophic fungal pathogen, *Botrytis cinerea*, is depressed in the *ssi2* mutant plant. In addition, loss of SSI2 activity also alters plant growth and development; the *ssi2* mutant plant is stunted and spontaneously develops lesions containing dead cells. The *ssi2* mutant is blocked in the desaturation of stearic acid (18:0) to oleic acid (18:1), resulting in the increased accumulation of 18:0 and lowered levels of 18:1. Second site mutations that suppress the *ssi2* phenotypes have been identified. Characterization of four complementation groups of suppressor mutants (*sfd*) indicates that the high level of 18:0 does not have a causal role in the *ssi2* phenotypes. Instead, all four groups of suppressor mutations have altered levels/distribution of hexadecatrienoic acid (16:3). ESI-MS/MS-based lipid profiling is underway to study the impact of the *ssi2* and *sfd* mutations on membrane lipid composition and to decipher the contribution of these changes to plant defense responses.

P-17

The Interface Between Bacterial Pathogens and Plants: Virulence Functions and Resistance Responses. D. MACKEY. Department of Horticulture and Crop Science, Ohio State University, Columbus, OH 43210. E-mail: mackey.86@osu.edu

Pathogens commonly secrete molecules that manipulate the host. *Pseudomonas syringae* secretes effector proteins directly into the cells of plants where they perturb signaling processes. These perturbations can enhance virulence of the bacteria. Alternatively, the innate immune system of the plant can be activated, causing induced resistance. Defense activation occurs if an effector is recognized by a resistance (R) protein of the plant. Multiple bacterial effectors target the Arabidopsis RIN4 protein. We hypothesize that these effectors enhance bacterial virulence by signaling through RIN4. Function of the R-proteins that recognize these effectors also requires RIN4. We hypothesize these R-proteins recognize perturbations produced by the virulence signaling of the effectors. Thus, depending on the status of a particular R-protein, signaling induced by a bacterial effector protein can cause enhanced virulence or induced resistance. The molecular mechanisms leading to these disparate outcomes will be discussed.

P-17A

Gene Identification in Wheat. CAMILLE M. STEBER, Janice M. Zale, and Lucia C. Strader. USDA-ARS, Washington State University, Pullman, WA 99164-6420. E-mail: csteber@yahoo.com

We are using chemical and transposon mutagenesis to identify genes in wheat that control seed dormancy and adult drought tolerance. Seed dormancy and drought tolerance are regulated by the plant hormone ABA. ABA induces seed dormancy during embryo maturation, and induces stomatal closure in response to drought stress. We have identified EMS-induced wheat mutants with both increased and decreased sensitivity to the plant hormone ABA. We will also eventually screen for ABA mutants generated by transposon mutagenesis. Transposon-based activation tagging is in many ways ideal for gene identification and cloning in wheat (*Triticum aestivum* L.). Identification of loss-of-function mutants and map-based cloning are complicated in allohexaploid wheat because of the large size (16,000 Mbp/1C) and high redundancy of the genome. We are developing an Ac/Ds-based tagging system for wheat that will enrich for gain-of-function mutants. As part of this project we tested the strength of various Ac transposase constructs in their ability to induce Ds transposition in callus. Ac constructs were cobombarded with a Ds/Lc excision marker and Ds transposition frequency was determined.

P-18

Risk Assessment of RoundUp® Ready Wheat Production in the Pacific Northwest. KIMBERLEE K. KIDWELL. Dept. of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420. E-mail: kidwell@mail.wsu.edu

Weed control is a primary concern for wheat producers in the Pacific Northwest (PNW) due to yield and crop quality losses associated with weed competition. The ability to control weeds through the use of herbicide resistant varieties is appealing to many producers, particularly for direct seed wheat production. RoundUp Ready® wheat will permit "in crop" weed control while maintaining the intrinsic environmental and economic benefits associated with direct seed management. The first adapted spring wheat varieties suitable for commercial production are projected to be available in the region in 2005. In spite of their imminent availability, questions concerning whether RoundUp Ready® wheat production will become a reality in the PNW have arisen. The effectiveness of the RoundUp Ready® system as a weed control measure is not in question. However, the asexual gene introgression technique that was originally used to introduce the herbicide resistance gene into wheat is "unnatural", which has generated concerns, primarily about food safety and the possible escape of transgenes into the environment. Controversies such as these lead to bans on the importation of transgenic crops into Europe and several Asian countries. Since more than 80% of the wheat produced in the PNW is exported to Pacific Rim countries, consumer acceptance, or lack thereof, is likely to determine the fate of RoundUp Ready® wheat production in the region. Inability to segregate transgenic from non-transgenic wheat may prohibit RoundUp Ready® wheat production if export customers are unwilling to buy transgenic crops. Furthermore, risks associated with incorporating Round-Up Ready® wheat into production systems have not been investigated to date. Agroecological changes resulting from over the top herbicide application may impact the productivity of RoundUp Ready® wheat. Grain yield advantages resulting from improved weed control may be lost due to increased soilborne pathogen activity on dying weeds within a RoundUp Ready® wheat crop. A study to proactively determine the risks of incorporating RoundUp Ready® wheat into direct seed production systems using near isogenic lines, with and without RoundUp resistance, was recently initiated. Preliminary results will be presented, and implications on the future of RoundUp Ready® wheat production in the PNW will be discussed.

P-19

Transgenic Plant-produced Cellulases for Biomass Conversion. E. E. HOOD. Plant Biotechnology Consulting, College Station, TX 77845. E-mail: EHA105@aol.com

Transgenic crops are ideal production systems for recombinant industrial enzymes. Seed is an efficient system that allows protein to be stably stored for months to years without loss of enzyme activity. For industrial enzymes, high levels of expression are required for commercial viability and consequently drive the technology to achieve high expression. The technology encompasses utilizing promoters with high functionality, targeting the protein to tissues and subcellular locations that promote efficient accumulation, and breeding the transgenic line into elite germplasm for selection of higher-expressing lines. Preparation of parity yielding agronomic material is a key piece of cost-effective production. By-product credits can be obtained for materials remaining after enzyme removal. Regulatory paths are a critical piece of the overall plan, but for industrial enzymes these will differ dramatically from requirements for pharmaceutical products. Industrial enzymes are large-volume, large acreage products with medium-range margins for which field production is the major cost. These principles will be illustrated with data for the industrial enzyme, laccase. Progress on the production of cellulases in transgenic maize will be presented. Independent transgenic events have been isolated for three genes, two cellulases and a β-D-glucosidase. Plants from these events are being cultivated in the greenhouse. Assays of first generation seed will begin in 2003. The seed-based expression of enzymes can be coupled with stover harvest and treatment from the same field to achieve cost-effective production.

P-20

The Impact of Feedstock Composition on Biomass Conversion Process Economics. S. R. THOMAS, M. F. Ruth, and B. R. Hames. National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401. E-mail: steven_thomas@nrel.gov

Corn stover (stalks+leaves+cobs) is currently employed in the DOE-funded Biofuels Program as a model feedstock for integration of enzymatic cellulose hydrolysis-based biomass conversion technology. The chemical composition of corn stover is quite variable from lot to lot, and there are also indications that different lots of stover exhibit differential reactivity to dilute acid pretreatment, as practiced at NREL. These observations are also likely to apply to other herbaceous feedstocks. Biomass composition and quality have therefore emerged as important issues in the development of integrated conversion technology. Since input feedstock chemical composition directly influences process yield, process economics can be dramatically affected by changes in feedstock quality. Our goal is to determine the range of compositional variation as well as the potential causes of variability in mature corn stover. Over 1000 specimens from the 2001 North American crop were obtained from a wide variety of sources and include a fairly broad cross-section of the maize germplasm, including commercial hybrids, inbreds, open-pollinated varieties, foreign accessions, primitive landraces, and related species. We report here on the statistical analysis of the composition of over 700 stover specimens measured using a calibrated near-infrared spectroscopic method developed at NREL. Results show that corn stover composition varies over a surprisingly wide range.

P-21

Economics and Opportunities for Improvements for Biological Conversion of Cellulosic Biomass to Ethanol. C. E. WYMAN. Thayer School of Engineering, Dartmouth College, 8000 Cummings Hall, Hanover, NH 03755. E-mail: Charles.Wyman@Dartmouth.edu

Biomass provides a unique feedstock for sustainable production of organic fuels and chemicals, and ethanol and other fuels made from cellulosic materials such as agricultural and forestry residues, major portions of municipal solid waste, and dedicated woody and herbaceous crops offer significant environmental, economic, and strategic benefits. Furthermore, quantities of cellulosic materials are potentially sufficient to meet the demand for transportation fuels, and the price of most cellulosics is competitive with petroleum. The primary challenge is to achieve low processing costs, and biological conversion can take advantage of the powerful tools of modern biotechnology to dramatically advance key processing steps. A pretreatment step is first applied to make the cellulose fraction accessible, and cellulase enzymes are then added to hydrolyze cellulose to glucose with the high yields vital to economic success. The five sugars in lignocellulosics - arabinose, galactose, glucose, mannose, and xylose - can be fermented efficiently to ethanol and other products as a result of developments in genetics. Over the last 20 years, these and other technical advances have dropped the cost of making cellulosic ethanol from more than $4.00/gallon to only about $1.20/gallon for biomass costing $42 per ton. At this cost, ethanol is competitive for blending with gasoline, and several companies are working to build the first commercial cellulosic ethanol plants. Although these initial facilities will be relatively small to capitalize on niche opportunities and manage risk, economies of scale appear to favor larger plants even though delivered biomass costs increase with demand, and coproduction of ethanol, chemicals, and electric power can provide important synergies. Advances in overcoming the recalcitrance of cellulosics are still needed to achieve competitive costs without subsidies, and strategic opportunities have been defined to reduce the cost of cellulosic ethanol sufficiently to become a low-cost pure liquid transportation fuel. In particular, pretreatment and the combined biological steps of cellulase production, cellulose hydrolysis, and sugar fermentations are the most expensive operations in the overall process, and advanced organisms and processing technologies can dramatically lower these costs. In addition, higher productivity cellulosic plants could reduce land use and drop feedstock transportation costs, and changes in plant composition could improve product yields.

P-22

Transgenic Turfgrasses and Issues with Gene Flow. E. K. NELSON[1], L. Lee[1], R. Harriman[1], K. Turner[1], and P. Christoffer[2]. [1]The Scotts Company, Marysville, OH, 43041 and [2]Washington State University, Pullman, WA. E-mail: eric.nelson@scotts.com

Gene flow can occur through seed movement, vegetative propagation or pollination. Potentials for intra-specific and inter-specific hybridization between grasses through gene flow has been recognized for decades. Isolation distance, border row removal and other cultural techniques have been effectively used to meet standards for production of identity-preserved seed of improved cultivars through oversight by state certification programs for over 40 years. Tolerance thresholds for off-types occurring in production fields and seed lots have been published and accepted by the seed industry. Cultivar developers and seed growers desiring to produce improved identity-preserved grass seed products have handled the responsibility of protecting their own crops using these proven means to limit adventitious presence of other crop, weeds and off-types in order to meet certification standards. Isolation methods and standards for certified seed production were developed from the results of pollen flow experiments employing traditional marker genes. Biotechnology has developed tools to readily examine intra-specific and inter-specific hybridization and further evaluate field isolation methods. Results from a recent pollen flow experiment that utilized herbicide tolerant creeping bentgrass (*Agrostis stolonifera*) confirmed the documented low frequency of hybridization with related bentgrass species and the efficacy of distance for identity preservation in bentgrass cultivars.

P-23

Gene Flow from Transgenic Crops to Wild Relatives—When Is It a Problem? A. A. SNOW. Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH 43210. E-mail: snow.1@osu.edu

Gene flow between crops and wild plants is often cited as a biosafety issue for transgenic crops. Gene flow occurs when pollen moves from a crop to a wild relative, or vice versa, and genes from their offspring spread *via* the dispersal of pollen and seeds. Crops such as canola, rice, wheat, sugar beet, sorghum, sunflower, squash, lettuce, and radish have sexually-compatible weedy relatives in the USA. Crops and weeds often exchange genes, but transgenes are potentially more diverse and more effective than genes used in conventional breeding. I will describe studies showing that crop genes can persist for many generations in weed populations, even when first-generation crop-wild hybrids have lower fitness than their wild counterparts. Using sunflower (*Helianthus annuus*) as a case study, I will show that a Bt transgene can be highly beneficial to wild sunflower populations. In the short term, the spread of transgenic herbicide resistance via gene flow may create logistical and/or economic problems for growers. Over the longer term, certain weeds are likely to benefit from transgenes that confer resistance to ecological factors such as herbivores, diseases, or harsh growing conditions.

P-24

Liability Issues Related to Transgene Flow in Agriculture. DREW L. KERSHEN. University of Oklahoma, College of Law, Norman, OK 73019-5081. E-mail: dkershen@ou.edu

An overview of the legal liability issues in torts and patent law that arise from the use of transgenic crops produced by agricultural biotechnology. Most emphasis is on tort liability claims for damage to property, persons, or economic interests (markets) from pollen flow. Tort theories discussed include trespass, strict liability, negligence, private nuisance, and public nuisance. Less emphasis on patent infringement cases in Canada and the United States involving farmers who used patented seed from agricultural biotechnology without the permission of the patent holder.

P-25

Inhibition of Plant Gene Expression Technologies for Fun and Profit. GREGORY P. POGUE, John A. Lindbo, and David Baulcombe*; Large Scale Biology Corporation, 3333 Vaca Valley Pkwy, Vacaville, CA 95688 and *The Sainsbury Laboratory, Norwich, UK. E-mail: greg.pogue@lsbc.com

A biological response to double-stranded RNA mediating resistance to pathogens and regulating the expression of proteins, known as RNA interference (RNAi) or post-transcriptional gene silencing, is conserved across many phyla, ranging from plants, non-segmented worms, insects and mammals. In plants, RNAi is implicated in the control of viral pathogens, control of certain developmental gene expression patterns, translational control and control of transposon activities. The study of plant and plant-pathogen systems has proven central to the discovery and understanding of the biology of RNAi since plant viruses can be both powerful elicitors and encode suppressors of RNAi. These viral systems provide biologists with key insight into the biology and regulation of RNAi in plants. Researchers have exploited the ability of plant viruses to elicit or suppress RNAi responses in order to either ablate target gene expression or enhance gene product accumulation. The manners in which these tools are being applied to biotechnology and plant functional genomics will be discussed.

P-26

Use of Transiently Expressed RNAi to Dissect Signaling Pathways in Plants. T. H. D. HO, R. Zentella, and J. Casaretto. Department of Biology, Washington University, St. Louis, MO 63130. E-mail: ho@biology.wustl.edu

Double-stranded RNAs (dsRNAs) can be used to silence the expression of target genes in a variety of organisms and cell types (e.g., worms, fruit flies, and plants). We have adopted this technique in a transient expression manner to study hormone signaling pathways in the aleurone cells of cereal grains. Specific RNAi molecules are generated in these cells via the biolistic introduction of transgenes containing inverted repeats of target sequences. It is shown that the gene silencing effect reaches a maximum within 4 hr of introducing the RNAi generating transgene, and one copy of the RNAi-generating transgene is capable of knocking down the expression of more than 10 copies of the target gene. In the cereal aleurone tissue, the phytohormone gibberellin (GA) induces, and another hormone abscisic acid (ABA) suppresses, the expression of alpha-amylases that are essential for the utilization of starch stored in the endosperm. Following the RNAi-mediated gene silencing approach, it is demonstrated that the transcription factor, GAMyb, is not only sufficient, but also necessary for the GA-induction of alpha-amylase. Another regulatory protein, SLN1, is shown to be a repressor of GA action, and knocking down the synthesis of SLN1 by RNAi leads to derepression of alpha-amylase expression even in the absence of GA. However, this effect is still suppressed by ABA. Although the ABA-induced serine/threonine protein kinase, PKABA1, is known to suppress the GA-induced alpha-amylase expression, PKABA1 RNAi does not hamper the inhibitory effect of ABA on the expression of alpha-amylase, indicating that a PKABA1-independent signaling pathway may also exist. We have also demonstrated that ABA induction and suppression of gene expression follow two distinct signaling pathways. Based on these promising results, we suggest that the generation of specific RNAi in a transient expression approach is a useful technique in elucidating the role of regulatory molecules in biological systems in which conventional mutational studies cannot be easily carried out.

P-27

Plant MicroRNAs and Their Targets. JAMES CARRINGTON. Oregon State University, Center for Gene Research and Biotechnology, 3021 ALS Building, Oregon State University, Corvallis, OR 97331-7303. E-mail: carringj@science.oregonstate.edu

Most eukaryotes possess a set RNA-based regulatory pathways to suppress invasive nucleic acids, guide modification of chromatin, and regulate gene expression at the transcriptional and posttranscriptional levels. The microRNAs (miRNAs, ~21 nucleotides) are formed by processing of precursor RNAs that adopt a fold-back structure, and function to inhibit expression of developmentally relevant genes. In plants, miRNAs function like RNAi-associated siRNAs to guide cleavage of target mRNAs. The target mRNAs belong to two functional classes. The largest class encodes a variety of transcription factors that control meristem identity, cell division, and cellular patterning of vegetative and reproductive organs. A smaller class encodes factors required for miRNA formation or activity, indicating that the miRNA pathway is under negative regulation through a feedback loop. Interestingly, regulation by miRNAs was also found to be strongly suppressed by viruses. Specifically, miRNA-guided cleavage functions are inhibited by virus-encoded RNA silencing suppressors. This explains why many plant viruses cause diseases that resemble developmental defects.

P-28

Somatic Embryo Development in Liquid Medium for Large-scale Propagation of Conifer Trees. P. K. GUPTA, R. Timmis, and W. Carlson. Weyerhaeuser Technology Center, G30 PO Box 9777, Federal Way, WA 89063. E-mail: pramod.gupta@weyerhaeuser.com

Micropropagation via somatic embryogenesis is currently applied to a large number of horticultural and forestry species. Many papers have been published and patents granted on somatic embryo development, maturation and germination for several conifer species. However, plantlet production via somatic embryogenesis is still expensive. Labor costs are said to account for 60% or more of the total production costs. Most of the somatic embryogenesis technology has been developed using semi-solid media. There are few reports on somatic embryo development and maturation from liquid medium (Gupta et al. 2000). Liquid culture technology saves cost in almost every component of expense, such as labor, time, space, media cost and material. Quality of the products is also improved in liquid medium compared to semi-solid medium. At Weyerhaeuser, somatic embryo production of Douglas-fir in liquid medium and manufactured seed delivery have been developed to reduce the labor costs and increase the efficiency for large-scale clonal production of conifers for reforestation. Embryonal suspensor masses (ESMs) of Douglas-fir have been scaled-up in 1L flasks and also in bioreactors. Over 300,000 somatic embryos have been produced from a large number of genotypes from several families for clonal field tests. Implementation of this technology for large-scale production will be discussed in this talk.

Gupta PK, Timmis R, Carlson W, Timmis K, Grob J, Welty E and Carpenter C. (2000). Advances in conifer tree improvement through somatic embryogenesis. In Challenges of plant and agriculture sciences to the crisis of biosphere on earth in the 21st century. Watanabe K & Komamine A (eds). Landes Bioscience publ. p. 303.

P-29

Liquid Systems for Micropropagation, Storage, Shipping and Acclimatization. J. W. ADELBERG. Department of Horticulture, Clemson University, Clemson, SC 29634. E-mail: jadlbrg@clemson.edu

Liquid media systems were designed to transfer solutes to stationary tissues, while shoots developed in gaseous headspace. Enhanced plant growth was achieved using high ratios of plant material/media volume on large, rectangular growth areas with efficient space utilization and energy transfer across the vessel. Two examples are described: 1) the thin film rocker - for micropropagation in sugar-containing media, and 2) the Acclimatron(TM) - for photoautotrophic acclimatization to bright, sunlit environments. Compared to agar, thin-film micropropagation on the rocker for several herbaceous crops (*Acorus, Colocasia, Hosta and Zingiber*) displayed higher rates of multiplication and greater uptake of sugar from media. With hosta on the rocker, more and larger plants were grown in less space on the culture bench with greater labor efficiency than agar. Sugar uptake was linearly related media sugar levels (1-7%) which resulted in larger shoots and roots, more plantlets, better viability in cold storage and larger plants following storage. Ancymidol reduced plant size, extended the multiplication phase, and increased plant density to a time when lack of sugar affected plant quality. Upon transfer to the Acclimatron(TM), dense two-dimensional arrays of shoot cultures grew in sunlight under temperature, humidity and biotic control. In the Acclimatron(TM) chamber, hosta had shoot and root biomass double approximately every 10-days under CO_2-enriched conditions. Light saturation of net photosynthetic rate (NPR) did not occur in the range of 100 to 1200 μM m^{-2} s^{-1} PPFD. Interaction with CO_2-enrichment at optimal levels (2800 μmol mol^{-1}) showed a quadratic increase of NPR with light intensity. Compact, acclimatized plug transplants were well suited for field planting. Both culture systems supported development of propagules further along the value chain, with fewer discrete interventions than conventional micropropagation.

P-30

Alternative Media for the Micropropagation of Plants. K. C. TORRES, L. Williams, N. Philman, and M. Kane. PhytoTechnology Laboratories, 7895 Mastin Drive, Overland Park, KS 66204. E-mail: info@phytotechlab.com

The use of Murashige and Skoog based nutritive culture media has been well documented over the past 30 to 40 years. Since the introduction of the Murashige and Skoog Medium in 1962, only a limited number of unique plant medium formulations have been introduced. Additionally, typical inorganic based medium such as Murashige and Skoog may not be suitable for the micropropagation of fruit and vegetable crops earmarked for the organic food industry. A total of fifteen organic media formulations were designed and tested for this purpose. This work concentrated on establishing and multiplying, fruit, vegetable and ornamental crops on the various media. Comparisons were made between the organic formulations and "optimized " inorganic formulations that had been cited in references or utilized in the industry for each crop. Shoot number and fresh weights of the cultures were determined for two to three generations. Results for this study will be presented and discussed.

VT-1

Gap Junctions, Homeostasis, and Epigenetic Toxicology. R. J. RUCH. Department of Pathology, Medical College of Ohio, Toledo, OH 43614. E-mail: rruch@mco.edu

Tissue homeostasis is the maintenance of cell number and differentiation and the coordination, and adaptation of cellular activities to new stimuli. This is highly dependent upon signaling or "communication " between and within cells mediated by secreted factors like hormones, growth factors, and cytokines (extracellular communication), second messengers and signal transduction cascades (intracellular communication), and across cell contacts and junctions (intercellular communication). Cellular communication is essential for tissue homeostasis and defects contribute to most, if not all, diseases. Gap junctions are aggregated channels that link the interiors of adjacent cells and permit the cell-to-cell diffusion of low molecular weight (<1 kDa) ions and molecules. This process, known as gap junctional intercellular communication (GJIC), helps maintain cellular ion, metabolite, and water balance (cellular homeostasis), and also regulates cell proliferation, apoptosis, differentiation, and coordination with other cells (tissue homeostasis). Gap junction channels are formed from protein subunits known as connexins; nearly twenty human connexins have been identified and most cells express one to three types. Many human diseases result from heritable mutations in connexin genes or alterations in connexin expression or gap junction formation and function. Only a small percentage of known human toxic agents cause DNA mutations (genotoxicity). Instead, most alter gene expression or gene product function (nongenotoxic or epigenetic toxicity), and often affect cellular communication, including GJIC. Such agents include synthetic (e.g., pesticides), plant-derived (e.g., phorbol esters) and endogenous factors (e.g., reactive oxygen species). Like heritable connexin mutations, the alteration of GJIC and other forms of cellular communication by epigenetic, toxic agents contribute to disease. Knowledge of the mechanisms will lead to less toxic agents and preventive strategies.

VT-2

Integrated Signaling Effects of Epigenetic Toxicants. B. L. UPHAM. Department of Pediatrics & Human Development and the National Food Safety Toxicology Center, Michigan State University, East Lansing, MI 48824. E-mail: upham@msu.edu

The epigenetic response of a cell to an environmental or food borne toxicant begins with the induction of intracellular signal transduction pathways that activate transcription factors, which ultimately regulate genes controlling the proliferation, differentiation and apoptotic events of cell. However, a cell within a tissue must coordinate not only physiological responses but also epigenetic events with their neighboring cells. Intercellular communication through gap junction channels also plays a major role in the expression of genes in response to endogenous and exogenous ligands, and aberrant gap junctional intercellular communication (GJIC) has been implicated in many human diseases. Therefore, GJIC probably plays a central role in coordinating intracellular signaling events between cells, and suggest common upstream events between GJIC and key signal transduction proteins, such as the mitogen activated protein kinases (MAPKs). The phospholipases are one class of signal transduction proteins involved in the early response of a cell to environmental and food borne toxicants, such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls. The release of membrane-derived second messengers, such as arachidonate, results in the inhibition of GJIC and activation of MAPKs. The closure of gap junction channels is hypothesized to be necessary in maintaining critical levels of low molecular weight intracellular-cofactors of signal transduction. Identifying the protein and non-protein cell signaling molecules involved in the epigenetic response of a cell to a toxicant will provide important mechanistic information needed to develop more accurate assessment of risk, the development of chemotherapeutic and preventative strategies, and potential nutritive or drug based intervention schemes. Support from NIEHS Superfund grant #P42 ES04911-07.

VT-3

Stem Cells, Cell-Cell Communication and Epigenetic Toxicants: Risk Assessment Implications. J. E. TROSKO. National Food Safety Toxicology Center, Michigan State University, East Lansing, MI 48824-1302. E-mail: james.trosko@ht.msu.edu

Many chemicals, capable of inducing multiple health risks are known to be non-mutagenic. The mechanisms by which chemicals can work to generate diseases include mutagenesis, cytotoxicity (either necrosis or apoptosis) or epigenetic toxicity. By an epigenetic toxic mechanism, chemicals can alter the expression of the genetic information at the transcriptional, translational or posttranslational levels in a stem cell, a precursor cell or a terminally differentiated cell. From conception, through embryogenesis, fetal and neonatal development to sexual maturation, adulthood and the aging process, homeostatic control of cell proliferation, differentiation, apoptosis, adaptive responses of the terminally differentiated cell and senescence, is actuated by three major and integrated forms of communication [extra-, intra- and gap junctional inter-cellular communication]. Epigenetic toxicants, such as environmental pollutants, natural products [TPA], drugs [Phenobarbital], food additives [saccharin], heavy metals [cadmium]; nutrients [unsaturated fatty acids]; and hormones [estrogen] and growth factors [EGF], can disrupt GJIC by triggering both receptor -dependent and independent signal transduction mechanisms which can down regulate GJIC at the transcription, translational or posttranslational levels. This can lead to abnormal cell proliferation, differentiation, apoptosis, adaptive responses or senescence of the stem, precursor or terminally-differentiated cells. Depending on the developmental stage at the time, duration, concentration and co-exposure of other chemicals, disruption of cell- cell communication can lead to birth defects, cancer, immunological problems, reproductive- and neurological dysfunctions. Knowing the concept of chemical modulation of GJIC into a biologically-based risk assessment model, based on acknowledging the differential toxicities of interacting stem, precursor and terminally-differentiation cells in tissues, should lead to accurate predictions of potential toxicities of chemicals.

VT-4

Ex Vivo Utilization of Dendritic Cells to Identify Vaccine Targets for Control of Infectious Biothreat Agents. KAMAL U. SAIKH. US Army Med. Res. Inst. Infect. Dis., Frederick, MD 21702. E-Mail: Kamal.Saikh@DET.AMEDD.ARMY.MIL

Rapid recruitment of dendritic cells (DC) is a hallmark of the acute inflammatory response to infectious diseases. These infected DC regulate the host defense system by direct or indirect induction of cytokine production; activation of natural killer cell (NK) cells, cytotoxic and T helper lymphocytes; immunoglobulin production by B cells; and T-cell tolerance. The differentiation of DC is driven by inflammatory stimuli or micro-environmental factors such as bacterial products, lipopolysaccharides, and locally produced cytokines, such as granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), and interleukin-1β (IL-1β). IL-15 is also produced by a wide variety of tissues in response to inflammatory stimuli. The major target cells for this cytokine are NK, memory T cells, and monocytes. Recently, we reported that monocytes treated with IL-15 are converted directly to mature DC and supported antigen-specific immune responses. The IL-15 activation pathway appears to be similar to other previously described methods for obtaining DC in culture, but may be more relevant to understanding the host immune response to infectious disease. With DC induced by IL-15, an *ex vivo* assay was developed for assessing primary human T-cell immunity to experimental vaccines for *Staphylococcus aureus*, *Bacillus anthracis*, *Yersinia pestis*, Ebola virus, and other infectious diseases of primary concern as biothreat agents. Our data suggest that this cell culture model may be a useful predictive method for assessing the potential clinical response to recombinant and nucleic acid based vaccines currently in development. New vaccine candidates may be identified for biothreat agents and emerging infectious diseases by using *ex vivo* cultures of IL-15 -induced DC and naïve T cells.

VT-5

Evaluating Potential Inhibitors of Anthrax Lethal Factor Protein. S. R. SIMON, S. Kocer, E. J. Roemer, and L. M. Golub. Depts. of Pathology, Biochemistry, and Oral Biology, SUNY at Stony Brook, Stony Brook, NY 11794-8691. E-mail: ssimon@notes.cc.sunysb.edu

Lethal Toxin produced by *Bacillus anthracis* is the agent responsible for most of the morbidity and mortality associated with anthrax. One component of this toxin, Lethal Factor (LF), is a zinc metalloproteinase which is known to cleave members of the mitogen-activated protein kinase kinase family (MAPKKs or MEKs). The human cell most sensitive to Lethal Toxin is the macrophage, which is activated to trigger a systemic inflammatory response that may progress to potentially fatal Acute Respiratory Distress Syndrome (ARDS). Although the relationship between MEK cleavage and the pathology of anthrax remains correlative, the proteolytic activity of LF is necessary for activation of macrophages. We have evaluated the inhibition of LF-mediated cleavage of members of the MEK family in macrophage lysates by two classes of zinc metalloproteinase inhibitors which can inhibit mammalian as well as bacterial metalloproteinases. Treatment of lysates of the human monocytoid cell line Mono Mac 6 with recombinant LF (List Biological Laboratories) resulted in reduction in molecular weight of immunoreactive Mek-2, followed by loss of immunoreactivity, consistent with multiple cleavages of the protein; we observed similar reduction in molecular weight of immunoreactive Mek-6 under the same conditions without loss of immunoreactivity, consistent with a single cleavage of the protein. To evaluate inhibition of LF-mediated MAPKK cleavage, we employed CMT-300 and CMT-308 (Collagenex Pharmaceuticals), two members of a family of nonantimicrobial chemically modified tetracyclines which are known inhibitors of matrix metalloproteinases, and Ilomastat, a low molecular weight hydroxamate metalloproteinase inhibitor of broad specificity. We achieved complete and lasting inhibition of LF-mediated cleavage of Mek-2 and Mek-6 with doses of all three inhibitors in the 5-20 microM range. In separate studies with collaborators at SUNY Syracuse we have shown that in multiple porcine models of ARDS, administration of CMT-300 prevents the otherwise fatal progression of lung damage while leukocyte respiratory burst activity and levels of leukocyte-derived inflammatory biomarkers are all diminished. We plan to evaluate the capacity of the CMTs to suppress the inflammatory response of LF-exposed Mono Mac 6 cells by introducing LF directly into the cytosol with a protein transfection agent (Chariot™, Active Motif Corp.). Because CMT-300 has already been shown to be safe in phase I trials with normal volunteers, if efficacy can be shown, it may potentially be rapidly deployed for protection in cases of possible anthrax exposure. [Supported by NIH (NIAID)].

VT-6

Morphological Expression of Mustard Gas-induced Skin Lesions *In Vivo* and *In Vitro*. JOHN P. PETRALI, Tracey A. Hamilton, Robert K. Kan, and Christina Pleva. Comparative Pathology Branch, Comparative Medicine Division, U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400. E-mail: John.Petrali@apg.amedd.army.mil

The weaponized chemical sulfur mustard (HD), known historically as mustard gas, gained notoriety as the major chemical weapon of World War I. Its use resulted in over 300,000 battlefield casualties. Since then its use in smaller conflicts has resulted in both military and civilian casualties. Regrettably, it remains today among the weaponized chemical agents of choice by some belligerent factions. Whole body exposure to HD causes immediate and delayed incapacitating injuries to ocular tissues, skin and respiratory airways. Human dermal exposure results in the delayed formation of fluid filled bullae that are progressive, persistent and slow to heal. Although the exact pathogenesis of mustard gas human skin toxicity remains investigatively elusive, anatomical and immunopathological data being gathered in controlled animal investigations and in *in-vitro* model systems are providing important clues as to approximate mechanisms. Summarized in this presentation are contributions made through the use of *in-vivo* models to include human skin-grafted athymic nude mice, hairless guinea pigs, and *in-vitro* systems such as cultured human keratinocytes, cultured skin explants, organotypic human skin equivalents. It is anticipated that pathological data gained through correlative *in-vivo* and *in-vitro* study will not only disclose HD toxicological mechanisms, but also lend direction to the development of prophylactic and therapeutic strategies specific for mustard gas toxicity.

VT-8

Dendritic Cell Immunotherapy of Cancer. REINER LAUS. Dendreon Corporation, Seattle, WA 98121. E-mail: rlaus@dendreon.com

Dendreon Corporation is developing immune based approaches towards the therapy of malignant tumors. Our clinically most advanced product, Provenge, a therapeutic prostate cancer vaccine, recently completed a phase III, double-blind, placebo controlled trial for treatment of men with hormone-refractory prostate cancer. Patients had metastatic prostate cancer that was progressive on hormone therapy. Endpoints of the study were time to disease progression and time to onset of cancer-related pain. Provenge proved to be safe and well tolerated. There was a significant benefit of Provenge on time to disease progression ($p=0.002$) as well as on delay on pain onset ($p=0.019$) in patients with Gleason Scores of 7 and smaller. This was the first randomized placebo-controlled immunotherapy trial in prostate cancer to demonstrate a treatment effect. Additional vaccines for treatment of B-cell malignancies, breast cancer, colon cancer and ovarian cancer are currently in phase I and phase II clinical studies. Thus far, the vaccines are safe with minimal side effects and promising preliminary efficacy data. We will discuss preclinical biology, clinical testing and monitoring as well as approaches towards commercialization of these vaccines.

W-1

Academic Institutions Response to the Staffing Needs of the Biopharmaceutical Industry. KAMAL A. RASHID. Biotechnology & Genomics Research Center, Utah State University, Logan, UT 84322. E-mail: krashid@cc.usu.edu

A major limitation to growth in the biotechnology industry is the shortage of well-trained personnel. The objective of this presentation is to highlight some of the efforts underway at Utah State University to extend the University's linkage to the biopharmaceutical industry to prepare it for the many challenges that the new revolution in life sciences has brought to the arena. Biotechnology programs and courses developed at academic institutions help strengthen both the local and national employment base and lead to economic growth and increased profitability for the industry. The biotechnology industry and bio-centers at academic institutions must maintain cooperation to address the needs and develop programs where there is a clear shortage of labor force. Periodic training and retraining of the workforce keep businesses viable and help maintain compliance with the FDA's regulatory process. At Utah State University we are offering several short intensive courses in the area of biotechnology and bioprocessing for industry personnel, as well as undergraduate courses that offer hands-on intensive curriculum designed to reflect industrial applications.

W-2

Industrial Mammalian and Microbial Cell Culture System for Biopharmaceutical Manufacture of Therapeutics. SARAD PAREKH. Cell Culture and Bioprocessing, Supply R & D, Dow AgroSciences, LLC, Indianapolis, IN 26268. E-mail: Skparekh@Dow.com

Process development for the production of biopharmaceutical and biologics manufacturing especially from various cell culture technologies and its validation from the laboratory to the market, however, is a very complex operation. This presentation will review overall work process and will focus on the areas critical from the regulatory and commercial manufacturing perspectives of therapeutics derived from microbes or mammalian cell culture system. Characterization cell lines, the SOPs for master cell banks, the final product and the essential documentation needs will be presented. Furthermore, how the production process parameters are optimized and validated, the pilot plant specifications defined including GLP enforced guidelines by FDA for equipment qualification and calibration to get yields and product, pure, safe and conforms with desired efficacy on packaging and formulation will be discussed. Included here, will also be the new innovations optimizing parameters for maximizing cell mass with mammalian cell culture, and its scalability and in process quality control methods employed by FDA for cGMP compliance requirements. Lastly, specific examples will be cited on methods and technologies developed for process controls, software data capture and analysis from information management perspectives, to support all aspect of biopharmaceutical manufacturing.

W-4

The Caco-2 Assay: An In Vitro Model for Prediction of Intestinal Permeability. D. A. LASKA. Discovery Drug Disposition and New Technologies, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285. E-mail:dalpsu@lilly.com

Prediction of intestinal permeability and ultimately systemic exposure or bioavailability of an ingested chemical or therapeutic agent has long been best evaluated through the use of animal models or during clinical trials. Recent advances in in vitro technology, understanding of membrane transporters and efflux pumps, and computer modeling programs has provided chemists, drug hunters, and toxicologists with innovative tools to assess or predict systemic exposure without the use of animal models. The application of the predictive in vitro models and basic membrane research have been coupled with defined SAR initiatives to allow rapid assessment of diverse chemical platforms and subsequent refinement of the in silico model. The Caco-2 human colon adenocarcinoma can be induced to differentiate to form a functional transporting epithelium that has been extensively used to evaluate intestinal absorption of chemicals and drugs. This presentation will describe the basic principles of intestinal transport and absorption as they apply to pharmaceutical development, as well as optimization and validation of in vitro models like the Caco-2 assay.

W-5

Hepatocyte Systems for Predicting First-pass Metabolism and Bioavailability of Chemicals In Vitro. EDWARD L. LECLUYSE. School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599. E-mail: ed_lecluyse@unc.edu

As our knowledge of the species differences in the regulation and substrate specificity of the mammalian drug-metabolizing enzymes has expanded significantly, the need for human-relevant *in vitro* hepatic model systems has become more apparent than ever before. Pharmaceutical scientists have attempted to utilize a number of liver-derived model systems to study drug disposition *in vitro*, including liver slices, immortalized cell lines, and primary hepatocytes. With the lack of phenotypic gene expression in nearly all immortalized cell lines and the limitations of liver slices, such as short-term viability and diffusional barriers, human hepatocytes have become the "gold standard " for evaluating hepatic metabolism and toxicity of drugs and other xenobiotics. They are used quite extensively for metabolic stability, metabolite identification, and assessing the potential of new drugs to be involved in serious drug interactions that are caused by the inhibition or induction of cytochrome P450 enzymes. There are important issues that must be considered when conducting *in vitro* drug testing utilizing human hepatocytes, including the effects of culture and study conditions. The maintenance of normal cellular physiology and intercellular contacts *in vitro* is of particular importance for optimal phenotypic gene expression and response to drugs and other xenobiotics when conducting enzyme induction experiments. Differential expression of the individual isoforms of the major CYP450 enzymes over time in cultured hepatocytes suggest that this model system must be used cautiously in its application for drug metabolism studies. Overall, primary human hepatocytes have proven to be an invaluable resource for the prediction of metabolic clearance and drug-drug interactions in humans.

W-6

Model Systems for Cytotoxicity Screening and Predicting Target Organ Effects. C. A. TYSON. SRI International, Menlo Park, CA 94025. E-mail: charles.tyson@sri.com

Pharmaceutical companies are intensively looking for alternative strategies for toxicity screening that will identify clinical leads with greater success, thereby reducing costs associated with animal and clinical testing of compounds doomed to fail in the clinic. *In vitro* systems will play a critical role because of the capability for screening large numbers and small amounts of candidate compounds at relatively low cost and because of the possibility of using cells and tissues from multiple species for better extrapolation of animal data to humans. Studies on structurally related compounds employ single cell or cell substituents for toxicity ranking; tiered-test approaches are required for more comprehensive assessments. The latter involves two or more test stages: (1) high throughput screening (HTP) for general cytotoxicity using cell lines, followed by (2) organ-specific primary cell systems or tissues for definitive testing of toxicity endpoints produced by prospective candidates. Although many organ-specific cell systems exist, few have advanced to validation and in many cases no tissue culture model has been developed at all. However, progress is being made. In this presentation background and concepts of tiered *in vitro* toxicity testing will be covered. Examples of HTP test paradigms developed for initial screening and of organ specific tests for myelosuppression, liver cell-specific injury, and predicting proarrhythmia will be discussed. The presentation concludes with an overview of future research directions needed to move the field forward.

P-1000

Development of an In Vitro Tuberization System for Sweet Potato Micro-storage Root Formation. MARCELINE EGNIN, Latrice Crawford, and Anne Sama. Tuskegee University, Tuskegee, AL 36088. E-mail: megnin@tusk.edu

Development of a micro-tuber storage root production system is necessary in biotechnology for quick in vitro transgene expression and yield assessments, which was previously limited to specific crops and field trials. In sweetpotato as well as other root crops, the formation and thickening of tuberous roots is one of the most economically important processes determining yield. It is thus important to understand the physiological mechanism responsible for the formation and thickening of roots critical to yield improvement. An in vitro system could provide a basic understanding of micro-tuber production and the underlying biochemical processes of the storage root system via visual and molecular screening, which would allow for crop improvement. This project evaluated factors affecting storage root production in vitro for sweetpotato. Several parameters were investigated to facilitate micro-storage root production: culture medium (Heller and Hoagland), elite sweetpotato genotype (Beauregard, Jewel, Mogamba, Rojo, TU-155 and Zapallo), micro-cutting length, hormonal type, carbon source, explant position, the use of charcoal, and increased darkness conditions. Explants were isolated from in vitro grown sweetpotato, and cultured for six months at 27° C under a 12:12 hour photoperiod. All explants from Beauregard and ROJO cultures exhibited 100% storage root initiation, thickening and bulking with root color change in Hoagland treatment in comparison to only 75% of explants from Jewel Tu-155. The same pattern was observed in 50% of explants from Mogamba and Zappallo. The explants cultured in Heller media showed overall better plant growth (vegetative system). The orientation of the cuttings on the nutrient medium was critical. Cuttings placed vertically inverted developed multiple micro-storage roots regardless of the culture medium or variety used. The addition of a two percent charcoal layer on the culture media in tubes wrapped in foil to increase darkness conditions promoted faster root initiation and bulking than those with the media exposed to light. Hoagland treatment was comparable to field conditions and superior to Heller cultures where root initiation was delayed with root showing slight thickening and bulking. Our results may provide novel approaches for furthering our general understanding of the fundamental microtuber production. Work Supported by NASA and USDA.

P-1001

Cryopreservation of Shoot tips of the Endangered *Asimina tetramera* by Encapsulation-Vitrification. B. L. PLAIR and V. C. Pence. Cincinnati Zoo and Botanical Garden Center for Conservation and Research of Endangered Wildlife (CREW), 3400 Vine Street, Cincinnati, OH 45220. E-mail: bernadette.plair@cincinnatizoo.org

Asimina tetramera, the four petal paw paw, is one of six dwarf species found in Florida. It is federally listed as endangered because of its limited range. Seedling shoot tips obtained from seeds sent by collaborators at Historic Bok Sanctuary were used to establish shoot cultures as part of CREW's Endangered Plant Propagation Program. Shoot tips from the resulting culture line were successfully cryopreserved using a modified version of the encapsulation-vitrification method described by Dai et al. (Euphytica 101:109-115, 1998). Shoot tips (< 1 mm in length) excised from cultures 40-45 days after transfer to fresh medium were pretreated for 48 hours on semi-solid nutrient medium containing .3 *M* mannitol + 10 u*M* ABA. Shoot tips were encapsulated in alginate beads containing 2 *M* glycerol plus 0.4 *M* sucrose and exposed to a plant vitrification solution (PVS2) for 2 hours at 0° C prior to immersing in LN. Shoot tips were rapidly thawed in a 38° C water-bath, rinsed with 1.2 *M* sucrose solution and placed on semi-solid medium for regrowth. Observations of growth were made after 2-8 weeks. Surviving tissue was removed from the alginate beads and transferred to fresh growth medium every week. Survival of shoot tips after LN exposure averaged 68%. Shoot tips of *A. tetramera* formed shoots directly after cryopreservation and shoots reestablished in tissue culture are now being rooted. These results suggest that encapsulation-vitrification of shoot tips may be useful for germplasm storage of this endangered species. This is particularly significant, since this species has recalcitrant seeds, and storing shoot tips may be the only method currently available for long term germplasm storage. A preliminary trial of this procedure on Rugel's paw paw, *Deeringothamnus rugelli*, a related species from Florida that is also federally listed as endangered, has resulted in 20% post thaw survival of LN treated shoot tips. Research supported in part by Institute of Museum & Library Services grants IC-00034-00 and IC-03-02-0130-02.

P-1002

In Vitro Collecting and Establishment of Tissue Culture Lines of Three Endangered Florida Pawpaws. V. C. PENCE and S. M. Charls. Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. E-mail: valerie.pence@cincinnatizoo.org

In vitro methods are being applied at CREW to the propagation of three federally endangered Florida pawpaws, *Asimina tetramera, Deeringothamnus rugelii,* and *D. pulchellus*, in collaboration with the Center for Plant Conservation (St. Louis, MO) and Historic Bok Sanctuary (Lake Wales, FL). Techniques were previously developed for the in vitro shoot propagation of *A. tetramera* and *D. rugelii*. In order to increase the number of genetic lines of these and to establish cultures of *D. pulchellus*, the technique of in vitro collecting was used to initiate cultures from wild plants at several sites in Florida and transport them to CREW. Multiple shoot tips and leaf pieces of 8, 10, and 11 plants were collected from *A. tetramera, D. rugelii*, and *D. pulchellus*, respectively. These were surface sterilized with 70% ethanol and placed into small vials containing 2.5 ml of Linsmaier and Skoog medium plus 3% sucrose, 0.22% Phytagel, and 0.5 mg/L BAP plus 0.5 mg/L NAA, with a fungicide and antibiotics. Tissues were transported to CREW and examined within a week of collecting. Contamination rates were very low, and clean tissues were transferred to several media within 7-9 days of collecting. Frequent transfer (every 7-14 days) of the tissues was critical in order to avoid excessive browning, with 17% and 4% of initial bud and leaf pieces, respectively, being lost to browning within a week after collecting. Only two of the 29 genotypes collected initiated outgrowth from a shoot bud. All other organized growth was from adventitious buds, which developed when tissues were transferred to a medium containing 0.66 mg/L TDZ, 0.2 mg/L BAP, and 0.22 mg/L IBA. Adventitious buds were initiated from 100% of the genotypes of *A. tetramera*, 60% of *D. rugelii*, and 63% of *D. pulchellus*. Vigorous shoot culture lines were established from 63%, 20%, and 27% of the genotypes, respectively. These are being used to produce plants for research and reintroduction, as well as for long-term germplasm storage. Research funded in part by Institute of Museum and Library Services Grants No. IC-00034-00 and IC-03-02-0130-02.

P-1003

Control of Bacterial Contamination in Large Scale Papaya Micropropagation. M. FITCH[1], T. Leong[2], N. Saito[2], G.Yamamoto[2], A. Dela Cruz[2], A. Yeh[2], S. White[2], S. Maeda[1], S. Ferreira[3], and P. Moore[1]. [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; and [3]University of Hawaii, 3190 Maile Way, Honolulu, HI 96822. E-mail: MFITCH@PBARC.ARS.USDA.GOV

Papayas were micropropagated for research projects for many years, and in 2002 scale up for commercial scale production was initiated. Problems associated with increased propagation were anticipated, but the discovery of slow-growing, cryptic bacteria in the culture population and stocks was, by far, the most devastating. The most serious bacterial pests were those that were barely visible without the aid of a dissecting microscope. Contaminated papayas in culture plates of proliferation medium, modified Murashige and Skoog (MS) medium containing 0.2 mg/L each of benzylaminopurine and naphthalene acetic acid, appeared green and healthy, but when shoots were placed in rooting medium, vermiculite moistened with ½ MS salts and 3% sucrose, the leaves senesced, root formation was slow and poor, and at temperatures above 28° C, the shoots sometimes bleached and died. Assays to screen stocks for contamination status were difficult because the bacteria grew very slowly on standard bacterial culture media, e.g., Luria broth, malt extract, or potato dextrose agar. The most dependable assay for contamination was visual scoring of each plate prior to subculture coupled with tests of stock lines to observe the amount of root development and shoot growth that occurred in rooting medium. If large, dense root masses and deep green, leafy canopies formed in 7-21 days, the stock cultures were presumed to be free of bacteria. Infected stock cultures were decontaminated either by treatment with the addition of carbenicillin at 500 mg/L and/or cefotaxime at 200 mg/L to the proliferation medium for one month or by agitating shoots in 20% commercial bleach for 10 min. Following decontamination, shoots with preformed roots were placed in the rooting medium and root mass and shoot development monitored. Contaminated shoots were placed in phytoauxotrophic rooting media devoid of sucrose. In contrast to senescent contaminated shoots in sucrose-containing media, the shoots in the phytoauxotrophic medium remained green and healthy in appearance. Contaminated shoots rooted about 1-2 months later than uncontaminated plants in the sucrose-containing medium. To control future problems with bacterial contamination, each culture plate was examined under a dissecting microscope prior to subculture and stocks were continually tested for rapid root and canopy formation in rooting medium containing sucrose. Although labor intensive, monitoring of all cultures helped minimize the incidence of contamination.

P-1004

Factors Affecting In Vitro Establishment of Cocoyam (*Xanthosoma sagittifolium* L Schott). A. SAMA, M. Egnin, and S. Zok. Tuskegee University, Tuskegee, AL 36088. E-mail: megnin@tusk.edu, annsama@yahoo.com

Cocoyam is an annual herbaceous plant that belongs to the Araceae family. It is a staple tuber crop grown in the tropics, and traditionally propagated asexually via suckers and whole or sections of corms and cormels. Reports of cocoyam micropropagation of have used apical shoot tips as the predominant explants. A single plant provides just one explant, although there exist several lateral buds as well. To test the potential of these buds in cocoyam micropropagation, several other factors were investigated for in vitro establishment: genotype, explant type, culture medium composition and physical state. Apical and lateral shoot tip explants of white and red cocoyam greenhouse-grown plants were initiated on three culture media either semi-solid or liquid and agitated on a rotary shaker. The culture media were composed of B5 basal salts and vitamins supplemented with 5 m*M* NAA, and B5 or MS basal salts and vitamins supplemented with 2 mg/l panthothenic acid, 100 mg/L arginine, 200 mg/L ascorbic acid, 20 mg/L putrescine and 20 mg/L gibberellic acid. Most explants remained viable although few were lost to contamination. The physical state of the medium was critical for cocoyam shoot tip establishment. Cultures on agitated liquid media developed faster than those on semi-solid media, and apical shoot tips grew larger than the lateral explants irrespective of the physical state of the medium. No significant difference in growth rate was found between the culture media at the initiation stage, but explants on the medium with MS basal salts were consistently bigger than those on the B5 based media. The B5 medium was very effective in producing plantlets with 3-5 large green leaves and several roots from the apical explants of the white genotype after eight weeks from initiation. This study demonstrates that cocoyam lateral buds are slow growing but can be efficiently employed as explants for micropropagation. Work supported by USDA and NASA.

P-1005

Somaclonal Variation Detection and Chimerism in Somatic Embryo-derived Cocoa. C. M. RODRIGUEZ LOPEZ, M. J. Wilkinson, and A. C. Wetten. School of Plant Sciences, University of Reading, Reading, Berkshire, RG6 6AS, United Kingdom. E-mail: c.m.rodriguezlopez@rdg.ac.uk

Genetic mutants that are chimeric for allele loss are difficult to detect by conventional PCR because the lost allele is still present in at least in some of the wild-type cell population. Such mutants are nevertheless likely to form a significant proportion of any regenerant population. Here, a protocol is described for the detection of genetic chimeras amongst somatic embryo-derived cocoa plants. The assay requires heterozygotic microsatellites and provides an accurate discrimination and quantification of the level of chimerism. Validation was performed using artificial chimeras created by mixing DNA templates prior to PCR. The system provided accurate results using less than 2.5 ηg of total DNA and can reliably detect very low proportions of mutant cells in a variable background of predominantly wild-type tissue. Overall, 18 SSR's were used to quantify and characterize the somaclonal variation induced by somatic embryogenesis in five different cocoa genotypes. The frequency of variant plants was approximately 35% and followed a second order polynomial regression with genotype and time in culture affecting X_{max}. No correlation was observed between allele size and the number of observed mutations, although this was probably due to small size range between the microsatellite loci used. The relation between time in culture and detected variation suggest that mutational events take place throughout callus development. Furthermore, the presence of both chimeras and wild-type plants arising from the same, restricted callus zone, suggests that mutations can occur during embryoid development.

P-1006

Encapsulation of Orchid - Phalaenopsis Shoot Tips for Storage and Exchange of Germplasm. W. T. P. S. K. SENARATH, Kui Jae Lee, W. H. Lee, and S. Rehman. Faculty of Bioresources Sciences, College of Agiculture, Chonbuk National University, Chonju 561-756, Republic of Korea. E-mail: senerath@yahoo.co.uk

The market for orchids is a large and valuable one for the horticultural industry. For breeders to maintain their regular supply to the Markey they must be able to produce novel cultivars regularly. Plants maintain in green houses are vulnerable to loss due to many reasons. Therefore a protocol was developed for the Cryopreservation of tips using dehydration/encapsulation method. In vitro propagated shoot tips from improved cultivars of Phalaenopsis violacea were used. Shoot cultured were maintained in semi solid V&W medium supplemented with fresh coconut water (SEM). All the plant materials were maintained at 25° C, with 16 hour day and the light was supplied by white fluorescent tubes (3-7 umol $m^{-2}S^{-1}$ PAR). Tips were excised under filter sterile anti-oxidant (0.2 *M* phosphate buffer (pH 5.7) + 50 g/l ascorbic acid and 15 g/l sodium borate) and were encapsulated in calcium alginate beads (30 g/l sodium alginate +14.7 g/l $CaCl_2.2H_2O$). Formed beads were blotted dried and were transferred into 5 cm petri dishes containing liquid SEM with different concentrations of sucrose (0.25-1.0 *M*). They were incubated in a shaker for 24 hours at 18° C in dark. Then they were dehydrated in silica gel for 1- 24 hours. Beads were transferred into small vials and were transferred for freezing (rapid or two stage). After that they were stored in liquid nitrogen for at least 24 hours. Samples were thawed at 40° C before place them on SEM for shoot growth. Shoot tips were excised from beads and cultured on semi-solid SEM and maintained at 25° C in dark and the growth was compared with non capsulated tips. Excised non-capsulated shoot tips exhibited shoot elongation and development after 28 days of incubation. Subsequently they developed into rooted plantlets. However encapsulated shoot tips showed less elongation after 28 days and after 60 days they were elongated up to the same length as non-capsulated tips. Excision of tips from beads before culturing showed the same growth as non-capsulated shoots. Dehydration of encapsulated tips in sucrose (0.25-0.5 *M*) does not show any significant effect on subsequent growth compared to untreated ones. Higher concentrations significantly reduced the growth as well as callus was observed at the base. Two hour exposure to silica gel has no effect on growth when encapsulated shoots pre-treated with 0.5 *M* sucrose but 3 hour exposure resulted significant decrease in number of shoots that grew. Two hour rapid freezing after pre-treatment with 0.5 *M* sucrose and 2 hour exposure to silica gel showed only 25% shoot growth. No viable material was recovered after longer or two step freezing.

P-1007

A Novel Disposable Film Culture Vessel for Photoautotrophic Micropropagation of *Epidendrum* Orchid. DAM THI THANH GIANG[1], H. Watanabe[2], M. Ujike[3], Y. Kume[4], and M. Tanaka[1]. [1]Fac. of Agric., Kagawa Univ., Kagawa 761-0795, Japan, [2]Mitsubishi Chemical Corp., Yokohama 227-8502, Japan, [3]Bio-U, Kagawa 765-0051, Japan, [4]Otsuka Techno Co. Ltd., Tokushima 771-5209, Japan. E-mail: dam_giang@yahoo.com

To overcome various disadvantages of conventional culture vessels, Tanaka *et al.* (1988) first developed a film culture system, the "Culture Pack" (CP) made of fluorocarbon polymer film (Neoflon® PFA film), which possesses superior properties such as thermal stability, high light transmittance and gas-permeability. Tanaka *et al* (1995) later developed the "Miracle Pack" (MP), the practical model of the CP. This MP system is found more suitable for the micropropagation of various plant species when compared to a conventional culture vessel. However, the MP made of PFA film and supported by a polycarbonate frame is still expensive due to the high price of the film and the frame, making it ill-suited for widespread use in commercial plant tissue culture laboratories. In order to reduce the cost of film culture vessels, a multiple-layered super cheap OTP® film, made of TPX and CPP, with similar characters as the PFA film has been used to make a novel disposable film culture vessel, namely the "Vitron" (Tanaka *et al.*, 2001). Since the frame of Vitron is made of polypropylene, its cost is remarkably reduced. In this study shoots with three leaves of *Epidendrum* (*E.* Rouge Magic x *E.* Joseph Lii 'Mother's Day Koto') were cultured photoautotrophically under high CO_2 conditions at a low photosynthetic photon flux density in three film culture systems: MP using PFA film, MP using OTP film and Vitron, with sugar-free liquid modified Vacin and Went medium and rockwool multiblock™ as substrates. The *in vitro* and *ex vitro* growth of *Epidendrum* plantlets cultured in the three film culture systems were almost equal, producing normal and vigorous plantlets. A 100% survival of plantlets was obtained after transferring to soil without any specialized *ex vitro* acclimatization treatment. Net photosynthetic rate of *in vitro Epidendrum* plantlets cultured in the three film culture systems were also equal. This study suggests that the novel Vitron film culture system could be used to replace the conventional culture vessel for photoautotrophic micropropagation of *Epidendrum* orchids.

P-1008

Transformation and Regeneration of Wetland Monocot *Juncus Accuminatus* Using Different Binary Vectors. L. CHEN, R. Nandakumar, P. K. Lai, and S. M. D. Rogers. Bioscience Department, Salem International University, Salem, WV 26426. E-mail: rogers@salemiu.edu

Our long-term goal is to express viral genes in wetland monocots whose gene products can be used as vaccines against infectious agents, such as the avian-transmitted West Nile Virus. The objective of the study presented here is to develop a protocol for establishing a model transformation system for the monocot *Juncus accuminatus* using different binary vectors residing in *Agrobacterium tumefaciens*. Three binary vectors, pTOK233 in *Agrobacterium* strain LBA4404, and both pCAMBIA-1201 and pCAMBIA-1301 in EHA105, were used to transform *J. accuminatus*. All vectors contained *uidA* and *hptII* genes within their T-DNA region. Seedling-derived calli were used as explants. Transformed calli were selected on MS media containing 40 or 80 mg/l hygromycin, regenerated and rooted on MS medium containing 5 mg/l of 6-benzylamino-purine (BA) and 0.1 mg/l of alpha-naphthaleneacetic acid (NAA), respectively. To date 20 lines of transformed plants have been selected and established in the greenhouse. Both transient and stable expression of the *uidA* gene was demonstrated by histochemical GUS assay in calli and in the plants. All parts of the transgenic plants show GUS activity to different degrees, depending on the plant line. Two plants from each line and 4 lines from each plasmid were analyzed for the presence of the reporter and marker genes. The presence of the *uidA* and *hptII* genes in transformants was confirmed by polymerase chain reaction (PCR). To our knowledge this is the first report on *J. accuminatus* transformation using binary vectors residing in *Agrobacterium*,and plant establishment in the greenhouse. In conclusion, the wetland monocot *J. accuminatus* appears transformable with *Agrobacterium tumefaciens*.

P-1009

An Efficient System for Biolistic Transformation and Plant Regeneration of Pearl Millet Using Spikelets Shaved from Immature Inflorescences. J. J. GOLDMAN*, W. W. Hanna*, G. Fleming, and P. Ozias-Akins. *Department of Crop and Soil Sciences and Department of Horticulture, P.O. Box 748, University of Georgia, Tifton Campus, Tifton, GA 31793. E-mail: jjg@tifton.uga.edu

Of the major cereals, pearl millet [*Pennisetum glaucum* (L.) R. Br.] lacks an efficient protocol to recover fertile transgenic plants. Tetraploid pearl millet is a close sexual relative of apomictic *P. squamulatum*. The ability to test putative cloned apomixis genes in sexual tetraploid millet should be possible with a transformation protocol. We initially tested the apical meristem, immature inflorescence, and immature embryo as suitable explants for biolistic transformation. Embryogenic tissue obtained from diploid and tetraploid genotypes was initiated from all explant sources on 2,4-dichlorophenoxyacetic acid (2,4-D)-containing medium. Efficient somatic embryo germination was induced on medium supplemented with thidiazuron (TDZ) and 6-benzylaminopurine (BA). Using the *bar* gene, phosphinothricin (PPT)-resistant plants were recovered from all explant sources at low frequencies. Florets shaved from immature inflorescences proved to be the most efficient explant for producing bombardment-grade tissue. The system was optimized using inflorescences from tetraploid plants. Key adjustments included : osmotic treatment (0.35 *M* sucrose), a modified gold particle coating procedure and 0.6-0.75 micron gold, bombarding at 1550 psi, delaying selection for 2 weeks after bombardment, and using 15 mg/L PPT for selection. The frequency of transformation increased using the adjusted conditions. Seven independent DNA/gold precipitations were used to bombard 52 plates, 29 of which produced an average of 5.5 herbicide-resistant plants per plate (155 total plants). The number of herbicide-resistant plants recovered per successful bombardment ranged from 1 to 28. Co-bombardment with a *gfp*-containing plasmid, combined with herbicide selection, resulted in plants that were both herbicide resistant and expressed GFP. The frequency of co-transformation ranged from 5-85%. Phenotypic (whole plant resistance to 500 mg/L glufosinate, GFP expression), Southern blot, and segregation data confirmed the integration, expression, and transmission of the foreign genes.

P-1010

Alleviation of Wheat Allergenicity Using the Thioredoxin System. H.-K. KIM, M.-J. Cho, H. R. Jung, Y.-B. Kim, S. Morigasaki, J. H. Wong, P. G. Lemaux, and B. B. Buchanan. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720. E-mail: hyunk-kim@nature.berkeley.edu

Wheat is one of the most important cereal crops for human food with annual production of over 500 million metric tons. It is also one of the major sources of food allergens, especially in developed countries like the U.S. It is estimated that approximately 15% of children under the age of three and 2% of adults suffer from food allergies. For unknown reaasons, the number of individuals plagued by this problem has been on the increase. Thioredoxin is a ubiquitous 12 kDa protein and contains a highly conserved catalytically active site, Cys-Gly-Pro-Cys, which reduces disulfide bonds of target proteins. Intramolecular disulfide bonds are the major components of many principal food allergens, which confer stability to proteins and protect against proteinases. It was shown that some food allergens, such as those in milk and wheat, are successfully reduced by thioredoxin *in vitro* and this reduction resulted in lowered allergenicity of a number of different preparations, including commercial wheat allergen extracts. Reduction of gliadins and glutenins by thioredoxin *in vitro* resulted in decreased allergenic responses in a canine allergenicity model that closely mimics humans. To determine the effect of thioredoxin on the mitigation of allergenicity in wheat grains *in vivo*, a commercially important bread wheat cultivar, Yecora Rojo, was transformed using a highly regenerable green tissue system. Three independent transgenic lines were generated: one line overexpressing wheat thioredoxin *h* and two lines overexpressing barley thioredoxin *h*. Thioredoxin *h* was expressed 10- to 20- times more highly in the endosperm of the transgenic wheat grains relative to controls. Allergenicity of the transgenic grain was tested in the canine model system and the results are consistent with a decrease in allergenicity as suggested by *in vitro* studies.

P-1011

Transgenic *Spirodela*: A Unique, Low-risk, Plant Biotechnology System. Marvin Edelman, Ron Vunsh, Jihong Li, Uri Hanania, Moshe Flaishman, Jane Vishnevetsky, and AVIHAI PERL. The Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel and The Department of Fruit Tree Sciences, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel. E-mail: perlx@int.gov.il

The monocot family *Lemnaceae* is composed of small, aquatic, floating plants. Two of its genera, *Lemna* and *Spirodela*, are distributed worldwide and have biomass doubling times of 48-72 h under controlled, axenic conditions. Reproduction is typically vegetative, with culture mass increasing exponentially until crowding sets in. *Lemna* species, however, can also flower and produce seeds while *Spirodela* rarely do so. *Spirodela oligorrhiza* proliferates exclusively in a vegetative manner. Its protein content approaches 50% of its dry weight and is well balanced in essential amino acids. Our group at the Weizmann Institute has successfully induced a tissue culture cycle (callus formation and plant regeneration), taking about 3 months, in *S. oligorrhiza*. Several *Agrobacterium* vectors were applied at the callus stage resulting in stable transformed plants. Four different binary plasmids fused to different leaders were successfully used as vectors for transformation. The transgenic lines are stable in growth rate, transgene expression, and activity. This was confirmed over a period of 18 months and more than 150 biomass doublings, by DNA-DNA and immunoblot hybridizations, fluorescence measurements and antibiotic resistance. Expression of transgene products ranged from 0.005% to about 3% of total protein. Expression level is a function of the plasmid construct used rather than transgene copy number. A significant fraction of native transgene product can be programmed for excretion, dependant on the construct leader. Low-risk advantages of the *Spirodela* transgene system are: enclosed growth facilities; seedless plants means no live transgenic material outside the growth facility; since *Spirodela* is edible, the whole, engineered, dried plant can be the final biotech product.

P-1012

Expression of a Synthetic Avidin Gene in Maize for Control of Corn Rootworm (*Diabrotica* spp.) and Other Insect Pests. SERENA B. MCCOY[1], Mitsuhiro Ueda[2], Karl J. Kramer[3], Subbaratnam Muthukrishnan[3], and Harold N. Trick[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, [2]Department of Applied Biological Chemistry, Osaka Prefecture University, Osaka, 599-8531, Japan, and [3]Department of Biochemistry, Kansas State University, Manhattan, KS 66506. E-mail: Trick@plantpath.ksu.edu

Corn rootworms cause millions of dollars worth of damage each year. There are currently two main methods of control that are used: crop rotation and insecticides. Crop rotation works well unless the species of corn rootworm employs extended diapause, which allows the eggs to remain in the soil for two winters and hatch the following spring. The use of insecticides for control creates concerns for environmental safety as well as health safety. Growers are looking for new management options. One potential option is the use of transgenic corn containing a synthetic avidin gene. Avidin, a glycoprotein found in egg whites, has a very high affinity for the vitamin biotin. Several insects including beetles (*Coleoptera*) and flies (*Dipetera*) have been reported to show stunted growth and mortality after consuming a diet containing avidin. We have introduced a synthetic avidin gene with maize-preferred codons into maize (HiII background) using three separate plasmids via particle bombardment. The three plasmids include the following: pRUBIAVD -rice ubiquitin promoter, rice chitinase signal sequence and avidin gene, and PinII terminator in pUC18 vector; pACAVD - rice actin promoter, rice chitinase signal sequence and avidin gene, and PinII terminator in pBluescript vector; and pACAVDBAC - rice actin promoter, rice chitinase signal sequence and avidin gene, vacuolar targeting signal and PinII terminator in pBluescript vector. Out of ten separate bombardments, 22 independent events were selected for regeneration. All events were grown to maturity and either selfed or backcrossed. Twenty events were PCR positive for the avidin gene. Further molecular analyses will provide integration and expression information necessary for future bioassay studies.

P-1013

Shoot Meristem: An Ideal Explant for *Zea mays* (L.) Transformation. D. AL-ABED, R. V. Sairam and S. L. Goldman. Plant Science Research Center, The University of Toledo, Toledo, OH 43606. E-mail: diaa29@aol.com

We report a rapid high frequency somatic embryogenesis and plant regeneration protocol for *Zea mays*. Maize plants were regenerated from complete shoot meristem (3-4 mm) explants via organogenesis and somatic embryogenesis. In organogenesis, the shoot meristems were directly cultured on a high cytokinin medium comprising 5-10 mg l^{-1} 6-benzyl aminopurine. The number of multiple shoots produced per meristem varied from 6 to 8. Plantlet regeneration through organogenesis resulted in just 4 weeks. Callus was induced in five days of incubation on an auxin modified MS medium. Prolific callus, with numerous somatic embryos, developed within 3-4 weeks when cultured on an auxin medium containing 5 mg l^{-1} 2,4-dichlorophenoxyacetic acid. The number of multiple shoots varied from 3 to 6 per callus. Using R23 (Pioneer), the frequency of callus induction was consistently in excess of 80% and plant regeneration ranged between 47-64%. All regenerated plantlets survived in the greenhouse and produced normal plants. Each transgenic plant produced leaves, glumes and anthers that uniformly expressed green fluorescent protein (GFP). The GFP gene segregated in the pollen. Based on this data it is concluded that the transgenics arose from single cell somatic embryos. The rate of T-DNA transfer to complete shoot meristems of *Zea mays* was high on the auxin medium and was independent of using supervirulent strains of *Agrobacterium*.

P-1014

Enhanced Fungal Tolerance in Transformed Banana (cv. 'Grand Nain') Plants Regenerated Through Somatic Embryogenesis. J. VISHNEVETSKY, Y. Cohen, L. Shlizerman, M. A. Flaishman, and A. Perl. The Department of Fruit Tree Sciences, The Volcani Center, P. O. Box 6, Bet-Dagan 50250, Israel. E-mail: jvishnev@hotmail.com

Enhanced regeneration system of banana (*Musa* spp. AAA group) cv. Grand Nain embryogenic suspensions was established and allowed us to develop a method of direct gene transfer by particle bombardment. Our construct contained 3 different genes. Two of them were shown to enhance fungal disease tolerance and the third one was shown to be involved in free radical protection. The initial plant material consisted of embryogenic white friable callus were obtained from immature male flowers. Cultures were maintained on Murashige and Skoog (MS) semi-solid medium supplemented with 44.5 g/l sucrose, 100 mg/l glutamine and malt extract, 1 mg/l biotin, 1 mg/l 2,4-dichlorophenoxyacetic acid (2.4-D), pH 5.3 (M2) in the dark with every 4 weeks subculture to the fresh medium. This callus was transferred to the M2 liquid medium, passed through the 1000 mm metal net mesh and cultivated on the shaker (100 rpm) in the dark before bombardment. First step of selection was performed in M2 liquid medium supplemented with the proper antibiotic in the dark. White cell aggregates (initial fresh weight doubled) were transferred to embryo development SH (Schenk and Hildebrandt) medium supplemented with 45 g/l sucrose, 15 g/l maltose, 100 mg/l glutamine and malt extract, 1 mg/l biotin, 230.2 mg/l l-proline, 0.2 mg/l a-naphthalene acetic acid (NAA), 0.05 mg/l Zeatin-riboside, 2.5 g/l Gelrite (Duchefa) in complete darkness. Aggregates multiplied 6-8 times from the initial quantity on the selection medium, produced embryos while part of them have enlarged and formed multiple meristematic centers on their surface during 3 months of culture. Those aggregates were subcultured to MS regeneration medium supplemented with 30 g/l sucrose, 0.2 mg/l 6-benzylaminopurine (BA), 0.05 mg/l Zeatin-riboside, 0.5 g/l activated charcoal for 2 months with subsequent subculture to MW (McCown's woody plant) supplemented with the same phytohormones. On this medium aggregates regenerated into plants resulting up to 20 different clones per 0.7 g of initial bombarded material during 6 to 10 months of incubation. Plants were rooted on MS medium supplemented with 0.2 mg/l BA. Molecular characterization of transformants demonstrated that the transgenes had been stably integrated into the banana genome.

P-1015

Heterogeneity In Terpenoid Gene Expression In Transformed Roots Of *Artemisia annua* L. Grown in Bioreactors. P. J. WEATHERS[1], F. F. Souret[1,4], Y. J. Kim[3], B. E. Wyslouzil[3], and K. K. Wobbe[2]. [1]Biology and Biotechnology Department, [2]Chemistry and Biochemistry Department, and [3]Chemical Engineering Department, Worcester Polytechnic Institute, Worcester, MA 01609 and [4]Delaware Biotechnology Institute, 15 Innovation Way, Newark, DE 19711. E-mail: weathers@wpi.edu

Artemisia annua L. synthesizes numerous bioactive molecules, including a potent anti-malarial sesquiterpene, artemisinin, making this plant an attractive model to study the accumulation of secondary metabolites. *A. annua* transformed roots have provided a valuable model system for assessing the scale-up production of natural products. The spatial heterogeneity in gene expression of key terpenoid biosynthetic enzymes was characterized in these transformed roots after growth in shake flasks, bubble column and mist bioreactors. Overall, higher 3-hydroxy-3-methylglutaryl coenzyme A reductase, deoxy-D-xylulose-5- phosphate synthase, deoxy-D-xylulose-5-phosphate reductoisomerase, and squalene synthase mRNA accumulation was found in the middle and top zones from both the wall and core regions of the bubble column reactor. In contrast, mRNA accumulation in roots grown in the mist reactor was generally higher in the wall region compared to the core. Similar to results observed in bubble column reactors, relative levels of mRNAs were greater in the middle and top zones of the wall region. Changes in oxygen level did not correlate with changes in terpenoid gene transcriptional induction throughout the different zones in both types of bioreactors. From our results, it appears that a combination of factors affecting gene expression in large-scale cultures might be involved.

P-1016

Utilization of RNA Interference to Confer Resistance to the Soybean Cyst Nematode, *Heterodera glycines*. RYAN M.STEEVES, Timothy C. Todd, and Harold N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. E-mail: rsteeves@plantpath.ksu.edu

The soybean cyst nematode, *Heterodera glycines*, is an important pest in soybean production throughout the United States and the world. Efforts to control soybean cyst nematode have traditionally focused on plant breeding. However, with the discovery of RNA interference (RNAi), new methods of control may be possible. In this study, we seek to utilize RNAi to provide broad-spectrum resistance against soybean cyst nematode by expressing double stranded RNA (dsRNA) in soybean roots that is homologous to essential mRNA transcripts found in *Heterodera glycines*. Three candidate genes were chosen from WormBase (www.wormbase.org) based on whether the genes loss of function resulted in sterility and/or embryonic lethality in gene knockout analysis on *C. elegans*. Degenerate primers were designed to amplify a portion of each of the candidate genes from *Heterodera glycines* genomic DNA and/or a cDNA library. 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. To determine whether the RNAi constructions would be lethal to the soybean cultures, transient assays were performed by co-bombarding soybean callus with GFP and the sense-antisense constructs. Results from the transient assay indicate that the constructs did not result in any noticeable silencing in the plant cultures. Transformation of soybean callus via particle bombardment was done with each of the three sense-antisense RNAi constructs. Twenty-two independent clones from one of the RNAi bombardments were PCR positive for the selectable marker. Of these, nineteen were identified as positive for the RNAi construct. Fertile transgenic plants from this construct have been regenerated. Molecular analysis and bioassays will be discussed.

P-1017

Genetic Transformation and Plant Regeneration in *Stevia rebaudiana* Using Microprojectile Bombardment. KAYE M. KNOWLES, Seema Dhir, M. Singh, and Sarwan Dhir. Center for Biotechnology, Fort Valley State University, Fort Valley, GA 31030. E-mail: dhirs0@.fvsu.edu

The purpose of this study was to develop a high frequency somatic embryogenesis system for *Stevia rebaudiana*; a perennial herb valued as a natural source of sweetener production. Multiple shoots (24-30 shoots/explants) were induced from shoot tips cultured on MS medium supplemented with 2.0 mg/L of BA + 0.1 mg TDZ. Green true shoots with fully developed leaves were observed in almost 70% of initial cultures. Maximum elongation of shoots was achieved with 2.0 mg/l of BA + 0.1 mg TDZ with 30 gm/L of maltose. Roots were induced in 30 d old shoots, upon transfer to MS medium individually supplemented with IAA or IBA (1-4 mg/L). Several plant growth regulator's 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,3,5-triodobenzoic acid (TIBA), alone or in combination with cytokinins (BA, KN or ZT) were tested for their capacity to induce somatic embryogenesis from leaf segments using micro propagated tissue culture raised plants. The leaf segments produced embryogenic callus on MS medium supplemented with 0.5 mg/l 2, 4-D and 0.3 mg/l BA in approximately 75% of explants (19-20 embryos/explants) cultured. Embryos at various developmental stages (globular, heart and torpedo shaped) were observed. Particle bombardment of leaf segments and embryogenic calli were performed at various levels of acceleration pressure (450-1800 psi). An average of 30 to 35% leaf segments and young embryogenic callus tissue expressed transient GFP gene expression at 1100 psi with a 6 cm distance from stopping screen to target tissue using gold particles. Leaf and embryogenic tissues bombarded with GFP gene were sub-cultured on embryo induction medium. Embryos at various developmental stages (globular, heart and torpedo shaped) expressing GFP genes are being recovered. Experiments on natural sensitivity and selection of transformed tissue under selective agents and PCR analysis of transgenic material will be presented.

P-1018

Shoot Formation on *Agrobacterium* Co-cultivated Tissues of Teak. S. N. WIDIYANTO, H. Rahmania, and S. Suhandono. Department of Biology, Institut Teknologi Bandung, Bandung - 40132, Indonesia. E-mail: srinanan@bi.itb.ac.id

Teak (*Tectona grandis* L.f.) is an important forest tree species for its high quality timber wood. A transformation procedure was applied by co-cultivating explants with *Agrobacterium tumefaciens* LBA4404 carrying the pBI121 binary vector. Prior to co-cultivating with *A. tumefaciens*, explants were placed on callus induction media consisted of woody-plant-medium supplemented with 1.0 μM thidiazuron and 0.01μM indole butyric acid for pre-conditioning. After co-cultivation, explants were cultured on callus induction media added with 100-200 mg/l kanamycin for recovery and selection. Results showed that selected tissues were resistant to kanamycin. Slow-growing selected tissues were becoming small visible callus clumps in 4-6 weeks. During the period of subcultures, selected callus clumps had performed its stable kanamycin resistance, persistently grown and produced larger callus clumps. The addition of 10 μM 6-benzyl adenine and 1.0 μM gibberellic acid-3 induced shoot formation on selected callus tissues after 4-6 periods of subcultures. Regenerated shoots were elongated and constantly performing its resistance to kanamycin on medium containing 100-200 mg/l kanamycin. Selected transformed teak shoots showed positive GUS staining results based on histochemical GUS assay.

P-1019

Towards a Mucosal Vaccine Against HIV. N. MATOBA, M. Bomsel, C. J. Arntzen, and T. S. Mor. Department of Plant Biology, Arizona Biodesign Institute, Arizona State University, P.O. Box 871601, Tempe, AZ 85287-1601. E-mail: Nobuyuki.Matoba@asu.edu

P1 peptide, corresponding to a portion of the gp41 envelop glycoprotein of the Human Immunodeficiency Virus (HIV), has been suggested to be a mucosal vaccine target against HIV which would be able to induce antibodies, especially secretory IgAs, to prevent transmission of HIV across the mucosal barrier (Alfsen et al., 2002). In this study, we are trying to create an efficacious mucosal HIV vaccine by constructing a fusion protein where the P1 peptide is genetically linked to the C-terminus of the well-known mucosal adjuvant as well as antigen carrier to mucosa-associated lymphoid tissue, cholera toxin B subunit (CTB). Recombinant CTB-P1 fusion protein expressed in *E. coli* BL21(DE3) by the use of the Novagen pET expression system was successfully shown to form pentamers by directing the protein transported to the periplasmic space as observed in the native CTB. Moreover, the CTB-P1 fusion was detected by the antibodies specific to CTB as well as the P1 peptide in the G_{M1} ganglioside-ELISA. Investigation is being conducted on the immunogenicity of the purified CTB-P1 fusion protein by oral immunization to a mice model. It has been demonstrated that edible plant vaccines are safe, efficacious and cost-effective alternatives to conventional vaccines. These findings prompt us to create transgenic plants expressing the CTB-P1 fusion protein as a candidate of mucosal HIV vaccines. Plant expression vectors under the control of cauliflower mosaic virus 35S promoter were constructed for the gene encoding CTB-P1 fusion as well as CTB. Tomatoes (MicroTom) were transformed with *Agrobacterium tumefaciens* LBA4404 harboring these vectors. Screening of the transformants is currently underway.

P-1020

Metabolic Engineering Phytoalexins from the *Vitaceae* Improves Antifungal Activity in Tropical Plant. Y. J. ZHU, C. S. Tang, and P. H. Moore. Hawaii Agriculture Research Center, Aiea, HI 96701. E-mail: jzhu@harc-hspa.com

Phytoalexins have been shown to be important natural components in the defense of plants against pathogen infection. Several fruit crops, including grapevine and peanut synthesize the stilbene-type phytoalexin, Resveratrol, (trans-3,4', 5-trihydroxy-stilbene) when attacked by fungal pathogens such as, *Botrytis cinerea* or *Plasmopara viticola* (Blaich, 1980). The level of resistance to *P. viticola* was positively correlated with the capacity of *Vitis spp.* to synthesize stilbene (Dercks, 1989). We conducted *in-vitro* pathogen inhibition assays to show that Resveratrol inhibited fungal pathogens of tropical plant, papaya (*Carica papaya L.*). Resveratrol at 1.0 m*M* in V8 agar culture medium inhibited mycelia growth of *Phytophthora palmivora* up to 50% of control. The compound was active against *P. palmivora* as low as 100 m*M*. Resveratrol was not as effective against the anthracnose pathogen, *Colletotrichum gloeosporiodes*. Further, we transformed papaya embryogenic cultures with the *stilbene synthase* gene cloned from grapevine and driven by its own inducible promoter along with the hygromycin resistance or kanamycin resistance gene under the control of a CaMV35S promoter. The presence of transgenes was confirmed by PCR and Southern blot analysis. Twenty lines of transgenic papaya plants were challenged with *P. palmivora* in greenhouse condition. Data from greenhouse studies showed that disease level in transgenic plant was reduced to 35% of the disease level in non-transformed control plants. This result indicated that metabolic engineering *Carica papaya* with heterologous phytoalexins either under its own inducible promoter or a constitutive promoter can improve plant defenses against fungal pathogens.

P-1021

A Novel Protocol for Regeneration of Soybean from Mature and Immature Cotyledon: Suitable for Genetic Transformation. G. FRANKLIN, E. Davis, S. Ismail, L. Carpenter, A. Hampton, B. Smith, S. Ibeji, J. McDougall, B. Sadia, M. Parani, S. L. Goldman, and R. V. Sairam. Plant Science Research Center, The University of Toledo, Toledo, OH 43606. E-mail: gfranklin71@yahoo.com

Genotypic independent regeneration of fertile soybean plants has been achieved from the proximal end of mature and immature cotyledons via organogenic nodule-like structures. Regeneration of shoots ranged from 69-84% in different cultivars. Induction of nodule like structures and their subsequent differentiation into shoot buds (4-19/explant) has been observed on modified MS medium containing TDZ and BAP. The regenerated shoot buds elongated and rooted on MS medium containing 0.1 mg/l GA3 and 0.5 mg/l NAA, respectively. Plantlets were transferred to greenhouse with 87% success, where they attained maturity and produced viable seeds. Preliminary experiments with *Agrobacterium tumefaciens* have shown a great promise towards developing a novel protocol for routine soybean transformation.

P-1022

Shoot Organogenesis in *Nicotiana* Species: Shoot Production per Responsive Leaf Explant Increases Exponentially with Explant Organogenic Potential. B.-C. LI, W. (Q. W.) Huang, and T. Bass. Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY 40546-0236. E-mail: bli2@uky.edu

As part of the effort to develop optimal plant varieties for plant-made pharmaceuticals (PMP) production and molecular farming, this study evaluated shoot organogenic potential of a total of 115 accessions, representing 53 *Nicotiana* species. To induce shoots, leaves from in-vitro-grown seedlings were cut into pieces, cultured on shoot-induction medium under low light for 3 weeks, and then subcultured onto the same medium for another 4 weeks under normal light. Statistical analysis detected significant differences among the 115 accessions for the percentage of leaf explants producing shoots and the number of shoots produced per responsive leaf explant. Importantly, regression analysis also found an exponential relationship between the number of shoots produced per responsive leaf explant and the percentage of leaf explants producing shoots. The number of shoots produced per responsive leaf explant increased rather slowly, ranging from zero to around five, as the percentage of leaf explants producing shoots increased from 0% to 80%, but the increase became dramatic as the percentage increased from 80% to 100%, reaching as high as 35 shoots per responsive leaf explant. To our knowledge, this exponential relationship is the first of its kind to be established in plant regeneration studies using either organogenesis or somatic embryogenesis systems. A possible mechanism governing this exponential relationship is discussed.

P-1023

Micropropagation of *Cordyceps sinensis* (Berk) Sacc., a High Value Medicinal Fungus Wildly Growing in Himalayan Region. NARENDRA KUMAR and P. S. Negi. Defence Research & Development Organization, Defence Agricultural Research Laboratory, Pithoragarh 262 501, INDIA. E-mail: dirdarl@sancharnet.in

Cordyceps sinensis or Caterpillar Mushroom is a high value medicinal, non-toxic, entomogenous fungus, which is found growing in the higher hills (1200 ft. To 16000 ft. Altitude) of Himalayas including Nepal, China, Tibet and India. The specimens of this fungus are very rare, occurring in the far reaches of high, cold and arid hills at an altitude between 12000 ft. to 16000 ft. above the sea level. This fungus is having a high medicinal value and is used in traditional remedies for various physiological disorders. Being a hormone stimulator, *Cordyceps* is an important anti-aging medicine. It also inhibits the formation of active monoamine oxidaze, an enzyme responsible for aging in man. The frequent use of this fungus may prevent the senile disorder. *Cordyceps* is found beneficial in case of climatic age illness, impotence, emission, neurasthemia, rheumatoid arthritis, cirrhosis, flabby waist and knee. *Cordyceps* has been in traditional use for the treatment of various diseases like chronic bronchitis, insomnia, hypertension, pneumonia, tuberculosis, pulmonary emphysema, anemia, night sweats and cough. Pure culture of high value medicinal fungus *Cordyceps sinensis* has been raised in the laboratory after culturing tissues from stromata portion of the living specimen. The culture of this fungus is possible on different types of the culture media like potato dextrose agar (PDA), beef extract dextrose agar (BEDA), casein hydrolysate dextrose agar (CHDA), soybean extract dextrose agar (SEDA) and rice extract dextrose agar (REDA). Optimum rate of mycelial growth was observed on casein hydrolysate dextrose agar medium followed beef extract dextrose agar, potato dextrose agar, soybean extract dextrose agar and rice extract dextrose agar. The fungus prefers acidic pH (5 to 5.5) of the culture media and low temperature condition between 10 to 15° C for its optimum growth under *in vitro*. With the result mycelia having numerous spores are observed under the microscope.

P-1024

Micropropagation Saves the Endangered *Musa* Germplasm in the FSM. P. C. Josekutty, H. ANDREW, N. H. Nena, R. A. George, T. N. Kilafwasru, and S. S. Cornelius. Micronesia Plant Propagation Research Center, College of Micronesia-FSM, Land Grant Program, College of Micronesia, PO Box 1000, FM 96944, F S Micronesia. E-mail: ptculture@mail.fm

Banana (*Musa troglodytarum*) varieties *"Kulasr and Kulundol"* are endangered species. These varieties, once abundant in the Micronesia became endangered probably due to the degradation of habitat, climate change, introduced diseases and pest and lack of organized cultivation. Traditionally people used them as baby food. Recent investigations in to the traditional wisdom associated with these bananas revealed high levels of carotene (precursor of vitamin A) in them. Federated States of Micronesia (FSM) population has severe vitamin A deficiency problem (VAD). Banana being a staple food in the FSM, availability of vitamin rich banana to the population could help reduce VAD. Therefore, micropropagation of these bananas were undertaken with duel objectives of saving this endangered species through rapid, large scale multiplication and field planting and addressing the VAD problem by making this VA rich fruits available to the FSM population. These banana varieties required low salt medium (1/2 strength Murashige and Skoog 1962 (MS) salts) and very little Benzylaminopurine (1 mg l^{-1} BAP) for multiplication. Culture initiation required longer time (100-120 days). Elevated levels of thiamin (1.0 mg/l) and potassium phosphate (250 mg/l) increased the growth and multiplication rate (7-10 shoots per explant). *Ex vitro* rooting was difficult but 1 mg l^{-1} indoleacetic acid on ½ strength MS medium containing 1% (w/v) activated charcoal resulted in 100% rooting in 7-10 days time. Hardening was effortless in a greenhouse with humidity and temperature control. The banana population in Kosrae, FSM is raised from less than 50 plants to over 1000 plants in one year demonstrating the power of micropropagation in saving endangered plants.

P-1025

Micropropagation and Field Performance Evaluation of Eight Micronesian Bananas. P. C. JOSEKUTTY, S. C. Cornelius, T. N. Kilafwasru, R. A. George, and N. H. Nena. Micronesia Plant Propagation Research Center, Land Grant Program, College of Micronesia- FSM, College of Micronesia, PO Box 1000, Kosrae, FM 96944, F S Micronesia. E-mail: ptculture@mail.fm

Eight varieties of Micronesian bananas (*Musa sp.*) namely *"kufwafwa, apat fussuse, apat regular, lacatan, usr wac, usr in yeir, usr kulasr and usr kulundol"* were micropropagated and evaluated for their field performance since 1999. Nutritional requirements and growth regulator requirements for growth and multiplication considerably differed among varieties. *Musa troglodutarum* varieties multiplied better in low salt (1/2 strength Murashige and Skoog 1962 (MS) medium) supplemented with just 1 mg/l^{-1} Benzylaminopurine (BA) compared to full strength MS medium and 3-8 mg l^{-1} BA for regular bananas with AAA and AAB genotypes. Considerable difference, regarding the time in the culture and origin and extend of somaclonal variation in the micropropagated plants existed among the varieties. Some of the variegated phenotypes developed normally, produced excellent bunches and reproduced variegated baby plants indicating a very stable genetic change.

P-1026

In Vitro Shoot Initiation of *Artocarpus heterophyllys* Lam. (Jak Fruit)—Effect of the Explant Type and the Season of Explant Collection. W. T. P. S. K. SENARATH*[1], K. A. H. K. Kasturiarachchi[1], and Kui Jae Lee[2]. [1]Department of Botany, University of Sri Jayawardhanapura, Nugegoda, Sri Lanka; [2]Faculty of Bioresources Sciences, College of Agriculture, Chonbuk National University, Chonju 561-756, Republic of Korea; and *Present address: Faculty of Bioresources Sciences, Chonbuk National University, Republic of Korea. E-mail: senerath@yahoo.co.uk

A method for rapid propagation of mature Jack fruit from apical meristem culture was developed. Four types of explants (mature embryos, apical meristems of young seedlings, apices from mature plants and nodal segments) were used in order to initiate shoots *in vitro*. The embryos from seeds soaked in water for 24 hours produced shoots after 8 weeks of incubation and the success rate was 70% while embryos from dry seeds only produced roots. There was no significant effect of cold storage (refrigeration) for 7 days on shoot initiation from mature embryos (65%) but the ability for shoot induction declines with time (55% after 21 days of cold storage). It has been found 88% of young apical meristems produced shoots in Campbell and Durzan (CD) medium compared to 60% in Murashige and Skoog (MS) medium. Only 1/3 of them produced multiple shoots. Shoot initiation from nodal segments was very rare. Mature apices produced callus. It has been observed that the callus production depends on the incubation temperature. Callus was induced at the base of the shoot by increasing the incubation temperature form $25\pm1°$ C to $30\pm1°$ C. Growth of the callus was retarded by accumulated phenolic compounds in the medium. Removal of the sheathing cover around the bud enhanced the shoot initiation from mature apices. Mature apical meristems were established in Modified Campbell and Durzan (CD) medium supplemented with 0.5 mg/l and IBA. Cultures were sub-cultured in every 4 weeks interval in order to reduce the accumulation of phenolic compounds. Reducing the accumulated phenolics at the base of the explant enhanced the growth rate. There was a significant difference in the growth performance of shoots and callus produced according to the period of the year in which explants were collected. 60% of the mature apices produced multiple shoots in November to December period. It was only 30% when the apices were cultured in April to May and decreased to 20% in June to July. The shoots produced in November to December period showed a higher vigour [in number of leaves per shoot (2.9 ± 0.15), mean leaf width (7.5 ± 1.1) and mean shoot length (26.0 ± 0.95)] than those produced in other months. Since jack fruit show seasonal changes in fruit bearing and shedding of leaves, it can be suggested that the difference in growth performances of tissues cultured in artificial culture media would have been effected by endogenous rhythms.

P-1027

Shoot Tip Culture: A Powerful Model System for In Vitro Flowering and Transformation Studies in Maize (Zea mays L.). SWATI BHARGAVA, Alok Shukla, and R. C. Pant. Department of Plant Physiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar-263145, India. E-mail: swati_gemini1@rediffmail.com

Seeds of maize (Zea mays L.) inbred lines namely, D003, D995, D994 and Gaurav were selected for present investigation. Seeds were surface sterilized with 0.1% mercuric chloride for 3 minutes with the addition of 2-3 drops of detergent for sterilization. After washing with sufficient amount of sterilized water the seeds were kept for germination on 0.5% agar at 28 degree centigrdae in a BOD incubator. Shoot tips were excised from 4 days old seedlings and were placed on MS medium supplemented with 2 mg/l benzylamino purine and 500 mg/l casein hydrolysate. They were then placed at 25 degree centigrade in tissue culture room. Shoot multiplication was observed in all the cultivars used for investigation. This system is highly reproducible efficient and can be exploited for in vitro flowering and transformation studies in maize.

P-1028

Micropropagation of *Papuacalia versteegii*, an Important Endemic Plant to Mount Jaya. ERLY MARWANI[1] and W. Sarosa[2]. [1]Dept. of Biology, Institute of Technology Bandung, Jl Ganesha 10, Bandung 40132, Indonesia and [2]Environmental Dept. PT Freeport, Indonesia. E-mail: erly@bi.itb.ac.id

Papuacalia versteegii is an endemic to sub alpine forest and shurblands of Mount Jaya. The populations of this shrub decrease drastically as a result of open mining exploitation. With goal to replanting the damaged land with native plant, mass production of *P. versteegii* was required. However, due to low seed viability and poor germination, *P. versteegii* was difficult to be propagated. Therefore, a protocol of *in vitro* culture for rapid propagation was investigated. Explants of shoot apices and axillary shoots were cultured on Murashige and Skoog (MS) media supplemented with various concentration of Benzylaminopurine (BAP), kinetin, either alone or in combination with Naphtaleneacetic acid (NAA). The highest number of shoots of 5.5 was regenerated from axillary shoot which cultured on MS supplemented with 0.01 m*M* NAA and 5.0 m*M* BAP, whereas, the shoot apex on that media only produced 3.0 shoots. The regenerated shoots rooted either on full or half-strenght MS media containing various concentrations of NAA and/or IBA (Indolebutyric acid). The shoots best rooted on half -strenght MS media with 0.01 m*M* NAA + 1.0 m*M* IBA. The regenerated planlets, when acclimatized in soil under greenhouse condition, showed 90% survival. Using this protocol, it would be possible to produce over 15.000 plants in the period of six months.

P-1029

Somatic Embryogenesis from Ovules of Kinnow (*Citrus nobilis* X *C. deliciosa*) for Elimination of ICRSV. G. RANI, B. Singh, S. Sharma, A. A. Zaidi*, V. Hallan*, A. Nagpal, and G. S. Virk. Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143 005, India and *Division of Floriculture, Institute of Himalayan Bioresources and Technology, Palampur, India. E-mail: gitarani_24@yahoo.com

An efficient procedure has been developed for inducing somatic embryogenesis from ovules of kinnow (*Citrus nobilis* Lour X *C. deliciosa* Tenora). The ovules excised from unopened flower buds were cultured on Murashige and Skoog's (MS) basal medium supplemented with various concentrations of kinetin (KN) or malt extract (ME). Maximum induction (31.94%) of embryogenic callus was noticed on MS medium supplemented with KN (9.29 μM). The transfer of embryogenic calli to similar media conditions resulted in somatic embryogenesis in all cultures (100%), with an average of 60 globular somatic embryos per culture. After 60 days of initial subculturing of embryogenic callus, the globular embryos turned to cotyledonary-shaped embryos (7.71 ± 0.08 per culture) which developed into complete plantlets using similar media conditions. Embryogenic callus induction, somatic embryo formation, maturation, germination and plantlet formation were achieved on MS medium supplemented with KN (9.29 μM) alone. The plantlets were transferred to sterilized soil and sand (1:1) mixture, where they were subsequently grown to maturity. Embryogenic callus and plantlets were screened for Indian citrus ring spot virus (ICRSV) elimination employing ELISA and RT-PCR, and found free of CRSV. A distinct feature of this study is the induction of somatic embryogenesis using only KN (9.29 μM) for all steps starting from embryogenic callus induction to plantlet formation, and the technique can be used to produce virus-free plants.

P-1030

Interactions Between Post-transcriptional and Transcriptional Silencing Pathways in *Arabidopsis thaliana* A. S. CALLAWAY, L. D. Jones, D. A. Sowinski, W. F. Thompson, and G. C. Allen. Department of Botany, North Carolina State University, Raleigh, NC 27695. E-mail: anton@unity.ncsu.edu

Gene silencing has a profound impact on the net level of transgene expression. Gene silencing has been operationally divided into two major forms: transcriptional gene silencing (TGS) whereby transcription initiation of the gene is blocked and post-transcriptional gene silencing (PTGS) which is manifested through the degradation of transcripts from the gene. Recent genetic data using silencing mutants of *Arabidopsis thaliana* suggest that these two silencing mechanisms are not entirely independent. Our study explores the interactions between TGS and PTGS using populations of transgenic Arabidopsis lines transformed with an intron-bearing luciferase reporter gene driven by either the CaMV 35S promoter or the octopine synthase promoter. The expression cassettes are either flanked by copies of the RB7 matrix attachment region (MAR) or not. The presence of flanking RB7 MARs has been shown to reduce gene silencing in transgenic plants. Our experiments are designed to test the hypothesis that transgenes initiating abundant transcript synthesis (e.g. driven by strong promoters and flanked by MARs) are more likely to be silenced at the post-transcriptional level than when transcript production is low. We are also comparing expression patterns at different developmental stages to determine when silencing occurs and following expression through several meiotic generations to assess the effect of MARs on the stability of transgene expression.

P-1031

A New Glyphosphate Tolerance Strategy in Transgenic Crops. MICHAEL W. LASSNER[1], Daniel L. Siehl[1], Rebecca Gorton[1], Sean Bertain[1], Hyeon-Je Cho[1], Donglong Liu[2], James Wong[1], Nick Duck[2], and Linda A. Castle[1]. [1]Verdia, Inc., Redwood City, CA 94063 and [2]Pioneer/Dupont, Johnston, IA 50131. E-mail: mike.lassner@verdiainc.com

Plants can be genetically modified to tolerate non-selective herbicides using two general strategies. In the first strategy, crop plants express a gene encoding a target site enzyme that is insensitive to the herbicide. This strategy is used in Roundup Ready(TM) crops that express a 5-enol-pyruvyl shikimate-3-phosphate (EPSP) synthase enzyme that is insensitive to glyphosate and in Clearfield (TM) crops that tolerate imidazolinone herbicides because they have an insensitive acetohydroxyacid synthase (AHAS) target enzyme. The second strategy involves inactivation of the herbicide either through degradation or modification. Examples of this strategy include phosphinothricin tolerant crops expressing the N-acetyltransferase genes, *pat* or *bar*, and bromoxynil tolerant crops expressing the *bxn* nitrilase gene. Detoxification of the applied herbicide not only protects the modified crop, it also removes herbicide residues from the environment.
Until recently, there were no known enzymatic activities that could detoxify glyphosate in a wide spectrum of host plants. Our advantage in developing novel glyphosate detoxification enzyme comes from the power of directed molecular evolution technologies. These gene improvement technologies obviate the need to discover enzymes exhibiting biological activity because we can evolve poor enzymes to improve their kinetic properties. Using a highly sensitive mass spectrometry method, we screened a microbial diversity collection to discover *Bacillus* glyphosate N-acetyltransferase genes (*gat*). The native *gat* genes are not capable of conferring a glyphosate tolerant phenotype to *E. coli* or plants. DNA shuffling was used to improve the enzyme activity more than 2000-fold. Expression of improved *gat* genes in tobacco, *Arabidopsis*, *Astragalus*, and maize confers glyphosate tolerance suitable for the selection of transgenic plants. Soil-grown plants tolerate high doses of glyphosate. Importantly, transgenic *gat* plants are morphologically normal and fertile. This new glyphosate tolerance strategy appears to be applicable to all plants and is being evaluated in several major crops.

P-1032

Profiling of Differentially Expressed Genes in Yam (*Dioscorea rotundata*) During Post-harvest Storage. S. KONE-COULIBALY, M. Egnin, and G. He. Biotechnology Laboratory, Tuskegee University, Tuskegee, AL 36088. E-mail: megnin@tusk.edu

Yam, Dioscorea spp., principally white Guinea yam (*Dioscorea rotundata* Poir) is an important staple food and source of carbohydrate for people in many developing countries of Asia, South America, and Africa, especially West Africa. The rampant increase in production and consumption of yam is, however, hampered by a lack of control of its dormancy. Dioscoreaceae dormancy is an important adaptative mechanism that helps to maintain organoleptic quality during storage, and at the same time creates a major problem for plant breeders. The duration of yam dormancy is predetermined and the inability to break yam dormancy limits not only the global commercial production on a large and small scale, but also its genetic manipulation for new available varieties. Attempts to artificially manipulate dormancy utilizing chemicals such as Gibberellic acid (prolongation) or 2-chloroethanol and 2-chloroethyl trimethyl ammonium chloride (breaking) have been achieved, however with little understanding of the physiological and molecular mechanisms that trigger the onset and the breakage of dormancy. Thus, elucidating the molecular changes taking place in yams during post-harvest storage will help understand the process of tuber dormancy. Total RNA were isolated from dormant yam tuber incubated at 32° C with relative humidity of 50% ±5% for 0 h, 2 days, 4 days, 7 days, and 15 days. cDNA generated from total RNA was subjected to cDNA-AFLP techniques to gain molecular insights and identify differentially expressed genes up-regulated and down-regulated during the dormancy in yam tubers. Two primer pairs have been identified that had equal potential for producing the same number of Transcript Derived Fragments (TDFs) in dormant yam samples. The resulting TDFs from post-harvest treated tubers will aid in the selection of putative up and down regulated fragments during the yam dormancy. Work supported By USDA and NASA.

P-1033

Chromatin Structure of T-DNA Integration Sites in Arabidopsis. K. E. FRANCIS and S. L. Spiker. Department of Genetics, North Carolina State University, Raleigh, NC 27695. E-mail: kefranci@unity.ncsu.edu

Controversy exists within the plant transformation literature regarding T-DNA integration into plant genomes. T-DNA integration has been suggested to occur randomly, and alternatively has been suggested to occur preferentially into transcriptionally active regions. Previous investigations of transgene integration may have been biased towards transgenes that have integrated into active chromatin, and have not adequately examined integrations into *in*active chromatin. Under selective conditions, only plants that are capable of expressing a selectable marker can be recovered. If a transgene integrates into transcriptionally silenced chromatin, that transgenic plant can be recovered only if transgene activity is not a prerequisite. Using a pooled-sample PCR strategy, we have identified transgenic plants without requiring selectable marker activity. Reporter gene activities in these "PCR-identified" plants and their progeny have been compared to those in "kanamycin-selected" lines. PCR-identified lines appear to have a higher occurrence of silenced transgenes, suggesting that some integrated transgenes are never expressed. Transgenic plants that show little or no transgene activity have been identified, and the chromatin structures at the sites of integration are being analyzed.

P-1034

SSR Markers as a Suitable Tool for Checking Recombination Events During Somatic Embryogenesis from Floral Explants in Grapes. L. MARTINELLI, M. S. Grando, J. Zambanini, V. Poletti, E. Maffettone, and R. Marconi. Istituto Agrario, 38010 San Michele all'Adige (TN), ITALY. E-mail: Lucia.Martinelli@ismaa.it

Anthers and ovaries are suitable explants for somatic embryogenesis induction in the *Vitis* genus (Martinelli & Gribaudo, 2001, Vitis 40:111-115); however, the occurrence of recombination, resulting from likely mejotic events, needs to be checked. The involvement of sexual cells in morphogenesis is usually assessed with histology and cytology, but the large number (38) and the small size (0.1μm) of the grape chromosomes are critical constraints. Besides, no information on the regenerant genotypes are achieved. A molecular typing of regenerant vines based on co-dominant simple sequence repeat (SSR) markers (Grando & Frisinghelli, 1998, Vitis 37:79-82) is an available tool for the recombinant line identification. Samples of somatic embryos and plantlets from embryogenic callus of both anthers and embryos and ovaries of the *V. vinifera* cv. Chardonnay, the rootstock Kober, and the accession *V. rupestris* du Lot were randomly chosen, and the SSR pattern analysis was performed. The genotype at polymorphic VVS2, VVMD5, VVMD7, VVMD27, ssrVrZAG62 and ssrVrZAG79 loci was produced and compared with reference varieties. In all the checked samples, no recombination events seemed to occur in the cells involved in the somatic embryogenesis induction, as all the samples showed the SSR marker pattern of the grape variety from which explants were taken, thus excluding the participation of cells of the sexual lines in the morphogenesis. (Chardonnay embryos were kindly provided by Dr. I. Gribaudo).

P-1035

Transgenic "Sweet Rice" Expressing a Thermostable Amylopullulanase in Seeds Leads to Starch Autohydrolysis and Production of Nutritionally-improved High-protein Flour. C.-M. Chiang, F.-S. Yeh, J.-F. Shaw, and S.-M. YU. Institute of Molecular Biology and Institute of Botany, Academia Sinica, Nankang, Taipei 11529, Taiwan, R.O.C. E-mail: sumay@ccvax.sinica.edu.tw

Commercial bioprocessing of starch and production of high-protein flour from plant storage organs uses thermostable microbial enzymes to accelerate starch hydrolysis and separation of protein from sugars. Transgenic rice seeds producing a thermotolerant and bi-functional starch hydrolase, amylopullulanase (APU), were generated to improve the cost effectiveness and efficiency of starch bioprocessing. No adverse effect on plant development, seed formation or seed starch accumulation was observed. Rice seed containing different amounts of amylose, which affects rice quality, was obtained by expressing various levels of APU. Starch in transgenic seeds could be hydrolyzed with optimal temperatures between 85-95° C. Seeds heated to 85° C resulted in complete conversion of starch into soluble sugars and production of high-protein rice flour within a few hours. This study establishes novel approaches to alter amylose content in seeds, accelerate bioprocessing of starch, and produce nutritionally-improved high-protein flour from rice seeds.

P-1036

Chemical Warfare Countermeasures: Expression of Human Acetylcholinesterase in Plants. S. P. FLETCHER[1], B. Geyer[1], M. Muralidharan[1], C. J. Arntzen[1], H. Soreq[2], and T. S. Mor[1]. [1]Plant Biology Department, Arizona State University, Tempe, AZ 85287 and [2]Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel. E-mail: spfletc@asu.edu

The use of plants as a production system for therapeutic proteins offers advantages in production scale, affordability, safety, and administration possibilities. Application of cholinesterases is of considerable interest as a potential efficacious medical intervention against cholinergic nerve agents, yet current production systems impede the safe delivery and affordability needed for their administration. Previously we have introduced transgenic plants as a potential system to produce human acetylcholinesterase (AChE); however, expression levels were rather modest. Here we report on the optimization of constructs encoding four human acetylcholinesterase isoforms, their synthesis *de novo* and their expression in transgenic *Nicotiana benthamiana* and *Lycopersicum esculentum* plants. Expression levels were dramatically improved using the optimized constructs as compared to their native counterparts. Our results further support the oligomerization of some of these isoforms. Interestingly, we observe the purported native plant cholinesterase activity, which can be biochemically distinguished from the recombinant human enzyme. Thus, we hope that transgenic plants over-producing human AChE may not only buttress chemical warfare defense, but also shed light on the little-studied plant cholinesterase function and regulation.

P-1037

Plant-based Production of a Subunit Mucosal Vaccine for Pneumonic Plague. BONNIE J. WOFFENDEN[1], Carole L. Cramer[1,2], and Fabricio Medina-Bolivar[1]. [1]Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, and [2]BioDefense Technologies, Inc., Blacksburg, VA 24061. E-mail: bwoffend@vt.edu

The threat of bioterrorism via pneumonic plague is significant due to the rapid spread of aerosolized *Yersinia pestis*, and rapid lethality (1-3 days) in nearly 100% of untreated cases. The whole-organism vaccine has safety concerns and is not proven efficacious for the pneumonic form of plague. Our goal is to develop a plant-based subunit vaccine for pneumonic plague to address key limitations of the current vaccine: mucosal efficacy, ease of delivery, rapid scalability, safety, and cost. A subunit vaccine based on a fusion protein of the plague antigens F1 and V administered parenterally with Alum adjuvant (non-mucosal) was recently shown to protect mice against multiple plague strains [Alpar et al., Adv. Drug Deliv. Rev. 51(2001):173-201]. However, mucosal delivery of antigen is expected to confer better protection against the pneumonic form of the disease. We have identified a plant lectin (MAC1) with utility as a mucosal adjuvant. Intranasal administration of MAC1:antigen fusions, purified from our tobacco hairy root expression system, was highly effective in stimulating mucosal immunity in mice [Medina-Bolivar et al., Vaccine 21 (2003):997-1005] and may have potential for human use. We have engineered tobacco to express a sequence-optimized F1:V fused to MAC1. Transgenic plant lines expressing high levels of MAC1:F1:V protein will be used to generate hairy root expression cultures. MAC1:F1:V protein purified from these lines will be used for intranasal immunization in mice. Stimulation of mucosal immunity to MAC1:F1:V in mice will be a first step in the development of an improved pneumonic plague vaccine.

P-1038

Statistical Analysis of Frequency and Quality of Transgenic Maize Production from Three Transformation Methods: *Agrobacterium*, Gunpowder Gun, Electric Gun. D. D. SONGSTAD, J. Nguyen, J. Brazeal, C. L. Armstrong, N. Bogdanova, E. Levine, J. Pershing, M. Pleau, G. Head, M. Kaniewska, F. Behr, M. Stoecker, and C. Hironaka. Monsanto, Chesterfield, MO 63017. E-mail: David.D.Songstad@Monsanto.com

An experiment was designed that compared three transformation methods (PDS-1000 gunpowder gun, electric discharge gun and *Agrobacterium*) where the following factors were controlled: genotype, selectable marker and construct. Genotype used was an Iowa stiff stalk elite inbred crossed by Hi-II, selectable marker was the glyphosate resistant EPSPS, CP4, driven by the rice actin promoter (Ract) followed by the rice actin-1 intron, EPSPS transit peptide and NOS 3' end. The construct was controlled in that the linear insert for the particle bombardments was the same sequence found between the right and left borders of the binary vector used in *Agrobacterium*. In a cis-orientation with the selectable marker was an insect control construct. Gene delivery methods were assessed based on transformation frequency, insect bioassay score, and frequency of single copy event production. Statistical analyses were performed using ANOVA or Chi-Square. *Agrobacterium* transformation provided a statistically significant advantage over the gunpowder or electric guns in terms of the frequency of events with the desired traits and frequency of single copy events. However, these transformation systems appeared to have no effect on the level of transgene efficacy. The electric gun gave the highest transformation frequency (20 to 45% of the bombarded calli produced transgenic events) but had the lowest frequency of single copy/insect bioassay positive events. The data indicates that *Agrobacterium tumefaciens* is the preferred transformation method under the tested conditions. Approaches to improve quality of gun-derived events in future transformations will also be discussed.

P-1039

Gibberellin Synthesis Inhibitors Improve Conifer Embryogenic Tissue Initiation. G. S. PULLMAN. Institute of Paper Science and Technology, Atlanta, GA 30318. E-mail: Jerry.Pullman@ipst.edu

Somatic embryogenesis (SE), the most promising technology to multiply high-value coniferous trees from advanced breeding and genetic engineering programs, is expected to play an important role in increasing productivity, sustainability, and uniformity of future U.S. forests. For commercial use, SE technology must work with a variety of genetically diverse trees. Initiation in loblolly pine (*Pinus taeda* L), our main focus species, is often recalcitrant for desirable genotypes. Initiation rates of loblolly pine, slash pine (*Pinus elliottii*), Douglas fir (*Pseudotsuga menziesii*), and Norway spruce (*Picea abies*) were improved through the use of paclobutrazol, a gibberellin synthesis inhibitor. In our media, paclobutrazol was effective at concentrations ranging from 0.85-10.2 μM. Using control media (no paclobutrazol) and 1.0 mg/L (3.4 μM) paclobutrazol, initiation rates in loblolly pine, slash pine, Douglas fir, and Norway spruce were improved from 22.5 to 35.2%, 19.3 to 28.5%, 16.4 to 24.4%, and 38.8 to 48.5% respectively. Initiation rates in loblolly pine were also improved through the combination of modified ½ P6 Salts, activated carbon at 50 mg/l, Cu and Zn adjusted to compensate for adsorption by activated carbon, 1.5% maltose, 2% myo-inositol (to raise osmotic level partially simulating the ovule environment), 500 mg/l case amino acids, 450 mg/l glutamine, 2 mg/l NAA, 0.63 mg/l BAP, 0.61 mg/l kinetin, 3.4 mg/l silver nitrate, 10 μM cGMP, 0.1 μM brassinolide, and 2 g/l Gelrite. Other gibberellin inhibitors such as flurprimidol, chloromequat-Cl, and daminozide also caused statistically significant increases in loblolly pine initiation when added to medium at concentrations of 0.32, 10.1, and 0.62 μM, respectively. No detrimental effects on subsequent embryo development were observed when 29 new initiations from medium without GA inhibitor and 28 new initiations from medium containing paclobutrazol were tracked through culture capture, liquid culture establishment, cotyledonary embryo development, and germination.

P-1040

A Comparative Account of the Studies on Somatic Embryogenesis and Organogenesis in Various Cultivars of Cotton (*Gossypium* spp.). TANVEER KHAN and R. C. Pant. Department of Plant Physiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar-263145, India. Email: tanu_tinkle@yahoo.co.in

In vitro studies on *G. hirsutum* L. and *G. arboreum* L. and two hybrid cultivars of cotton were performed. Different cultivars were screened for their ability to regenerate via somatic embryogenesis and callus organogenesis. Embryogenesis was observed in cv. BD-1, BD-6, Sarvottam, Jawahar Tapti *(G. arboreum)* and SH-131 and LH-900 (*G. hirsutum*) when cultured on MS medium with different phytohormone combinations. Globular somatic embryos were observed in all the cultivars, heart shaped and torpedo stages were also observed in cv.BD-1, BD-6 and SH-131. Use of botanical microtechnique of these cultivars showed different stages of embryo development and the presence of bipolar structures confirmed somatic embryogenesis. Organogenesis was observed in cultivars BD-1, BD-6, Sarvottam, Jawahar Tapti, Hybrid H-8 and JK-Hybrid. Small succulent leaves regenerated from the callus cultured on different combinations of NAA, Kinetin and BAP. BAP was effective in shoot organogenesis while 2iP was effective for the induction of somatic embryos. Studies are being performed to confirm various factors responsible for polarity development and further regeneration in cotton.

VT-1000

Differentiation Kinetics of In Vitro 3T3-L1 Preadipocyte Cultures. K. C. O'CONNOR, H. Song, K. D. Papadopoulos, and D. A. Jansen. Tulane University and School of Medicine, New Orleans, LA 70118. E-mail: koc@tulane.edu

Engineering autologous adipose constructs from cell culture is a promising strategy to overcome limitations of conventional soft-tissue implants. A methodology is presented to experimentally determine and mathematically model the differentiation kinetics of in vitro 3T3-L1 preadipocyte cultures that can aid in construct design. Relative rates of morphological and interfacial events during adipogenesis were compared. Model results suggest that maturation of an intermediate multilocular phenotype was the rate-limiting step in morphological differentiation and had an intrinsic rate of 0.012 per day. Dislodgment of multilocular fat cells was the primary mechanism of cell loss during adipogenesis. The maximum rate of lipid droplet nucleation was predicted to precede that of coalescence by 10 days and to be three times faster. Coalescence probability was estimated to decrease from 33% to 11% for 4 and 8 micrometer diameter droplets, respectively. Fluid drainage and the cytoskeleton between droplets could have impeded coalescence. The kinetic analysis suggests that droplet ripening was the dominant mechanism of lipid production. Applications of this research include engineering of an adipose construct and predicting surgical outcome of patients requiring soft-tissue augmentation.

VT-1001

Characterization of I-11.15, an Immortalized Murine Splenic Macrophage Cell Line. A. DELAFOULHOUSE, C. Calumpong, and D. B. Drath. Department of Biological Science, California State University, Fullerton, P.O. Box 6850, Fullerton, CA 92834-6850. E-mail: ddrath@fullerton.edu

A retrovirus free colony stimulating factor 1-dependent cell line, I-11.15, originally derived from an immortalized BALB/C splenic macrophage colony was cultured and examined for the expression of several innate and adaptive effector functions typical of normal macrophages. All properties were assessed and compared to either freshly isolated or 2-day-old cultured mouse macrophages harvested from both the peritoneal cavity and the spleen. The ability to release antimicrobial and tumor killing factors including nitric oxide (NO) and tumor necrosis factor (TNF) was determined by spectrophotometric analysis. Phagocytosis was assessed by measuring the amount of ingested fluorescein-conjugated *Escherichia Coli* K-12 particles. The results indicate that I-11.15 cells have the ability to release comparable amounts of NO and TNF. Preliminary microscopic studies showed that I-11.15 cells are also capable of phagocytizing bacteria in a manner equivalent to normal mouse macrophages. The release of superoxide anion and the chemotactic abilities of I-11.15 are still under investigation. The ease by which I-11.15 cells can be grown and the stable expression of distinct effector functions make the I-11.15 cell line a particularly valuable model for study. Should any defect be found with respect to I-11.15 activity, then the cell line will take an additional value as means for understanding the molecular processes of that function.

VT-1002

Improving the Embryonic Stem Cell Test (EST) by Establishing Molecular Endpoints of Tissue-specific Development. R. BUESEN, A. Seiler, A. Visan, B. Slawik, E. Genschow, and H. Spielmann. Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET), Federal Institute for Risk Assessment (BfR), Berlin, Germany. E-mail: r.buesen@bfr.bund.de

Blastocyst-derived pluripotent embryonic stem cells (ES cells) of the mouse can be induced to differentiate *in vitro* into a variety of cell types, including cardiac muscle cells. The embryonic stem cell test (EST) is based on the capacity of murine ES cells (cell line D3) to differentiate into contracting myocardial cells. It has been developed to assess the embryotoxic potential of drugs due to evaluating the inhibition of differentiation which can be detected by microscopical analysis of beating heart muscle cells. Applying linear analysis of discriminance, a biostatistical prediction model (PM) was developed to assign test chemicals to three classes of embryotoxicity. In an international validation study the EST predicted the embryotoxic potential of drugs and other chemicals in the same manner as *in vitro* embryotoxicity tests employing embryonic cells and tissues from pregnant animals. In a joint research project with German drug companies we are attempting to improve the EST by establishing molecular endpoints of differentiation in cultured ES cells. We have studied the expression of tissue-specific proteins in ES cell cultures in the presence of embryotoxic chemicals by immunofluorescent antibody techniques, e.g. FACS analysis. The results obtained recently using molecular endpoints specific for differentiation into cardiomyocytes and neuronal cells employing intracellular flow cytometry will be presented. The inhibition of differentiation of ES cells into specific tissues by FACS analysis may provide a more objective endpoint for predicting the embryotoxic potential of chemicals than the validated microscopical analysis. In the present project the other groups are focusing on endogenous gene expression in early development by RT-PCR methods or the DNA microarray technique. As a further improvement of the EST molecular endpoints hold promise to be a suitable foundation for a target automation. *The project is funded by the German Federal Ministry for Education and Research.*

VT-1003

Growth and avb3 Expression in OPC1 Cells Grown on Thermanox® Plastic and Collagen Substrates. J. MOORE, C. Davitt, and H. Hosick. School of Biological Sciences, Electron Microscopy Center, Washington State University, Pullman, WA 99164. E-mail: moore@mail.wsu.edu.

The extracellular matrix (ECM) in mature bone is composed primarily of collagenous proteins. Of the ECM proteins present in bone, type I collagen is the most abundant. The remaining 10 percent of ECM proteins are the non-collagenous proteins, including osteocalcin and osteonectin (1,2). We have investigated substrate effect on growth and subsequent avb3 expression by a human osteoblast cell line OPC1 (6) at 16 and 36 hours, growth of OPC1 increased on collagen. At 16 hours on collagen, cell number was approximately equivalent to cell numbers on 36 h Thermanox® plastic. Electron microscopy (TEM) demonstrated possible secretory granules in the cytoplasm. Scanning electron microscope (SEM) showed surface features consistent with osteoblasts grown in vitro: filopodia, membranous extensions and flattened adhered appearance on both Thermanox® plastic and collagen. However, the OPC1 cells on collagen appeared to have higher numbers of filopodia. During early proliferation of OPC1 cells, integrin avb3 is expressed; however, avb3 appears 16 hours sooner on the collagen substrate than on Thermanox® plastic. It is known that overexpression of avb3 increases proliferation, but inhibits mineralization (4). OPC1 cells grown on collagen may begin to differentiate sooner than those grown on plastic. Because of a decrease in avb3 expression in 36 hc as compared with 16 hc, we may expect to see earlier differentiation via expression of bone matrix proteins.

VT-1004

The Extracellular Matrix Laminin, Fibronectin and Collagen IV in Green Sea Turtle Gonadal Cell Cultures. ANGGRAINI BARLIAN, S. Sudarwati, L.A. Sutasurya, and H. Hayashi. Department of Biology, Institute of Technology Bandung, Bandung 40132, West Java, Indonesia. E-mail: aang@bi.itb.ac.id

Green sea turtle, *Chelonia mydas*, is a reptile of the tropical area including Indonesia, and belongs to animals with temperature-dependent sex determination (TSD). The cell lines originated from differentiating male and female gonads have been established, and the cultures were maintained in appropriate temperatures. This *in vitro* system was used as a cellular model to study the mechanism of TSD. The aim of this research was to localize the extracellular matrix Laminin (Lm), Fibronectin (Fn) and Collagen IV (Coll IV) in turtle gonadal cells and to find whether male and female turtle cell cultures have different subunits of those extracellular matrix compared to mammalians. Male gonadal cell culture was obtained from differentiating male gonads and was maintained in 25° C, while female gonadal cell culture was obtained from differentiating female gonads and were maintained in 31° C. Lm, Fn and Coll IV were localized by immunocytochemistry using the monoclonal antibodies from mammalians, and the subunits of those extracellular matrix were determined by immunoblotting. The results of immunocytochemistry and confocal microscopy observations showed, that Lm, Fn and Coll IV were synthesized and secreted by turtle gonadal cells. The results of immunoblotting showed that subunits differences was found between the three extracellular matrix and the presence of subunit Lm (Mr 300 kDa) and Fn (Mr 200 kDa) that belongs to green sea turtle cells. The results of this research could give a new insight in cellular models of the possible role of extracellular matrix in the mechanism of sex determination and differentiation.

E-2000

The Effect of Trace Metals on Bioluminescent Dinoflagellates. P. D. CLARK. St. Andrew's Episcopal School, Ridgeland, MS 39157. E-mail: clarkp@gosaints.org

The purpose of this project was to determine whether the trace metals Iron, Copper, and Magnesium, usually present at low concentrations in seawater, added to the marine environment in varying PPM concentrations as Ferrous Sulfate ($FeSO_4$), Cupric Sulfate ($CuSO_4$) and Magnesium Sulfate ($MgSO_4$) will affect the bioluminescence of *Pyrocystis lunula* Dinoflagellates. It was hypothesized that the trace metals of Iron, Copper, and Magnesium would affect the bioluminescence of *Pyrocystis lunula* Dinoflagellates. The Dinoflagellates were cultured in 21 different flasks. Bioluminescence of the Dinoflagellates was measured in lux with a light sensitive probe. Of all the Dinoflagellates tested, the trace metals added in varying PPM concentrations did affect the bioluminescence of *Pyrocystis lunula* Dinoflagellates. After running a T-Test on the data, the differences were determined not to be significant. The effects were, however, measurable. Group H (1 PPM $MgSO_4$) had the highest overall average while group C (0.1 PPM $FeSO_4$) had the lowest overall agerage. G2 (0.01 PPM $CuSO_4$) on Day 2 had the highest average of 5.3 lux. There were many flasks on several days that tied for the lowest average in a single test. The lowest average was 0.3 lux.

E-2001

Identification of Drug Resistant Loci in Breast Cancer Cells by RAPD-PCR Fingerprinting. RAYMOND MAILHOT[a], Kamleshwar P. Singh[b], Satya Narayan[c], and Deodutta Roy[b]. [a]The Altamont School, Birmingham, AL 35213; [b]Department of Environmental Health Sciences, University of Alabama, Birmingham, AL 35294; and [c]Shands Cancer Center, University of Florida, Gainsville, FL 32610. E-mail: kasingh@uab.edu

The contribution of common genetic variation to cancer risk is an emerging focus in the study of genetics and disease. In this study, we have screened the genome to identify the loci that differentiate the various genotypes of breast cancers as well as the drug resistant breast cancer cells from the drug sensitive ones. Seven random ten-mer primers were used for Random Amplified Polymorphic DNA (RAPD)-PCR fingerprinting of breast cancer cells. Of these, five primers produced reproducible and scorable amplification fingerprints. One of the primers, OPA17, revealed noticeable intensity differences of RAPD amplification products between DNA samples of estrogen-responsive breast cancer cells (MCF-7) and estrogen-independent breast cancer cells (MDA-MB). Most interestingly, the DNA fingerprint generated by primer OPAA02 from drug resistant breast cancer cells was distinctly different from other breast cancer cells. The novel findings of this study are (1) RAPD fingerprinting is an efficient method for detecting mutations in the genome of breast cancer cells. (2) Several mutations resulting into insertions/deletions or intensity changes of gene loci in the breast cancer cells were identified. (3) These mutations may be responsible for the conversion of estrogen-dependent breast cancer cells into estrogen-independent or drug sensitive breast cancer cells into drug resistant breast cancer cells. Further characterization by cloning and sequencing of these mutated gene loci would be helpful in designing any preventive and therapeutic strategies by targeting these loci.

E-2002

Some Effects of Plant Growth Regulators on Mammalian Cell. K. C. CAWLEY, R. Aleman, M. Blackledge, C. Campbell, J. Cohen, B. Lindsey, M. Kondapalli, J. Marsh-Haffner, N. Myint, and K. Taylor. Biotechnology Department, Portland Community College, Portland, OR 97280.

Plant growth regulators have been shown to influence the growth of mammalian cells in culture. Some studies have demonstrated growth promoting effects, and others have suggested inhibitory or anti-tumorigenic effects. In this study, the effects of kinetin are explored in both NIH 3T3 cells and in early passage dog fibroblasts, looking at changes observed in growth rate over 9 days.

E-2003

Plant Tissue Culture and Plant Conservation: In Vitro Methods for Introducing Concepts to the Classroom. B. L. PLAIR, V. C. Pence, and S. M. Charls. Cincinnati Zoo and Botanical Garden Center for Conservation and Research of Endangered Wildlife (CREW), 3400 Vine Street, Cincinnati, OH 45220. E-mail: bernadette.plair@cincinnatizoo.org

Tissue culture propagation is a central focus of the plant conservation research program at CREW. In vitro methods are used both for collecting plants and for propagating rare species. Students can be introduced to tissue culture techniques as well as issues of plant conservation through the use of an "In Vitro Collecting Kit" and "Test Tube Plants". In vitro collecting (IVC) is the technique of starting tissue cultures in the field. CREW has developed an IVC Kit and manual of experiments for use by teachers and students. The kit contains tissue culture media and tools as well as instructions for collecting and sterilizing plant leaf tissues for culture. Using the kit, students can practice sterile technique in initiating plant tissue cultures outdoors or in the classroom without the need for a laminar flow hood or laboratory setting. They can test different media to examine their effects on controlling contamination and stimulating growth and development. In addition, teachers can use IVC to introduce and explore concepts in the areas of plant conservation and endangered species preservation. The IVC Kit provides students with the opportunity to gain hands-on tissue culture experience without the need for a traditional tissue culture laboratory. Workshops conducted for students and teachers both locally and nationally have demonstrated the use of IVC Kits and Test Tube Plants as flexible learning tools that can be adapted to a variety of educational and research uses. Test Tube Plants illustrate the tissue culture techniques used at CREW to propagate endangered species. Information on the plant's habitat and on issues of plant conservation related to each species is included with each plant. One species, the Rupicola African Violet, is critically endangered in the wild and is being used at CZBG as a "flagship" species for plant conservation. Both the IVC Kits and the Test Tube Plants allow teachers to introduce concepts on the science of plant tissue culture as well as on issues of germplasm collection and plant conservation.

E-2004

Investigating the Use of Oil Degradation Microbes in the Secondary Science Classroom. CAROL A. HARRISON. Booker T. Washington High School and Tuskegee University, Tuskegee, AL 36088. E-mail: megnin@tusk.edu

Booker T. Washington High School, through Tuskegee University's Southern AgBiotech Consortium for Underserved Communities (SAC-UC), had been able to offer a variety or enrichment activities for its students. The program has provided funds for equipment, materials and teacher training in an effort to promote an awareness of biotechnology in minority and low socio-economic students. One such enrichment activity involves the use of Ward's Oil Spill Kit. The kit allows the student an investigative approach as to what can be done in the event of an oil spill. Through its use, students will study the role of microorganisms in eradicating a spill and compare it with the physical methods that are usually used. This activity involves three investigations that can be performed separately or concurrently. Two types of microorganisms used in the investigations are Pseudomonas species and Penicillium species. Both are naturally occurring and are non-pathogenic to marine life as well as humans and animals. Investigation #1 compares the physical characteristics of oil before and after microbial exposure as well as the growth and oil degrading ability of a fungus and a bacterium. Investigation #2 simulates and evaluates a bioremediation oil spill clean-up in water, determines the limitations of bioremediation and explores adverse shore oil spill and compares the bioremediation methods to physical methods which are presently most widely used to clean-up coastal oil spills. Work Supported By SACUC/USDA.

I-2001

Effect of Bt Proteins on the Viability of Selected Insect Cell Lines. C. L. GOODMAN[1], H. Nabli[2], J. Baum[3], T. Malvar[3], B. Isaac[3], Y.-J. Lee[3], A. H. McIntosh[1], and S. J. Phipps[2]. [1]USDA, ARS, BCIRL, 1503 S. Providence Rd., Columbia, MO 65203; [2]University of Missouri, Department of Entomology, 1-87 Agriculture Bldg., Columbia, MO 65211; and [3]Monsanto, 700 Chesterfield Parkway, St. Louis, MO 63017. E-mail: goodmanc@missouri.edu

Bt proteins (Cry proteins) are anti-insect proteins that are generated by the bacterium *Bacillus thuringiensis*. These proteins are commonly used as a means to control pest insects (either by direct application or by insertion of their genes into plant genomes). Although many studies have been undertaken to determine their mechanism(s) of action and their species specificities, much variation exists between proteins. Therefore, *in vitro* studies were undertaken to aid in the evaluation of these biocontrol agents. The responses to selected Bt proteins of cell lines from the corn earworm/cotton bollworm, *Helicoverpa zea* (Lepidoptera: Noctuidae), a major pest of corn and cotton, were determined using a viability assay. Additionally, the degree to which the proteins bound to the cells was evaluated by Western blot. The cell lines tested included those from the following tissues: larval midgut (BCIRL-HzMG8, RP-HzGUT-AW), larval fat body (BCIRL-HzFB33), and pupal ovaries (BCIRL-HzAM1). The Cry proteins used in our studies included the lepidopteran-specific protein Cry1Ac and the coleopteran-specific protein Cry3Bb. For each protein, no differences were seen in the degree to which they bound to each lepidopteran cell line (e.g., Cry1Ac bound similarly to each cell line). However, binding differences were noted between proteins (i.e., Cry1Ac bound to a greater degree than Cry3Bb). In the viability studies, none of the lepidopteran cell lines exhibited changes in viability when exposed to Cry3Bb, whereas many of these lines exhibited dose-dependent reductions in viability when exposed to Cry1Ac. Effects of pH on cell viability and cell-protein interactions were noted as well, with the latter being minimally affected by changes in pH.

I-2002

Insulin-like Peptides Stimulate Midgut Stem Cell Proliferation of Lepidopteran Larvae *In Vitro*. SHINTARO GOTO[1], Marcia J. Loeb[2], and Makio Takeda[1]. [1]Graduate School of Science and Technology, Kobe Univ., Kobe, Hyogo, 6578501, Japan and [2]IBL, USDA, ARS, Beltsvile, MD 20705. E-mail: 981d840n@y00.kobe-u.ac.jp

The mechanisms that control the growth rate of internal tissues during postembryonic development are poorly understood in insects. The midgut is the largest organs in lepidopteran larvae, and histologically studied well compared to other tissues. The cell mass increase during molting period, but the factors remodeling the midgut were difficult to study until *in vitro* system of midgut cells was established. Using the *in vitro* system, some factors that promote midgut differentiation were isolated from the conditioned medium. However no peptide factors that promote midgut cell proliferation have been reported. Since insulin-like immunohistochemical reaction was observed in the molting midgut, it was expected that an insulin-like peptide might promote cell proliferation in the midgut. Bombyxin is an insulin-like peptide in lepidopteran insects. To clarify the function of bombyxin and the other insulin-like peptides in midgut stem cell proliferation, these peptides were added to the culture of midgut stem cells and the number of the cells were counted. Insulin, IGF-1, IGF-2 and bombyxin stimulated proliferation of midgut stem cells *in vitro*, the most effective being bombyxin that induced a maximum effect at $10^{-12}M$. The highest number of cells was observed 3 days after the addition of bombyxin. Although one time addition of bombyxin could not keep the maximum effect thereafter, the second addition made 3 days after the first addition retained effect. It suggests that the decline of the effect observed was not due to the loss of sensitivity of the cultured cells but the growth factor added to the culture lost the effect.

I-2003

Effects of Insect Hormone Actions, 20E and JH, on Midgut Stem Cells of Lepidoptera. G. SMAGGHE[1,2], W. Vanhassel[1], C. Moeremans[1], K. Elsen[1], and M. Loeb[3]. [1]Department of Biology, Free University of Brussels, Brussels, Belgium; [2]Laboratory of Agrozoology, Ghent University, Ghent, Belgium; and [3]Insect Biocontrol Laboratory, USDA-ARS, Beltsville, MD 20705. E-mail: gsmagghe@vub.ac.be

With midgut stem cell cultures *in vitro*, addition of the two principal insect hormones, 20-hydroxyecdysone (20E) and juvenile hormone (JH3) separately to the medium stimulated cell proliferation in a concentration-dependent manner. Stem cells were obtained from larvae of an economically-important pest insect, the cotton leafworm (*Spodoptera littoralis*; Lepidoptera: Noctuidae). We also tested two metabolically-stable insect growth regulatory compounds, an ecdysteroid agonist, RH-2485, and a juvenile hormone analogue pyriproxyfen, and compared the *in vitro* effects with an aqueous fat body extract from tobacco hornworm (*Manduca sexta*) pupae, FBX, and two purified factors, MDF2 for cell differentiation and MP for cell proliferation. Further, with whole insect larvae, we evaluated the effects of RH-2485 and pyriproxyfen on the midgut physiology, and on larval growth and development. Typically, RH-2485 induced premature molting and finally larval death, and pyriproxyfen provoked a supernumerary larval stage. From this study, we can report that the dramatic symptoms of midgut hyperplasia with RH-2485 is consistent with the high increase in stem cell number *in vitro* induced by 20E and RH-2485. Surprisingly, similar effects occurred in the presence of FBX. The data discuss the action of the insect hormones and the interrelationship with other factors in the development and metamorphosis of the insect midgut.

I-2004

Who Controls Midgut Stem Cell Differentiation—The Stem Cell or the Environment? R. S. HAKIM[1], M. Loeb[2], and J. Young [1]. [1]Dept. of Anatomy, Howard University, College of Medicine, 520 W St. NW, Washington, DC 20059 and [2]Insect Biocontrol Laboratory, U.S. Department of Agriculture, Bldg 011A, Rm 214, BARC West, Beltsville, MD 20705. E-mail: rhakim@fac.howard.edu

In *H. virescens*, as in *M. sexta* and other lepidoptera, midgut development proceeds through the sequential proliferation and differentiation of the midgut stem cells. In this process, in larvae, the stem cells repeatedly differentiate to goblet, columnar, and to a lesser extent endocrine cells of the midgut; a reserve population of stem cells are retained for the next molt, as well as being available for differentiation in midgut repair. In the last larval molt, in *H. virescens*, the stem cells differentiate to a simple cuboidal epithelium with a characteristic vesiculate cytoplasm, and a brush border. Larval epithelium is eliminated in the gut lumen. Adult epithelial cells, of similar height, but narrower presumably form from differentiation of these same cells. These observations are of particular interest when one considers the capabilities of stem cells in culture. Stem cell cultures exposed to synthetic midgut differentiation factors (MDF 1-4), differentiate to recognizable larval midgut goblet and columnar cell types. Cell-free conditioned media taken from cultures of mixed prepupal and pupal cells induced development of mature prepupal and pupal cell types from stem cells collected from larval midgut. However, use of vertebrate growth factors have resulted in cultures producing a much wider variety of cell types than seen previously, including epidermal epithelium; This evidence demonstrates that while, as a group, the midgut stem cells possess a wider range of potential than is expressed in vivo, actual stem cell fates are controlled by their local environment.

I-2005

Ecological Monitoring of the Ai River (Ural, Russia) Under the Influence of Oil Spills: Hydrobiont's Adaptations & Reaction. A. I. VAGAPOV. Laboratory of Ecology, Municipal Ecological Lyceum of Chelyabinsk. E-mail: anton_v@inbox.ru

The subject of the present work is the effect of oil pollution in rivers on aquatic organisms. There are several oil-trunk pipelines that spill great quantities of crude oil in our region. Moreover, many of these pipelines were built more than 40 years ago. Therefore, the ageing of the metal pipelines results in many oil spills. Unfortunately, the oil will often spill into small and large rivers. I was fortunate to have investigated one of such oil spills in 1999. The present work is quite original because of the fact that the investigation of the river took place both before as well as after the oil spill. Thus, I've obtained interesting data and I am able to estimate objectively the influence of oil spills upon organisms inhabiting rivers. I divided the organisms into groups based on their reaction to the crude oil pollution. I also identified several examples of adaptations that animals made in order to survive in their polluted environment. I divided the fish into 3 groups based on their reaction to the oil pollution. For example, the grayling (*Thymallus thumallus*, Salmoniformes) and the bullhead (*Cottus gobio*, Scorpaeniformes) were placed among the most sensitive Pisces because of their species-specific characteristics. I have proposed a theory of generic behaviour prototypes based on these data. In the future, I plan to continue this work by investigating the types of defense mechanisms fish have to oil pollution on the cellular and tissue levels, working both in field and in laboratory. I thank Mr. Eugen Chibilev (Arkaim Scientific Reserve) for his invaluable help in this work.

P-2000

Transformation of Barley with Two Antifungal Genes. L. S. DAHLEEN and M. Manoharan. USDA-ARS, Fargo, ND 58105 and Univ. of Arkansas, Pine Bluff, AR 71601. E-mail: dahleenl@fargo.ars.usda.gov

Insertion and expression of multiple antifungal genes has the potential of increasing resistance to a variety of fungal diseases. Using particle bombardment and bialaphos selection for the *bar* gene, multiple barley plants containing both a rice thaumatin-like protein (*tlp*) gene and a rice chitinase (chi) gene were regenerated from three transformation events. Southern analysis confirmed integration of the transgenes into the barley genome. Northern analysis of T_0 plants indicated that event 1 did not contain RNA from either gene. Plants from event 2 expressed both genes at high levels, while plants from event 3 showed small amounts of *tlp* RNA but no chi RNA. Western analysis of T_1 progeny confirmed that event 2 lines expressed both genes and event 3 lines expressed only *tlp*. Homozygous T_2 lines have been identified and are being examined for gene expression levels for both transgenes.

P-2001

Regeneration and Genetic Transformation of Durum Wheat. M. MANOHARAN[1] and L. S. Dahleen[2]. [1]Department of Agriculture, University of Arkansas, Pine Bluff, AR 71601 and [2]USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58105. E-mail: Manoharan_M@uapb.edu

Durum wheat (*Triticum turgidum* L.) is an important cereal crop used for making pasta and semolina. Efforts are in progress to improve durum wheat through gene transfer technology for characteristics such as disease resistance, especially for Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe). A major constraint is the lack of an efficient, reproducible and reliable method of genetic transformation of durum wheat. We have established an efficient and reproducible regeneration system with the cv. Monroe. Murashige and Skoog (MS) medium with different concentrations (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) of picloram (4-amino-3,5,6-trichloropicolinic acid) or 2 mg/L 2, 4, 4-dichlorophenoxy acetic acid (2,4-D) were used to culture immature embryos for their morphogenetic response. Embryogenic calli proliferated on 2.0 mg/L picloram but was less frequent on 2,4-D containing media. Picloram at 2.0 mg/L also regenerated more plants than either 2,4-D or the other picloram concentrations. For genetic transformation, the calli were bombarded with the pathogenesis-related gene thaumatin-like (*tlp*) from rice, and a modified *Tri101* gene, along with the *bar* gene for selection. PCR and Southern analysis indicated the regenerated plants contained the transgenes and the Western analysis confirmed the expression of the *tlp* in the durum wheat cv. Monroe.

P-2002

Effects of UV-B on the Development of In-Vitro Propagated African Violet Chimera (*S. ionantha*). W. Y. LEUNG, P. Castillo-Ruiz, E. Belanger, and S. Taylor. Biology Department, Champlain Regional College Saint-Lambert, Saint-Lambert, QC, CANADA, J4P 3P2. E-mail: wai-yei.leung@sympatico.ca

The Alps is a selected cultivar of African violets (*Saintpaulia ionantha*) with pinwheel flowers. The attractive bicolor pattern (white with blue stripes) of the flowers makes the Alps cultivar an interesting subject for in-vitro propagation. In this study, plant tissue culture was used to propagate the Alps cultivar from leaf cuttings and to observe the general development of the plant after exposing the callus to UV-B radiation. It is hypothesized that the Alps chimera plant is more sensitive to UV-B radiation than the non-chimera *S.ionantha* hybrid used as control, therefore showing greater instability in its overall pattern of development. Before radiation, it was observed that the growth of the Alps' cultivar explants was relatively slow since the first leaves of this cultivar appeared approximately 15 days later than those of the control. An overall sensitivity of the Alps chimera explants to radiation was also observed and seemed to depend on the developmental stage of the callus. The Alps' callus, at an early stage, was more affected by these radiations. Days following the UV exposure, they showed a bright yellow color. UV-B radiation appeared to have a direct effect on the chloroplasts as indicated by a change in the color of leaves of the Alps that developed from the exposed callus. Further research is needed to establish the actual cause of these effects on the chimera. Some possible explanations could include effect at the gene expressions, mRNA transcript, and/or chloroplast protein level.

P-2003

Overexpression Antioxidant Gene in Tomato Increases Tolerance to Heat and Chilling Stress. YUEJU WANG[1*], Michael Wisniewski[2], Lailiang Cheng[3], Richard Meilan[4], Minggang Cui[1], and Leslie Fuchigami[1]. [1]Dept. of Horticulture, Oregon State University, Corvallis, OR 97331; [2]USDA-ARS, 45 Wiltshire Road, Kearneysville, WV 25430; [3]Dept. of Horticulture, Cornell University, Ithaca, NY 14853; and [4]Dept. of Forest Science, Oregon State University, Corvallis, OR 97331. E-mail: wangyu@bcc.orst.edu

Ascorbate peroxidase (APX) plays an important role in the metabolism of H_2O_2 in higher plants and protects plant against oxidative stresses. The effect of overexpressing a cytosolic ascorbate peroxidase (cAPX) gene derived from pea (*Pisum sativum*) in transgenic tomato (*Lycopersicon esculentum*) plants on temperature stress resistance was studied. Transformants were selected using Kanamycin resistance and confirmed by PCR, Southern, Northern, and Native gel analysis. Total APX enzyme activity of transgenic plants is 3- to 6-fold higher than wild-type plants as determined by native PAGE. In some lines, APX activity of transgenic plants was several fold greater than that of the wild-type (WT) plants under non-stressed conditions. Several independently-transformed lines were evaluated for resistance to oxidative stresses resulting from exposure to low-and high-temperature. The transgenic lines tested were more resistant than the wild-type tomato plants to both chilling (4° C) and heat (40° C) stress. Injury was assessed by electrolyte leakage. Seeds obtained from the transgenic plants germinated at low temperature (9° C) in the dark whereas seeds from WT plants could not. The level of APX activity in leaves of the transgenic plants was 25 fold higher than in the wild-type plants under chilling stress and 5 fold higher than wild-type plants under heat stress. These results suggest that increased level of ascorbate peroxidase in the transformants was important for resistance of tomato plants to high and low temperature stresses.

P-2004

Expression of the *Arabidopsis* CBF1 Gene in Poplar Confers Elevated Freezing Tolerance. YONGJIAN CHANG, Jeffrey S. Skinner, and Tony H. H. Chen. Department of Horticulture, 4017 ALS Building, Oregon State University, Corvallis, OR 97331. E-mail: changy@science.oregonstate.edu

The *Arabidopsis* CBF1 gene driven by the CaMV 35S promoter was transformed into hybrid poplar (clone 717-1B4) by *Agrobacterium*-mediated transformation. Kanamycin-resistant transgenic poplar plants were analyzed by genomic PCR and Northern blot analysis. Of 19 lines verified to be transformed with the 35S:CBF1 operon, only two displayed detectable transcript levels. Transgenic lines positive for CBF1 expression displayed reduced rooting ability, retarded shoot growth, and shorter internodes when grown *in vitro*; transgenic lines negative for CBF1 expression were unaffected for these traits. However, these lines resumed normal growth within 2-4 weeks following transplantation to soil and growth in a greenhouse. The freezing tolerance of one of the transgenic lines was evaluated via electrolyte leakage following controlled freezing experiments; evaluation of the second line is currently underway. The freezing tolerance of leaves and stems for the transgenic line was significantly higher (3.3° C and 2.6° C, respectively) than those of wild type plants. Following one week of cold acclimation at 2° C, leaf freezing tolerance increased 3.9° C and 2.3° C for wild type and transgenic plants, respectively. Our results indicate that constitutive expression of *Arabidopsis* CBF1 can confer elevated freezing tolerance in poplar.

P-2006

An Approach for Fiber Improvement in Kenaf Using a Gibberellin Oxidase Gene. M. M. YOUNG and N. A. Reichert. Department of Plant and Soil Sciences, Box 9555, Mississippi State, MS 39762. E-mail: myoung@pss.msstate.edu

Kenaf (*Hibiscus cannabinus* L.) is grown for its vegetative fibers extracted from the stem. The two distinct stem fiber types, the outer bast and inner core, are used in the manufacture of different products. Elite genotypes, improved through classical breeding efforts demonstrate adequate, but not substantially better, fiber yields. An *Arabidopsis thaliana* cDNA clone encoding the GA 20-oxidase gene (AtGA20ox1) was obtained from a researcher who demonstrated this gene enhanced vegetative growth in transgenic hybrid aspen. The cDNA was cloned into a vector with expression controlled by the 35S promoter from cauliflower mosaic virus. The vector also contained a chimeric neomycin phosphotransferase II gene for transgenic kenaf tissue selection. Kenaf leaf explants from cv. Tainung 2 were transformed via biolistics- and *Agrobacterium tumefaciens*-based procedures. Optimized parameters for biolistics included a preculture time of 7 days and a rupture pressure of 900 psi, and for *A. tumefaciens* included preculture and inoculation times of 2 days and 10 minutes, respectively. Post-transformation, tissues were placed on selection media containing 10 mg/l geneticin. Preliminary experiments indicate that greater numbers of explants survived on selection media post-bombardment vs. post-inoculation with *A. tumefaciens*. Confirmed transgenics will be analyzed via various methods to determine the overall effect on kenaf when expressing an additional GA oxidase gene.

P-2005

Kanamycin Resistant Alfalfa Has a Point Mutation in the 16S Plastid rRNA. D. Rosellini[1], P. BARONE[1], P. LaFayette[2], F. Veronesi[1], and W. A. Parrott[2]. [1]Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, Università degli Studi di Perugia, Borgo XX giugno 74, 06100, Perugia, Italy and [2]Center for Applied Genetic Technologies, University of Georgia, Athens, GA. E-mail: roselli@unipg.it

Kanamycin, an aminoglycoside antibiotic, is one of the most used tools for obtaining transgenic plants, by inserting a gene that detoxifies it as the selectable marker in gene constructs. Kanamycin resistance mutations have not been reported in higher plants, whereas two point mutations in the decoding region of the plastid 16S ribosomal RNA, each conferring kanamycin resistance, have been found in the unicellular green alga *Chlamydomonas reinhardtii*. In attempting to transform the plastid genome of alfalfa using a kanamycin resistance gene as the selectable marker, somatic embryos were obtained after a minimum of two months of culture of leaf explants in the presence of 50 mg l^{-1} kanamycin. Kanamycin resistance was confirmed by 4 to 5 regeneration cycles on kanamycin-containing media using mature somatic embryos to start each cycle. PCR and Southern analyses demonstrated that the kanamycin resistance gene was not present in the resistant embryos. Resistant plants produced 85% less biomass than controls and did not flower. Their leaves were white as they formed and slowly became green. When leaf explants from resistant plants were used for regeneration, they produced less callus and embryos than control leaves on both kanamycin and kanamycin-free media; kanamycin presence resulted in slower regeneration and further reduced callus and embryo production, indicating that kanamycin resistance was not complete. Sequencing of the plastid DNA region corresponding to the decoding site of the16S rRNA in 10 independent resistance events revealed a A→C transversion at position 1357 of the 16S plastid rDNA, the same site at which an A→G conversion confers kanamycin resistance to *C. reinhardtii*. All the progeny of the resistant plants obtained through somatic embryogenesis in the absence of kanamycin had the mutated phenotype, indicating that the mutation was homoplasmic. We propose that kanamycin resistance in alfalfa requires homoplasmy for the described rDNA mutation, which reduces binding affinity of the plastid ribosome for the antibiotic and adversely affects plastid translation.

P-2007

A Poplar Promoter Functions in Potato and is Induced by Wounding and Fungal Infection. DMYTRO P. YEVTUSHENKO, Rafael Romero, William W. Kay, and Santosh Misra. Department of Biochemistry & Microbiology, University of Victoria, Victoria, British Columbia, V8W 3P6, Canada. E-mail dmytro@uvic.ca

Regulated transgene expression has distinct advantages for engineering of disease/pest resistant plants. We constructed plant transformation vectors using the beta-glucuronidase (*GUS*) reporter gene system and compared the spatiotemporal transcriptional activity of two wound-inducible proteinase inhibitor promoters, an 823 bp fragment of the *win3.12* promoter from hybrid poplar (*Populus trichocarpa x P.deltoides*) and a full-length *pin2K* promoter from potato, in transgenic potato plants in response to mechanical wounding, elicitor treatment and fungal infection. We demonstrate that a truncated poplar promoter (designated *win3.12T*) is sufficient to confer wound-regulated transgene expression in potato, both within the damaged tissue and throughout the plant. Strong positive correlation between transgene copy number and wound-induced GUS expression was observed in the leaves of *win3.12T* plants, whereas in the plants with *pin2K* promoter higher number of transgene insertions had, in general, an inhibitory effect on total GUS accumulation. In addition, the *win3.12T* promoter had strong systemic activity in aerial parts of potato in response to fungal infection (*Fusarium solani*): up to three times higher than even in local response to mechanical wounding. It indicates that the poplar promoter has regulatory elements that are responsive to pathogen invasion in the heterologous host, and the same signal molecules mediate remote wound/infection responses in both angiosperm species. These data will be used to study the components of signal transduction pathways in plants and to select the appropriate promoter for the gene transfer applications.

P-2008

Screening *Arabidopsis thaliana* and *Chlamydomonas reinhardtii* for Their Phenotypic Response to 2,4,6-trinitrotoluene (TNT). NRUPALI PATEL and C. Neal Stewart, Jr.
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It is estimated that approximately 0.82 million cubic meters of soil at former military and ordnance plants throughout the US is contaminated by explosives; major contaminants often include 2,4,6-trinitrotoluene (TNT) and its degradation products. *Arabidopsis thaliana* ecotype Columbiaand *Chlamydomonas reinhardtii* strain 89 (Utex) are genomic models systems that have the potential to be exploited for the use of sensing explosives. The effects of TNT on germination and early seedling development of *Arabidopsis* were determined. There was no significant variation in the speed and probability of germination with an increase in TNT concentrations (0 μM - 30 μM). Studies of early seedling development of rosette diameter, root length and leaf color demonstrated some variation with an increase in TNT concentration. At low concentrations (1 μM - 5 μM) there was no significant difference in rosette size however at higher TNT concentrations (10 μM - 30 μM) the rosette diameter decreased linearly. Root length decreased with an increase in TNT. Leaf color was observed and showed no significant color change however at the 30 μM leaves were yellow. The algal species, *Chlamydomonas reinhardtii* was grown in a range of TNT concentrations from 1 μg/ml to 5 μg/ml. A growth response curve was conducted over one week. Results from the growth response indicated that TNT concentrations above 3 μg/ml are toxic to the algae.

P-2009A

Reactivation of a Disabled Virus Vector by the Utilisation of an Inducible Recombination System in Tobacco Plants. A. TREMBLAY[1,2], C. Beauchemin[2], V. Bougie[2], A. Séguin[3], J.-F. Laliberté[2].
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The ability of viruses to self-multiply could be an interesting tool to exploit for the large-scale production of recombinant proteins in plants. With this in mind, potyvirus vectors have been developed that enable in frame insertions of a foreign sequence into an appropriate site within a complete copy of RNA genome (cDNA). The devastating effects of these viruses on plant development is however a major disadvantage of their utilisation. To circumvent this, we have produced a transgenic tobacco line with an inactivated copy of the Turnip mosaic virus (TuMV) genome. The viral genome will be turned on only upon contact with an inducer at the appropriate stage of plant development. The virus vector has been inactivated by the insertion of two LoxP sites between a translation terminator that prematurely stops the viral expression. We have shown by *in vitro* test tube experiment directly on vector that excision of the translation terminator occurs following the addition of the recombinase Cre. The recombination event leaves only one LoxP site and reactivates the viral expression. We are currently constructing transgenic tobacco plants that contain the Cre gene under the control of an inducible promoter to evaluate the inducible conditions and soon, produce transgenic tobacco line for inducible virus activation. This work is supported by the Natural Sciences and Engineering Research Council of Canada.

P-2009

DNA Fingerprinting of Orchardgrass (*Dactylis glomerata* L.) cv. Persist for Plant Variety Protection. J. K. McDANIEL and B. V. Conger.
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Molecular marker technology has been used for plant variety protection (PVP) of clonally propagated and annual self pollinated seed propagated species. It has been used to a lesser extent for PVP of synthetic cultivars of perennial, obligate outcrossing, polyploid species, such as forage grasses. The objective of the current investigation was to determine if DNA fingerprinting (DAF) could be used to differentiate 'Persist' orchardgrass from eight currently grown commercial cultivars. Fifteen 8-nucleotide primers were used to generate profiles of the six parent clones. The parental profiles were compared to a pooled parent profile and to those of S1, S2, and S3 generations of Persist. Consistent banding patterns were found in each of the three generations and pooled parent profiles that were present in the parent clones. The same primers were used to generate profiles of the eight commercial cultivars. These profiles had many bands in common to Persist. However, two primers produced bands in Persist that were not present in the other cultivars. The results indicate that DAF, and perhaps other PCR techniques, can be used for identification and protection of highly hererogeneous, polyploid, outcrossing synthetic cultivars.

P-2010

Positive Correlation Occurs Between Cytosine Methylation and Adventitious Shoot Induction in Petunia. P. P. KUMAR[1], A. P. Prakash[1], A. Kush[2], and P. Lakshmanan[3].
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The DNA methylase inhibitors, 5-azacytidine and 5-aza-2'-deoxycytidine inhibit adventitious shoot induction in petunia leaf cultures. Cytosine methylation status at CCGG sites in shoot- and callus-inducing culture treatments was analyzed by coupled restriction enzyme digestion (*Hpa*II or *Msp*I) and random amplification. Two differentially methylated genomic DNA bands from the PCR products, OPU9-1 and OPU9-2, showed similarity to *CDC48* and MADS-box genes, respectively. Cytosine methylation was restored at CCGG sites when the leaf explants were transferred from medium containing the drugs to medium without the drugs, concomitantly recovering the ability to develop adventitious shoot buds. Furthermore, combined bisulphite treatment and restriction analysis revealed differential methylation of CGCG sites in the drug-treated and control cultures. These results demonstrate that cytosine methylation at CCGG and CGCG sites within a MADS-box gene and a CDC48 homologue, among others, is necessary for adventitious shoot bud induction in petunia leaf explants.

P-2011

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A Plant Transformation Ctr. (PTC), is being implemented at Michigan State University under auspices of the Agricultural Experiment Station. The PTC has the mission to develop efficient gene transfer and crop regeneration systems to support Michigan agriculture. The focus is on 1) increased agricultural efficiency, 2) support of sustainable agro-ecosystems, 3) engineer crops that lead to decreased pesticide use, 4) increased field and post-harvest resistance to pests & diseases, and 5) value-added traits for consumer products. On campus researchers, as well as visiting scientists, students and technicians are provided with services, supplies, equipment and training. At this time, visiting scientists with interests in celery, blueberry, sour cherry, dry bean, and selected forest species are particularly encouraged to review center opportunities at the PTC web site- http://www.ptc.msu.edu/

P-2012

Aluminum Tolerance in Alfalfa with the Citrate Synthase Gene. P. BARONE[1], D. Rosellini[1], J. H. Bouton[2], P. L. LaFayette[2], M. Sledge[3], F. Veronesi[1], and W. A. Parrott[2]. [1]University of Perugia, Italy; [2]University of Georgia, Athens, GA; and [3]Noble Foundation, Ardmore, OK. E-mail: pib8@uga.edu

Alfalfa is very sensitive to acid soils, in which Al^{3+} ions become soluble and toxic to roots, severely reducing yield (Bouton and Parrott, 1997). Transgenic tobacco and papaya plants overexpressing a citrate synthase (CS) gene from *Pseudomonas aeruginosa* (Donald et al., 1989) were reported to have an enhanced tolerance to Al (de la Fuente et al., 1997). We explored a similar approach in alfalfa. Regen-SY alfalfa was transformed with the CS gene under control of the *Arabidopsis act2* constitutive promoter (An et al, 1996) or the tobacco TobRB7 root-specific promoter (Yamamoto et al., 1991). The *nptII* gene under the control of the potato ubiquitin 3 promoter (Garbarino JE, Belknap WR.,1994) was used for selection. Seven transgenic plants were obtained containing the Actin-CS construct and 14 with the TobRB7-CS construct, with each plant having insertions of the CS gene at 1 to 4 loci. Root length from transgenic somatic embryos germinating in the presence and absence of Al was measured. All had slightly longer roots than the control in the presence of aluminum. A greenhouse Al tolerance trial with rooted cuttings (Dall'Agnol et al., 1996) was used to identify the most resistant transformants. Three of the four most resistant plants have the CS gene driven by the *act2* promoter. Of these four, two had shown reduced hematoxylin root apex staining, which indicates a reduced Al uptake by the root tip. These results are in contrast to those of Delhaize et al. (2001), who claimed that transformation of alfalfa with CS did not improve tolerance to aluminum toxicity.

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P-2013

A Non-antibiotic Marker for the Selection of Transformed Plants. P. R. LAFAYETTE, P. M. Kane, and W. A. Parrott. Department of Crop & Soil Sciences, Center for Applied Genetic Technologies, University of Georgia, Athens, GA 30602. E-mail: plaf@uga.edu

The possibility of obtaining more efficient selection of transgenic cells, along with concern over perceived risks from the presence of antibiotic genes in transgenic plants is leading to selection strategies which do not use antibiotic resistance genes as selectable markers. The *atl* operon, which permits the use of arabitol as the sole carbon source, is present in *Escherichia coli* strain C. The arabitol dehydrogenase gene, *atl*D, converts D-arabitol into D-xylulose, which is metabolizable by plants. We optimized the *atl*D gene for expression in plants by synthesizing the gene using plant-preferred codons and by removing cis-active DNA motifs. The synthetic *atl*D was placed behind the potato ubiquitin 3 promoter and inserted into a binary vector containing a GUS gene and a CaMV35S-driven *nptII* gene. Tobacco leaf discs were transformed using *Agrobacterium* and selected with either kanamycin or arabitol + sucrose combinations. Transgenic shoot regeneration frequencies were similar for both arabitol selection and kanamycin selection. Parameters being optimized include the ratio of arabitol to sucrose employed during selection and the time for transfer of leaf discs from arabitol-containing media to sucrose media. *In vitro* digestibility experiments with *atl*D expressed in *E. coli* show the protein is degraded rapidly when incubated with simulated gastric or intestinal fluids. Digestibility is one of the tests required to evaluate the allergenicity of novel plant proteins.

P-2014

Co-ordinate Expression of β and δ Zeins in Transgenic Tobacco. JENNIFER RANDALL[1], Dennis Sutton[1], Soumitra Ghroshroy[2], Suman Bagga[3], and John D. Kemp[1]. [1]GeneLab, EPPWS, New Mexico State University; [2]EM Lab, Biology, New Mexico State University; and[3]Agronomy and Horticulture, New Mexico State University. E-mail: jrandall@nmsu.edu

Two classes of alcohol soluble seed storage proteins found in the endosperm of maize contain unusually high levels of cysteine and methionine. These two proteins, the beta and delta zeins, have been introduced into plants with the expectation of improving the sulfur nutritional content of various plants. Traditional methods of expressing multiple transgenes in plants include crossing transgenic plants which contain the genes of interest, and co-transformation of the transgenes. Co-ordinate expression of transgenes is not always successful with these traditional methods. We have attempted to co-ordinately express the beta and delta zein proteins with the use of a synthetic self-hydrolyzing 2A polyprotein utilized by a number of viruses (Donnelly et al, 2001). The beta and delta zeins were fused with a 20 amino acid synthetic 2A polypeptide between them. This beta zein-2A-delta zein construct was introduced into tobacco. Western analysis indicates that tobacco plants containing this transgene accumulate both beta zein and delta zein protein. The 2A polypeptide cleaves correctly allowing the beta zein protein and the delta zein protein to accumulate. Protein bodies have been observed in these transgenic plants. The morphology of the protein bodies is altered when compared to the rosette protein bodies observed in beta and delta zein crossed plants. The morphology of the protein bodies may be altered due to the increase of amino acids at the c-terminal end of the beta zein. This technology allows two genes to be expressed in one cassette, under the control of the same promoter, eliminating the traditional need for crossing or co-transformation.

P-2015

Characterizing the Plastid Ribosomal Region Among 25 Diverse Angiosperms: An Early Step Towards Universal Plastid Transformation Vectors for Plant Expression.
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The burgeoning field of plant protein production systems (GM organisms) has remarkable potential to address a wide range of important military, clinical and environmental and bioengineering problems. The integration of expression cassettes into the plastid genome (plastome) utilizing RecA-mediated homologous is an exciting instrument to generate transgenic plants, particularly when the goal is high levels of protein production. A transformation construct consists of an expression cassette containing the gene-of-interest and a selection factor with plastid-specific *cis*-regulatory sequences flanked by lengthy regions with significant homeology to the plastid genome (ideally cloned from the species to be transformed). The efficacy of integration into any given plant species is determined by: (a) the degree of conservation (both percent identity and distribution of non-conserved bases) between the flanking arms and the plastid genome, (b) the cellular environment of the target species, and (c) the genetic environment of the target plant species. In permissive environments, the efficacy of RecA-mediated recombination is dependent on the degree and nature of identity between the transformation vector and target plastome. Utilizing flanking DNA 100% identical to the target plant is labor intensive, but probably the most efficient mechanism to transform plastids. Conversely, a small set of vectors with significant homeology (similar to, but not identical) to the plastome may transform a wide range of plant species with nearly equal efficiency. We are characterizing the potential for a limited set of transformation vectors to integrate into a wide range of angiosperms. Hypothesizing that the plastid ribosomal region will be highly conserved, and therefore a good target region, we have sequenced 2.7 kilobases of the ribosomal region from 25 dispersed angiosperms, characterizing PCR conditions and primers, the degree of similarity among the plant species as well as potential stumbling blocks in the formation of chimeric rRNAs. Sequences will be submitted to Genbank.

P-2016

DNA Methylation and Transgene Silencing in Wheat Transformed with the Wheat Streak Mosaic Virus Coat Protein Gene.
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A spring wheat line 566B was co-transformed with the *bar* gene and wheat streak mosaic virus coat protein gene (*WSMV-CP*) by the biolistic method. Transgene silencing was observed after analyzing transgene expression over several generations. Seedlings from subsequent selfed generations of 566B, from T_1 to T_3, were screened for expression of the *WSMV-CP* by polymerase chain reaction (PCR), Southern blot and Western blot analysis. Seedlings of different generations were inoculated with WSMV at the three-leaf stage and analyzed by ELISA. Results indicated that all 566B T_1 plants containing the *WSMV-CP* expressed WSMV coat protein, and all showed strong resistance to WSMV. While the *WSMV-CP* was carried through to the T_2 and T_3 generations, all transgenic plants in these generations showed transgene silencing. Expression of *WSMV-CP* could be restored, at least temporarily, in most of these silenced plants by treatment with 5-azacytidine (5-AzaC). Results indicated that transgene silencing of 566B was at transcriptional level and was directly caused by DNA methylation.

P-2017

RAPD Analysis Concerning Genetic Variability of *Phytophtora infestans* in Potato.
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One of the most important potato diseases in Romania is the late blight, caused by the pathogen Phytophthora infestans. At the moment it is impossible to perform an accurate identification of Phytophthora species and its pathotypes, based on their morphological characteristics. The RAPD marker technique could be beneficial for revealing of the genetic variability of different pathotypes. Recently we have initiated new molecular genetic experiments in potato starting with isolation, multiplication and in vitro culture of *Phytophthora infestans* collected from different local genotypes and cultivars. For in vitro culture we used five potato cultivars: Desiree, Condor, Sante, Sieglinde, Nicola and three culture media: MS0; MSP1 (with AIA 1.5 mg/l; BAP 1.5 mg/l) and 2.4D (with 2.4D 2.5 mg/l; AIA 1.0 mg/l). *Phytophthora infestans* isolation was performed from infected leaves and multiplied on Zea and Pisum selective media with antibiotics, Vancomycin (200 ppm) and Nystatin (400 ppm). DNA amplification was performed with six primers: P9- GGTGCGGGAAA; P-10-GTTTCGCTCC; P11-GTAGCCCGT; P12AACGCGCAAC; P13-AACGCGCAAC; P14-CCCGTCA-GCA. The best medium for in vitro cultivation of potato was MSP1 on which the biggest number of vigorous plantlets was obtained. The isolation of *Phytophthora infestans* was a difficult task due to other infections like Alternaria. Up to now, in our experiments, the isolation and multiplication of *Phytophthora infestans* has succeded only for Condor cultivar. DNA amplification from infected and uninfected tubers revealed a significant polymorphism. For the P11 primer there was noticed a significant polymorphism between uninfected and infected material, only in Condor variety. For the P12 such a polymorphism was noticed for all the three varieties. The polymorphic bands identified in infected material belong, probably, to the phytopathogenic agent. The polymorphism identified among the three potato infected cultivars is due, probably, to the genetic variability of different potato late blight pathotypes.

P-2018

Expression of GFP Reporter Gene in *Arundo donax* Following Microprojectile Bombardment.
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Arundo donax L is a non-food woody perennial crop plant which has been considered important due to its use for phyto-remediation purposes. The jellyfish green fluorescent protein (GFP) provides a new tool to monitor gene expression in plants. Expression of GFP can be observed over an extended period of time in living tissues following UV or blue excitation. The objectives of this study were to introduce the GFP as a reporter gene in *Arundo donax*, to evaluate transient and stable expression over time, and to examine the effect of prolonged blue light exposure on GFP activity after microprojectile bombardment. Embryogenic calli were induced on Murashige and Skoog (MS) media supplemented with 2.0 mg/L of 2,4-D were bombarded with 1.0 μM gold particles coated with a plasmid DNA vector containing GFP reporter gene fused to 35S constitutive gene promoter. The GFP gene was expressed physiologically in embryogenic cultures as early as 12 hrs. Peak expression was seen at approximately 24 hrs (approximately 600 spots/bombardment). Prolonged exposure of high intensity blue light did not alter the number of transient events. The effect of different parameters such as types of tissues, distance, varying pressures on stable expression of GFP in embryogenic callus tissues will be discussed.

P-2019

Cryopreserved Storage of Hops (*Humulus* L.) Germplasm. BARBARA M. REED, Carolyn Paynter, Jeanine DeNoma, Jeff D'Achino, and Nese Okut. USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Rd., Corvallis, OR 97333-2521. E-mail: reedbm@bcc.orst.edu

The USDA-ARS National Clonal Germplasm Repository (NCGR) stores the global diversity of *Humulus* for the US Plant Germplasm System. The primary collection of hops is stored as trellised plants in a field genebank. To insure the safety of the collection, a subset of the field collection was established as virus-free *in vitro* cultures stored at 4° C and as potted plants grown in screened houses. In this study we characterized the response of diverse *Humulus* genotypes to cryopreservation and stored 100 meristems of each in liquid nitrogen. Shoot tips (0.8-1.0 mm) of cold-acclimated *in vitro* plantlets were dissected and precultured on 5% DMSO medium for 48 h under cold-acclimating conditions. Following cryoprotection, shoot tips were frozen to -40° C at 0.1° C/min in a programmable freezer and immersed in liquid nitrogen. Control vials were thawed in 45° C water for 1 min, then in 23° C water for 2 min. Recovery ranged from 30 to 80%. The cryopreserved meristems will be stored at NCGR Corvallis and at the National Center for Genetic Resources Preservation in Ft. Collins, CO.

P-2020

Elimination of Grapevine Virus A (GVA) By Cryopreservation of *In Vitro*-grown Shoot Tips of *Vitis vinifera* L. Q. WANG[a, b], M. Mawassi[a], P. Li[a], R. Gafny[a], I. Sela[b], and E. Tanne[a]. [a]Agricultural Research Organization, The Volcani Center, Department of Virology and [b]The-Tolkovsky Laboratory, Bet Dagan 50250, Israel and [b]The Hebrew University, Faculty of Agricultural, Food and Environmental Quality Sciences, Institute of Plant Sciences, Virus Laboratory, Rehovot 76100, Israel. E-mail: qiaochunw29@hotmail.com

Methods for the cryopreservation of *in vitro*-grown shoot tips of grapevine were recently developed. The present study demonstrates that grapevine virus A (GVA) can be successfully eliminated from naturally infected grapevine by cryopreservation of *in vitro*-grown shoot tips. The various steps taken before freezing in liquid nitrogen did not, by themselves, eliminate GVA. However, the freezing step resulted in 97% GVA elimination. The size of the shoot tips used for cryopreservation influenced their survival rate, while viral eradication was independent of their size in the range of 0.5 to 2.0 mm. In comparison, plant regeneration from meristems increased with size, and meristems of 0.1 mm completely failed to regenerate. Regeneration from 0.4-mm meristems reached 100%, but none of the regenerated plantlets were GVA-free. Meristems of 0.2 mm resulted in only 12% GVA-free plants. Frequency of GVA elimination was not affected by the cryopreservation procedure, be it encapsulation-dehydration or vitrification. Leaf morphology of plants regenerated from cryopreserved shoot tips was similar to that from control shoot tips. Results from the present study suggest cryopreservation of shoot tips as a simple and efficient method for eliminating GVA from infected grapevine plants.

P-2021

In Vitro Selection of Somaclones through Phosphate Starvation in Maize (*Zea mays L.*). ANSHU MIGLANI, Alok Shukla, and R. C. Pant. Department of Plant Physiology, G. B. Pant University of Agriculture & Technology, Pantnagar-263145, U. S. Nagar, Uttaranchal, India. E-mail: anshum2002@rediffmail.com

Phosphorus is the crucial component of biologically active compounds and is one of the most important macronutrient that is least accessible to plants. It significantly affects plant growth and metabolism. Rocks containing phosphate are limited and mines will be depleted by 2090 according to available reports. Phosphorus starved somaclones were selected to enhance phosphate uptake under phosphorus starvation in maize (*Zea mays* L.) inbred line Gaurav. About 15-30 days old embryogenic calli, initiated on MS medium supplemented with 2 mg/l 2,4-D, were taken. Somatic embryos were induced in such calli by consecutively transferring them to MS basal medium containing different concentrations of phosphorus in the MS medium (i.e. 38.6 mg/l, 30 mg/l, 20 mg/l and 10 mg/l) in the form of KH_2PO_4. Phosphorus starved calli were selected on the basis of callus growth and anthocyanin pigmentation. Somatic embryos were confirmed by microtomy. Different stages of somatic embryos obtained were pro-embryo, globular, ovoid and post-globular. Calli containing 30 mg/l phosphorus were found to be the best for survival. Protein profiling exhibited an over-expression of ~27 kDa protein in all the phosphorus-starved calli. It can be further exploited to locate the gene/s responsible for the enhancement of phosphate uptake under phosphorus starvation in higher plants. These genes can be cloned and used for transformation studies. This will be beneficial to maintain sustainability in maize improvement when treated with phosphate fertilizers.

P-2022

The Use of Clontech Coral Reef Proteins as Tools in Plant Transformation. ALLAN WENCK[1], Celine Pugieux[2], Mark Turner[2], Martha Dunn[1], Cheryl Stacy[1], Annalisa Tiozzo[2], Erik Dunder[1], Emiel van Grinsven[1], Rafiqul Khan[1], Marina Sigareva[1], Wen Chung Wang[1], Janet Reed[1], Shanaaz Tayab[2], Paul Drayton[2], Duncan Oliver[2], Gaston Legris[2], Helen Rushton[2], Hugh Trafford[2], Karen Launis[1], Yin-Fu Chang[1], Dong-Fang Chen[2], and Leo Melchers[1]. [1]Syngenta Biotechnology Inc. 3054 Cornwallis Road, Research Triangle Park, NC 27709 and [2]Jealott's Hill Research International Research Station, Jealott's Hill Nr, Bracknell, Berks RG42 6EY. UK. E-mail: allan.wenck@syngenta.com

Marker genes are of great use in transformation technology in order to facilitate development of new transformation techniques. Several marker genes are currently available for use, but each has specific problems such as background, need for a substrate or not useful with viable tissue. Green Fluorescent Protein (GFP), isolated from jellyfish, has improved the reporter gene field, as it doesn't need a substrate or external co-factor to emit fluorescence and can be tested *in vivo* without destruction of the tissue under study. Its use, though, can be limited in plants due to a high level of background fluorescence mainly from green tissues using UV illumination wavelengths. In addition, GFP and mutant forms have significantly overlapping excitations and emission wavelengths making them not useful for use in the same cell. Recently, 5 Novel Fluorescent Proteins (NFPs) were isolated from non-bioluminescent species of reef coral organisms and have been made available through ClonTech (BD Biosciences). They are: AmCyan, AsRed, DsRed, ZsGreen and ZsYellow. We have evaluated them in a large range of both monocots and dicot plants and our results indicate that they are valuable reporting tools for transformation in a wide variety of crops. We report here their successful expression in wheat, maize, barley, rice, banana, onion, soybean, cotton, tobacco and tomato. Transient expression could be observed as soon as 24 h after transformation in some cases allowing for very clear visualisation of individually transformed cells. Stable transgenic events were generated, using mannose, kanamycin or hygromycin selection. Transformed plants were phenotypically normal, showing a wide range of fluorescence levels, and were fertile. Expression of AmCyan, ZsGreen and AsRed were visible in maize T1 seeds, allowing a more than 99% accurate visual segregation. The excitation and emission wavelengths of some of these proteins are significantly different enough that it was possible to visualize cells transformed with more than one of the fluorescent proteins. These proteins will become useful tools for transformation optimisation studies and other studies in the plants demonstrated. The wide variety of plants successfully tested demonstrate that these proteins will potentially find broad use in plant biology.

P-2023

Growth Response of In Vitro Cultured *Campanula* Plantlets to Trophic Phase, Photosynthetic Photon Fluxes, and Temperature. GYEONG HEE KIM, Nam Hee Choi, Mi Young Lim, Young Hoe Kim and Byoung Ryong Jeong. Dept. of Horticulture, Division of Applied Life Science, Graduate School, Gyeongsang National University, Jinju 660-701, Korea. E-mail: nany96@hanmail.net

Effect of trophic phase (photoautotrophic vs. photomixotrophic) and two photosynthetic photon fluxes (PPF, 45 vs. 90 μmol·m^{-2}s^{-1}) on growth of in vitro cultured *Campanula punctata* Lam. var. rubriflora Mok and *Campanula takesimana* Nakai plantlets was investigated. Plantlets were grown for about four weeks on the MS medium in environment controlled rooms under the 16 h per day photoperiod. Growth of both species under photoautotrophic environment (without sucrose and with supplemental CO2 at 1000 μmol·mol^{-1}) was significantly promoted as compared to that under photomixotrophic environment (with sucrose and without supplemental CO). Especially, petiole length, and fresh and dry weights of shoot and root were affected by interaction of trophic phase and PPF. Effect of two temperatures (15 vs. 25° C) and two photosynthetic photon fluxes (45 vs. 90 μmol·m^{-2}s^{-1}) on photoautotrophic growth of both species was also investigated. Growth in 15° C was significantly suppressed as compared to that in 25° C. The Hunter's 'a' value was greater while 'b' value was smaller in 15° C than in 25° C, and 'L' value was not significantly different. Plantlets growth was enhanced the most under a photoautotrophic phase at 25° C and under 90 μmol·M^{-2}s^{-1} PPF.

P-2024

Monitoring Phytohormone-induced Changes in Cotton Ovule Culture Gene Expression Using Real-time, Reverse-Transcription Polymerase Chain Reaction. H. J. Kim[1] and B. A. TRIPLETT[2]. [1]Department of Biological Sciences, University of New Orleans, [2]USDA-ARS, Southern Regional Research Center, New Orleans, LA 70124. E-mail: btriplet@srrc.ars.usda.gov

Many genes expressed during cotton fiber development are transcriptionally regulated. Two phytohormones, auxin and gibberellic acid, are known to be essential for maximal fiber production *in vitro*, however the effect of these and other plant hormones on transcript accumulation of fiber specific genes is unknown. Primers were designed to nine cotton fiber genes whose expression levels had previously been monitored by Northern blot analysis. Three of the genes are preferentially expressed during the cell elongation phase of fiber development: germin-like protein1, expansin, and alpha-tubulin 5. One of the genes is preferentially expressed during the transition from cell elongation to secondary wall synthesis: Rac 13. Two genes are expressed during secondary wall synthesis: cellulose synthases, CesA1 and CesA2. Three of the tested genes are constitutively expressed: alpha-tubulin 4, actin, and ubiquitin-conjugating protein. Ten day old cotton ovule cultures were exposed to seven different phytohormones for two days and total RNA was extracted. The reverse transcription reaction was conducted using random hexamer primers. An ABI-7900HT sequence detection system was used for real-time PCR with SYBR Green I. Relative expression was calculated using 18S rRNA as a normalizer. Expression of the two cellulose synthase genes was up-regulated by the addition of kinetin to the medium and down-regulated by the addition of auxin and brassinosteroid. Ethylene and gibberellic acid stimulated the accumulation of Rac13. Salicyclic acid down-regulated transcript accumulation of all genes normally expressed during the cell elongation and transition phases of development. Collectively, these experiments show that real-time PCR is a sufficiently sensitive method for monitoring changes in transcript accumulation in cotton ovule cultures and will be useful for identifying the molecular events responsible for the transition from cell elongation to secondary cell wall production.

P-2025

Accumulation Profiles of Lipid and Phenolic Antioxidant Compounds During a Growth Cycle of Suspended Cells of *Salvia officinalis* L.*. Paulo S. C. Braga[a], Paula C. Santos-Gomes[a], Rosa M. Seabra[b], Paula B. Andrade[b], and MANUEL FERNANDES-FERREIRA[a]. [a]Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; [b]ICETA/CEQUP - Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Porto, R. AnÔbal Cunha, 4050 Porto, Portugal. E-mail: mfferreira@bio.uminho.pt

Cells suspensions from *Salvia officinalis* L. were established in liquid MS medium supplemented with an auxin and a cytokinin and the growth profiles were determined during a growth period of 18 days. Samples were taken at the beginning of the subculture, after 1 day, and thereafter with intervals of 2 days. Some samples were used for determination of fresh and dry biomass cell density, other samples were used for extraction and GC and GC-MS analyses of lipid compounds and other ones were used for extraction and HPLC-DAD analyses of phenolic antioxidant compounds. Thirteen fatty acids besides squalene, campesterol, and b-sitosterol were identified in the lipid extracts. Five phenolic acids, four phenolic diterpenes, and the flavonoid genkwanin were identified in the phenolic extracts. The accumulation of all the identified compounds in the *S. officinalis* suspended cells was followed during the growth cycle of the respective batch suspension cultures. The specific accumulation of the main fatty acids, namely, palmitic, linoleic, and linolenic acid started rising abruptly 2 days before the beginning of the exponential biomass growth phase and started decreasing several days before the end of that phase. Rosmarinic acid, which during the first seven days was not detected, appeared at ninth day, increasing abruptly at eleventh day and keeping rising up to the end of the culture cycle. *This work was supported by FCT through the Project POCTI/AGR/43482/2001.

P-2026

Stable Transformation of *C. annuum* and *C. baccatum* Explants Inoculated with *A. rhizogenes* and *A. tumefaciens*. LUIS VALERA and Gregory C. Phillips. Molecular Biology Program, New Mexico State University, Las Cruces, NM 88003. E-mail: grphilli@nmsu.edu

Chile pepper and Bell pepper are vegetable crops growing in importance in the USA and the world. Therefore, the requirement of a reliable genetic transformation method for this species to speed its breeding is unquestionable. Nevertheless, pepper is one of the most difficult species to be transformed, probably due to regeneration problems among other factors. Publications have pointed to cotyledons and leaves as the most promising explants for transformation with *Agrobacterium*. We tested these explants together with half seeds, zygotic embryos, organogenetic callus, inverted hypocotyls and 'hooks' (bent hypocotyls at the time of germination). Three hundred explants of each kind from *C. annuum* (cv 'NM6-4') and *C. baccatum* (cv 'Campanita') were tested with two *Agrobacterium* species: *A. rhizogenes* strain K599 and *A. tumefaciens* strain EHA105. Stable transformation was recorded through histochemical GUS test performed on regenerating structures 60 days after co-cultivation. *C. baccatum* hypocotyls and hooks showed 15.3 and 23.3%, respectively, of explants having transformed shoot buds with EHA105. The results dropped to 1.7 and 1.0%, respectively, with strain K599. On the other hand, *C. annuum* hooks, cotyledons and embryos showed 8.3, 4.0 and 4.0% transformation, respectively, with the strain EHA105. The same explants showed 0.3% or less transformation when the strain was K599. Additional experiments for increasing the transformation efficiency with selected explants are underway. Large-scale transformation of hypocotyls, hooks and cotyledons have also been initiated to estimate the probability of obtaining transformed complete plants.

P-2027

Herbicide Resistant Celery (*Apium graveolens* L.) Plants Produced by Agrobacterium-mediated Transformation Using the Bar Gene. A. LOSKUTOV and K. C. Sink. Plant Transformation Ctr., Michigan State University, E. Lansing, MI 48824. E-mail: loskutov@msu.edu

Transgenic celery plants were produced using an Agrobacterium tumefaciens-mediated transformation system. Strains EHA105 or GV3101 each carrying a binary vector containing the bar gene under NOSP (pGPTV-BAR) or under 35SP (pDHB321.1) were used. Leaf explants were inoculated and co-cultivated for 2 d. Callus tissues were induced from the explants on MS + 2,4-D 0.5 mg/l + kinetin 0.6 mg/l + timentin 300 mg/l (CM medium). Three-four weeks after inoculation, a two-step selection method of calli started on CM + 0.25-0.35 mg/l glufosinate ammonium (GS), and was increased after 3-4 weeks to 1.0-2.5 mg/l GS. During this 2-3 month period, selected callus clones were tested for resistance to 5, 10, 50, 100 mg/l GS. Shoots were regenerated from resistant callus clones on B5 + 2-iP 1.0 mg/l + NAA 0.3 mg/l and rooted on MS. For EHA105:pGPTV-BAR, four GS-resistant plants were confirmed transgenic by PCR. Using GV3101:pDHB321.1 or EHA105:pDHB321.1 four and one plant(s), respectively, were confirmed by PCR. To date, Southern's of five of these 9 plants indicated a range of single to 3 copies of bar. Five of the transgenic plants tested positive for the chlorophenol red assay. Greenhouse tests of plants to GS are underway, and plants of each transgenic line are being grown to larger size for seed production and genetic analysis. This is the first report of transgenic celery plants with a newly introduced agricultural important trait.

P-2028

Transgenic Chrysanthemum (*Dendranthema grandiflora* cv. Subangyuk) with *LS Like* Gene Expresses Branchlessness Habit. S. Y. LEE, B. H. Han, H. J. Yoo, H. K. Shin, I. G. Mok, J. G. Woo, E. J. Suh, and Y. P. Lim[1]. Horticultural Biotechnology Laboratory, National Horticultural Research Institute, Rural Development Administration, Suwon 441-440, Korea and [1]Horticultural Division, Chungnam National University, Daejeon 305-764, Korea. E-mail: lsy0504@rda.go.kr

Commercial chrysanthemum cultivars having many lateral branches developed caused high labor and cost consuming for farmers. To develop without or less lateral branches variety, we introduced *ls like* gene constructs into cv. Subangyuk which is one of the most popular chrysanthemum cultivar in Korea. *Ls like* gene was related to control lateral suppression and a partial-length gene, and was cloned from chrysanthemum breeding line 95B1-49, having branchlessness habit, treated with 4 mM silverthiosulfate. It was constructed into pCAMBIA2301 vector, containing marker gene (neomycin phosphotransferase 2 (*npt*2), as sense or antisense direction. These constructs were used for *Agrobacterium*-mediated transformation chrysanthemum cv. Subangyuk. We used C58C1 as *Agrobacterium tumefaciens*. As a results of PCR and Southern analysis with the neomycin phosphotransferase 2 and *ls like* gene, 55 plants were confirmed as transformants. Twenty plants among them were transformed with sense construct, but the rest were antisense construct. Northern analysis revealed that they expressed detectable *ls like* gene. They generally showed branchlessness habit although the strength was weak.

P-2029

Phloem-specific Expression of the β-Glucuronidase Reporter Gene in Transgenic Strawberry (*Fragaria vesca* and *F. x ananassa*). Q. LIU[1,2], Y. Zhao[1], and R. E. Davis[1]. [1]Molecular Plant Pathology Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD 20705 and [2]Shandong Institute of Pomology, Taian, P. R. China, 271000. E-mail: zhaoy@ba.ars.usda.gov

Phytoplasmas are cell wall-less, mycoplasma-like organisms that are restricted to sieve elements in the phloem tissue of diseased plants. Infection of strawberry (*Fragaria ssp*) by phytoplasmas can cause heavy fruit loss as well as plant death. An objective of our program is to develop strategies for improving strawberry resistance to phytoplasma disease *via* genetic engineering. One such strategy involves phloem-specific expression of anti-apoptotic/anti-microbial peptide genes in engineered strawberry plants. To achieve efficient phloem-specific expression in strawberry, we tested the promoter of an *Arabidopsis* sucrose-H^+ symporter (AtSUC2) gene, which directs companion cell-specific expression of AtSUC2 in wild-type *Arabidopsis* plants and was confirmed by others to be active in the phloem of transgenic tobacco. Transgenic lines of diploid (2n=2x=14) 'Alpine' strawberry *F. vesca* FRA197 and FRA198, and of octoploid (2n=8x=56) *F. x ananassa* ever-bearing cultivars 'Hecker' and 'La Sans Rivale' were obtained by a modified *Agrobacterium*-mediated leaf segment transformation protocol using a PBI101-derived expression cassette with a T-DNA encoding the β - glucuronidase reporter gene (*gus*) under the control of the AtSUC2 promoter. The integration of the *gus* gene into transgenic strawberry plants was confirmed by PCR and Southern blot analysis. Histological GUS activity was found specifically in the vascular system of major and minor veins, petioles, and roots of all tested transgenic plants. Numerous independent transgenic lines were recovered from the above four strawberry genotypes. Diploid strawberries exhibited much higher transformation rates (61.5% and 66.1% for FRA197 and FRA198, respectively) than octoploid strawberries (10.4% and 7.4% for 'Hecker' and 'La Sans Rivale', respectively). Based on these results, the AtSUC2 promoter and the modified transformation protocol maybe useful for engineering phytoplasma-resistant transgenic strawberry plants.

P-2030

Agrobacterium-mediated Transformation and Plant Regeneration from Leaf Segments of Sweet Cherry Dwarf Rootstock 'Gisela 6' (*Prunus cerasus X P. canescens*). Q. LIU[1,2], Y. Zhao[1], R. W. Hammond[1], H. Zhao[2], and R. E. Davis[1]. [1]Molecular Plant Pathology Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD 20705 and [2]Shandong Institute of Pomology, Taiwan, P. R. China, 271000. E-mail: zhaoy@ba.ars.usda.gov

As a part of our project to develop protocols aimed at improving cherry rootstock resistance to viral and bacterial diseases *via* genetic engineering, studies were conducted to examine the influence of medium composition and growth regulators such as cytokinins on formation of adventitious shoots from leaf segments of *in vitro* propagated Gisela cherry dwarf rootstocks (*Prunus cerasus X P. canescens*). An optimal condition was established and applied to efficiently regenerate genetically modified cherry plants following *Agrobacterium*-mediated transformation. Leaf segments from rootstocks Gisela 5, 6, and 7 were cultured on the modified woody plant medium (WPM) supplemented with 22.0 μM 6-BA and 2.5 μM IBA. Leaf segments from all three sources responded to the induction medium (WPM), and shoot regeneration occurred within two to three weeks. Regeneration frequencies reached 55.8% and 77.7% for Gisela 5 and Gisela 6, respectively. Under the same conditions, the frequency of shoot organogenesis from Gisela 7 was much lower, with only 16.7% of the leaf segments forming shoots. Gisela 6 was chosen as a host for further experiments. Modified binary Ti plasmids with a T-DNA carrying the β-glucuronidase reporter gene (*gus*) under the control of either the constitutive CaMV 35S promoter or the phloem-specific *Arabidopsis* sucrose-H^+ symporter (AtSUC2) gene promoter were introduced into the cherry rootstock by use of *A. tumefaciens* EHA105-mediated transformation. Six independent transgenic lines were recovered from a total of 3700 inoculated explants. Integration of the *gus* gene into the regenerated cherry plants was confirmed by PCR and Southern blot analysis. GUS activities were detected in all leaves sampled from each transgenic plant.

P-2031

Transformation of Rice with Bacterial Artificial Chromosome (BAC) DNA. B. H. PHAN[1], C. Topp[1], C. Zhong[1], U. Akoh[1], J. Jiang[2], R. K. Dawe[1], and W. A. Parrott[1]. [1]University of Georgia, Athens, GA 30602-6810 and [2] University of Wisconsin, Madison, WI 53706. E-mail: baophan@uga.edu

Plant transformation with BAC DNA containing high-molecular weight DNA can be useful to help characterize gene function. Thus far, the only transfer of high-molecular weight DNA into a plant has been *Agrobacterium*-mediated transformation of tobacco. The use of microprojectile bombardment has been limited to the introduction of YAC DNA into cultured cells of tobacco and tomato, without subsequent plant recovery. In this study, microprojectile bombardment was used to transform rice with a BAC containing an 90-kb insert of centromeric satellite repeat elements from rice. pCAMBIA1305-2, which contains *hph* for hygromycin resistance, was co-bombarded with the BAC DNA. Molar DNA ratios of 1:1, 1:2, 1:6, and 1:10 (pCAMBIA1305-2 : BAC) were tested, along with total DNA concentrations of 800, 500, or 125 ng per bombardment. Approximately 1% of the immature embryos bombarded yielded callus colonies transgenic for the BAC. All independently transformed plants containing the rice BAC, but none of the plants engineered with only pCAMBIA1305-2, have a distinctive altered morphology. Further analyses of these plants may provide an insight into the function of the genetic elements found on the centromeric DNA present in the BAC.

P-2032

Agrobacterium-mediated Genetic Transformation of Pigeon Pea with Hemagglutinin Nuraminidase (HN) Gene of *Peste des Petits Ruminants Virus* as a Source of Edible Vaccine. V. V. SATYAVATHI, V. Prasad, K. J. M. Valli, K. Abha, M. S. Shaila, and G. Lakshmi Sita. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India 560 012. E-mail: valluri.satyavathi@ndsu.nodak.edu

Hemagglutinin neuraminidase (HN) geneof *Peste des petits ruminants virus* (PPRV) has been expressed in pigeon pea as a possible source of vaccine (antigen) against PPRV. *Pestis des petits ruminants* virus is the causative agent of a highly contagious disease of sheep, goats, and wild ruminants with a high mortality rate. It is endemic in parts of West Africa with occasional outbreaks in Eastern Africa, the Middle East and South Asia. The major drawback of the currently used vaccine is its risks of contamination and heat labile nature. As an alternative source, expression of antigens in plant tissue as 'edible vaccines' provides a heat-stable environment and is ideal for mass immunization. Here, we present our results in developing transgenic pigeon pea (*Cajanus cajan* (L.) Millsp.) plants expressing the *hemagglutinin neuraminidase* (HN) protein of the PPR virus. A 2-kb fragment containing the coding region of the HN protein was cloned into a binary vector pBI121 and mobilized into *Agrobacterium tumefaciens* strain GV3101. Cotyledonary nodes from germinated seeds of pigeon pea were used for *Agrobracterium* mediated-transformation. The presence of transgenes *nptII and* HN in the plants was confirmed by PCR using specific primers. The expression of HN protein in the transgenic lines was confirmed by Western blot analysis using polyclonal monospecific antibody to HN and more importantly plant-derived HN protein was shown to possess neuraminidase activity. Expressed protein in leaves and seeds could be used as a source of edible vaccine for the wild bovids.

P-2033

Vaccinium angustifolium Cell Cultures: An Alternative Method of Studying the Anti-Cancer Potential of Wild Blueberries. TRISTAN F. BURNS KRAFT, Chris Knight[1], Barbara Schmidt[1], Randy Rogers[1], David Seigler[2], and Mary Ann Lila[1]. [1]Department of Natural Resources and Environmental Sciences and [2]Department of Plant Biology, University of Illinois, Urbana, IL 61801. E-mail: tkraft@uiuc.edu

Vaccinium angustifolium Ation (wild blueberry) fruits have been intensively researched for potential health benefits and are rich sources of antioxidants. They have been shown to contain compounds that inhibit initiation, promotion, and proliferation stages of carcinogenesis, as shown through cycloxygenase, quinone reductase, ornthine decarboxylase, and hepatocyte bioassays, as well as others. Wild blueberry fruit fractions have demonstrated 77% inhibition against cycloxygenase-2, exhibited doubling of quinone reductase activity at 3.5 μg/mL, and provided 94% inhibition in the hepatocyte bioassay. ^{1}H-NMR spectra and thin-layer chromatography indicate that active fractions are often rich in proanthocyanidins and other flavonoids. Blueberry fruits, however, contain many sugars and pectins, which impede the efficiency of extraction and can introduce artifacts in routine bioassays. Plant cell culture is an alternative method for producing phytochemicals without many of the associated interfering compounds, in a highly controlled environment that can be manipulated to influence the types and amounts of active compounds. Wild blueberry suspension cultures were grown in the dark on a 7 day cycle at 25° C on a Gyrotary shaker set at 150 rpm in a solution culture medium. These cultures have been maintained in culture for 1.5 years and were initiated from seedlings germinated *in vitro*. Spectrophotometric assays compared 70% aqueous acetone extracts of 10 and 14 day old blueberry cultures to determine the harvest date that optimizes production of bioactive proanthocyanidins and other phenolics. Results of the Folin-Denis and the acid-butanol assays, which test for phenolics and proanthocyanidins respectively, revealed significantly higher amounts of these compounds in 10 day old cultures compared to 14 day old cultures. Therefore, 10 day old *in vitro* cultures provided the best yield of bioactive chemopreventive phytochemicals from wild blueberry germplasm. Cell culture extracts produced in our lab have demonstrated equal or greater potency than fruit extracts in a range of bioassays and permit quantitative assessment of the phytomedicinal value of these natural components.

P-2034

Differential Accumulation of Flavonoids in Cell Cultures of *Vitis vinifera*: Implications for Pharmacological Research. SINEE P. KOPSOMBUT, K. A. Marley, R. A. Larson, and M. A. Lila. University of Illinois, Department of Natural Resources and Environmental Sciences, Urbana, IL 61801. E-mail: kopsombu@uiuc.edu

Thorough analysis of the contribution of biologically-active natural plant compounds in human health maintenance usually requires that individual phytochemicals must be isolated and tested in controlled bioassays. Epidemiological studies as well as laboratory analyses have suggested that bioactive flavonoid compounds in grape (*Vitis vinifera*), including anthocyanin pigment complexes and proanthocyanidins, have broad therapeutic benefits. Anthocyanins have been linked to anticarcinogenic, anti-inflammatory, antioxidant, and cardioprotective properties, whereas proanthocyanidins share similar beneficial qualities and are closely linked to the anthocyanin biosynthetic pathway. Many flavonoids, however, are unstable and/or easily degraded during the process of isolation, and complex multi-unit proanthocyanidin oligomers and polymers of flavan-3-ol units can deteriorate and therefore are difficult to extract and quantify from fruits. Cell cultures, however, can frequently be induced to produce the same range of bioactive phytochemicals, without interferences to extraction/fractionation that are inherent in fruit. In this study, the flavonoid profiles from continuous cell cultures of two grape genotypes, Bailey Alicant (a hybrid) and Merlot, were compared in order to assess their utility as resources for phytochemical extraction. Cell cultures were extracted after 6 days in liquid suspension medium and extracted in 50% aqueous methanol. The acid butanol assay was used to quantify proanthocyanidins, based on the oxidative cleavage of proanthocyanidin molecules to produce anthocyanidin chromophores. The anthocyanidin abosorbance levels were measured using a spectrophotometer (λ=550 nm). Whereas cultures of Bailey Alicant produced copious yields of both anthocyanins and complex proanthocyanidins, the Merlot cultures produced only proanthocyanidins, which provided an advantage in terms of clean isolation of only proanthocyanidin oligomers for bioassays. Bailey Alicant had a proanthocyanidin concentration of 0.051 mg/L of proanthocyanidin per 0.5 g/mL of extract. Merlot extract (0.5 g/mL) produced 0.057 mg/L of proanthocyanidin exclusively. Further results with biotic and abiotic elicitors, including sodium acetate ($NaC_2H_3O_2$), are underway to determine the extent to which flavonoid profiles can be skewed to favor production of specific classes of bioflavonoids.

P-2035

Transformation of Carnations with Flavonoid Biosynthesis Related Genes. B. J. AHN, K. H. Hwang, B. H. Min, and H. Y. Joung. College of Bioresources Science, Dankook University, Cheonan 330-714, Korea; Nong Woo Bio Co. Ltd., Yeoju, Kyunggido 469-880, Korea; and National Horticultural Research Institute, RDA, Suwon 440-760, Korea. E-mail: bjahn@dankook.ac.kr

Carnation is a major cut flower plant and improved cultivars are plentiful through rigorous breeding efforts. Still desirable cultivars possessing disease tolerances and other useful traits like true blue flower color or environmental tolerances are expected to be bred. To produce transgenic carnation plants expressing modified flower colors, we tried to insert several flovonoid biosynthesis related genes stepwise. Four cultivars of red flowered carnation including 'Desio' were transformed with a DFR gene encoding dihydroflavonol 4-reductase and a CHI gene encoding chalcone synthease isomerase was used to transform three yellow flowered cultivars. Explants of leaves, stems and shoot tips were excised and infected with *Agrobacterium tumefaciens* strain LBA4404 harboring a binary vector pGA748/DFR or pGA748/CHI. After 10 days of coculture, the tissues were cultured on MS media supplemented with 1 mg/L BAP, 0.1 mg/L NAA, 250 mg/L cefotaxime/carbenicillin, 500 mg/L kanamycin. Kanamycin resistant putative transformants were selected and regenerated through over six times of subcultures only among the cultures derived from shoot tip explants. More than 50 plants were confirmed to contain the transgenes through PCR, Southern and Northern blot analyses. They were established in soil and multiplied through cuttings. Morphological deformities were not detected at juvenile stages and are being grown to bloom to examine their phenotypes.

P-2036

Development of a Robust Tissue Culture System: Ideal for *Agrobacterium* Mediated Transformation in Sorghum. B. SADIA, W. Alaiwi, J. MacDougall, K. Meeker, B. Smith, S. Ibeji, G. Franklin, M. Parani, S. L. Goldman, and R. V. Sairam. Plant Science Research Center, The University of Toledo, Toledo, OH 43606. E-mail: bsadia@utnet.utoledo.edu

We report a highly efficient protocol for multiple shoot formation in three sorghum hybrids (NC+6C21, NC+6B50 and NC+262) using shoot meristems. Of the six different modified MS media tested, medium supplemented with 3 mg/l BAP and 1 mg/l TDZ was found to be the most effective for multiple shoot formation in all three sorghum hybrids. The highest number of shoots recorded in NC+ 262 was 68 ± 2.00/explant. Here we also report for the first time transient expression in sorghum via *Agrobacterium* using immature inflorescence. Putatively transformed calli showed 30-50% GUS activity, 21d post transfer to selection medium. Molecular analysis of the regenerated plantlets is in progress.

P-2037

Biolistic Transformation and Expression of Fertile Soybean Transgenics for Chitinase and Glucanase. JUNG-HOON LEE[1], William Schapaugh[1], Subbaratnam Muthukrishnan[2], and Harold N. Trick[3]. [1]Department of Agronomy, [2]Department of Biochemistry, [3]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. E-mail: hhl6644@ksu.edu

Our long-term goal is to control the soybean fungal disease charcoal rot and soybean cyst nematode (SCN) with the use of biolistic transformation. Our strategy for plant protection is to over-express several pathogenesis-related (PR) proteins in a gene pyramid or a "stacked" gene approach. The genes we have used in this study included wheat glucanases (AF112965 and AF112967), wheat chitinases (AF112963 and AF112966), a rice chitinase (D16221), and a synthetic, insect-derived chitinase (*Manduca Sexta* chitinase, *msc* AAC04924). All PR genes are under the control of constitutive CaMV35S promoter and linked to *hpt* gene as selectable marker. Immature embryos of soybean cultivars 'Chapman', 'Jack', and 'Fayette' were used for particle bombardment. Several combinations of these genes were introduced into the soybean cultures via co-bombardment. Nineteen independent events with different combinations of the transgenes were selected on hygromycin-containing media and regenerated into plants. All events were fertile. To confirm integration and copy number, Southern blot analysis were performed with specific probes that were derived from non-homologous regions of the transgenes. Northern and Western blot analyses as well as bioassays are in progress and will be discussed.

P-2038

Genetic Transformation of Chilli (*Capsicum annuum*. L, var Pusa jwala and California Wonder) via *Agrobacterium tumefaciens* with Coat Protein of Pepper Vine Binding Virus (PVBV). K. J. MARAGATHA VALLY[1] and G. Lakshmi Sita[2]. [1]Texas A&M University, Entomology Department, Heep Center, College Station, TX 77843-2475, and [2]Indian Institute of Science, Dept. of Microbiology and Cell Biology, IISc, Bangalore, Karnataka 560012, INDIA. E-mail: Vally@tamu.edu

Direct transformation was established in Chilli plants via *Agrabacterium* mediated transformation avoiding tissue culture. *Agrobacterium tumefaciens* strain LBA 4404 harboring the binary vector pBAL2 carrying the reporter gene GUS intron (GUS-INT) and the marker gene neomycin phosphotransferase (NPTII) was used initially for transformation. Chilli seeds were soaked for 12 hours in overnight grown culture of LB4404 diluted to half strength for transformation, and transferred to sterilized solirite after drying in filter papers prior to potting. Plants were watered supplemented with 50 mg/l Kanamycin. Putative transgenics plants were recovered in 12-16 weeks from the time of gene transfer to establishment in pots. Molecular analysis of the field established plants were carried to confirm the transgenic nature. The histochemical GUS assay and PCR analysis are used to verify the presence of GUS andNPTII genes respectively in putative transgenic plants. The GUS and PCR data is used to standardize conditions. Using the above protocol transformation was done with *Agrobacterium* (LB4404) harboring the coat protein of Pepper Vine Binding Virus coat protein (PVBV cp) along with the marker gene neomycin phosphotransferase (NPTII). Putative transgenics plants were initially screened with PCR analysis using NPTII and PVBV (cp) primers. Subsequently to confirm the transgenic nature, molecular analysis was done with field grown plants. The presence of NPTII gene in the transgenic plants was verified by RT-PCR analysis and PVBV coat protein with Western blot analysis. Molecular analysis will be presented.

P-2039

Using Leaf Disk for Guayule Transformation. NIU DONG and Katrina Cornish. USDA/ARS/WRRC/CIU, 800 Buchanan St., Albany, CA 94710. E-mail: ndong@pw.usda.gov

Guayule (*Parthenium argentatum*), a shrub in the desert area of Texas, New Mexico, Arizona, and California, is a new crop candidate for rubber industry. Transformation guayule with genes of agronomic traits is necessary. However, the existing transformation method using shoot node is time consuming and tedious. We have developed a new method using leaf disks as explants for guayule transformation. A BAR selective marker and an intron containing GUS reporter gene has been introduced into the guayule. Transgenic guayule plants with this method show resistance to the herbicide glufosinate and positive GUS staining. Southern blot confirmation of the transformation is underway.

P-2040

Increased Transformation and Rooting Efficiencies in Canola (*Brassica napus* L.) Using *Agrobacterium* Mediated Transformation. VINITHA CARDOZA and C. Neal Stewart, Jr. Department of Plant Sciences and Landscape Systems, 2431 Center Drive, Rm. 252 Ellington Plant Sciences, University of Tennessee, Knoxville, TN 37996-4561. E-mail: vinu_cardoza@hotmail.com

Canola (*Brassica napus* L.) is one of the most important oilseeds in the United Sates and Canada. It is widely being exploited to engineer various desired traits. However, the transformation efficiency is still low in canola and there is a need to develop efficient transformation methods to improve canola varieties. An efficient protocol for the production of transgenic *Brassica napus* cv. Westar plants was developed by optimizing two important parameters; preconditioning time and co-cultivation time. *Agrobacterium tumefaciens*-mediated transformation was performed using hypocotyls as explant tissue. Two variants of a GFP-encoding gene, mGFP5-ER and eGFP, both under the constitutive expression of the cauliflower mosaic virus 35S promoter, were used for the experiments. Optimizing the preconditioning time to 72 h and co-cultivation time with *Agrobacterium* to 48 h increased the transformation efficiency from a baseline of 4% to 25%. With mGFP5-ER the transformation rate was 17% and with eGFP it was 25%. Transgenic shoots were selected on 200 mg/l kanamycin. Rooting efficiency was 100% on half strength Murashige and Skoog medium with 10 g/l sucrose and 0.5 mg/l indole butyric acid in the presence of kanamycin.

P-2041

Utilization of Glufosinate Selection and Cysteine in *Agrobacterium*-mediated Cotyledonary Node Transformation in Twelve Soybean Cultivars. M. M. PAZ, Z.-B. Guo, Z.-Y. Zhang, A. K. Banerjee, and K. Wang. Department of Agronomy, Iowa State University, Ames, IA 50011-1010. E-mail: mmpaz@iastate.edu

Agrobacterium-mediated transformation of soybean cotyledonary node explants was conducted using glufosinate or bialaphos selection *in vitro*. Glufosinate selection enhanced soybean transformation as compared to bialaphos. The use of 6 mg L^{-1} glufosinate yielded higher final transformation efficiency ranging from 2.0% to 6.3% while bialaphos at 4 to 6 mg L^{-1} gave 0% to 2.1% efficiency. Based on independent experiments using different soybean cultivars and constructs derived from the base vector pTF101.1, final transformation efficiency ranged from 0.61% to 2.86% when cysteine and dithiothrietol (DTT) were present during co-cultivation and from 0.25% to 0.89% without these compounds. Also, the addition of cysteine and DTT to the cocultivation medium improved T-DNA transfer as indicated by enhanced transient GUS expression. Shoot regeneration was attained in twelve soybean cultivars belonging to maturity groups I-VI. These cultivars may be amenable to genetic transformation and may provide a valuable tool in soybean improvement programs.

P-2042

Use of a Site Directed Recombination Strategy for Selectable Marker Removal in *Glycine max* (Soybean). JOANNE EKENA[1], Michael Petersen[1], and Larry Gilbertson[2]. [1]Monsanto Company, Agracetus Campus, 8520 University Green, Middleton, WI 53562 and [2]Monsanto Company, 700 Chesterfield Pkwy, Chesterfield, MO 63198. E-mail: joanne.l.ekena@monsanto.com

An essential aspect of transgenic plant production is the use of a selectable marker for the generation of transformed lines. A variety of strategies exist for removal of this selectable marker after transformation including two T-DNA Agrobacterium transformation, markerless bombardment, co-bombardment and a number of recombinase based systems. We have adapted a site-directed recombination technology for use in marker removal in Soybean (*Glycine max*). We tested the use of the Cre recombinase for its ability to excise a marker gene flanked by lox sites. We will discuss the use of a crossing strategy, whereby a plant containing the Cre recombinase gene and a plant containing a marker gene flanked by lox sites are sexually crossed to demonstrate heritable excision of the selectable marker in the F2 generation.

P-2043

Selection of Transgenic Papaya Seedlings Using Kanamycin and DMSO. THOMAS W. ZIMMERMAN and Nina St. Brice. University of the Virgin Islands Agricultural Experiment Station, RR2 Box 10,000, Kingshill, VI 00850. E-mail: tzimmer@uvi.edu

Papaya Ringspot Virus (PRV) is a widespread disease throughout the Caribbean. It limits the production of papayas by causing deformed mottled leaves and fruits with the characteristic ringspots. Resistance to PRV has been obtained through the development of transgenic papaya incorporating the PRV coat protein. However, transgenic or non-transgenic papaya seedlings cannot be determined from appearance. The transgenic papaya also contains the neomycin phosphotransferase (NPTII) gene used for kanamycin selection in tissue culture for the transgenic papaya cells that were regenerated into plants. Applications of kanamycin at 0, 100, 250, 500 or 1,000 mg/L were applied in combination with dimethyl sulfoxide (DMSO) at 0, 1 or 10 ml/L. The treatments were sprayed on two transgenic papaya lines (HBX-4-99 and HYX-5-99) and a control (Wash x 356-3) line. Kanamycin was used to detect papaya seedlings lacking the NPTII gene, while DMSO was used to enhance the permeability of the plant cell wall and membrane to the kanamycin. Seedlings lacking the NPTII gene developed yellow areas on the leaves following the application of kanamycin. DMSO enhanced the effect of kanamycin susceptible plants. No yellowing of plant tissues was evident in papaya line HBX-4-99 following applications of kanamycin indicating a homozygous line for resistance. A combination of 100 mg/L kanamycin and 1 ml/L DMSO can be used successfully to screen transgenic papaya seedlings.

P-2044

High-frequency Transformation of Undeveloped Plastids in Tobacco Suspension Cells. C. L. LANGBECKER, Guang-Ning Ye, Peter T. J. Hajdukiewicz, Charles W. Xu, Charles L. Armstrong, and Jeffrey M. Staub. Monsanto Company, 700 Chesterfield Parkway, West, Chesterfield, MO 63017. E-mail: camri.l.langbecker@monsanto.com

Although chloroplast transformation technology was developed more than a decade ago, no reports exist of stable transformation of undeveloped plastids or other specialized plastid types, such as proplastids, etioplasts or amyloplasts. In this work we report development of a dark-grown tobacco suspension cell model system to investigate the transformation potential of undeveloped plastids. Electron microscope analysis confirmed that the suspension cells carry plastids that differ significantly from the leaf cell model in size, localization and developmental state. Using antibiotic selection in the light, we demonstrated that both plastid and nuclear transformation of these cell suspensions is efficient and reproducible. Homoplasmic plastid transformants are readily obtained in cell colonies, or in regenerated plants, providing a more consistent and versatile model than the leaf transformation system. Because of the uniformity of the cell suspension model, we could further show that growth rate, selection scheme, particle size and DNA concentration influence the frequency of plastid transformation. Our results indicate that the novel plastid transformation system reported here will be useful for characterizing the events that lead to homoplasmic plastid transformation.

P-2045

Somatic Embryogenesis and Transformation in Alfalfa Using Gene Gun. KAYE KNOWLES, Seema Dhir, and S. K. Dhir. Center for Biotechnology, Fort Valley State University, Fort Valley, GA 31030. E-mail: dhirs0@mail.fvsu.edu

We are attempting to generate transgenic alfalfa plants, as a potential source of edible vaccine that will synthesize antigen to the Cholera toxin of *Vibrio cholera*. Hyper expression of foreign protein (up to 46% of total soluble protein) has been accomplished via chloroplast genetic engineering. Hyper expression of antigen to the Cholera toxin subunit would facilitate oral delivery of this protein, thereby paving the way for production of edible vaccine. As a first step towards chloroplast transformation, we optimized parameters for microprojectile bombardment transformation of alfalfa. The plasmid construct, PIL TAB 380 which contains β-glucoronidase (GUS) gene, and AtZFPI: GFP alone or in combination were coated onto gold particles and leaves were bombarded. GUS and GFP gene expression in each leaf was observed as isolated blue spots after staining with X-gluc or under UV blue light after 24 hours of culture. We tested several conditions to maximize the number of blue or fluorescent green spots in each leaf. These conditions included: pressure (1100, 1300, and 1550 psi), fresh leaf vs. calli derived from leaves, and gold particle sizes (0.6 and 1.6 microns). Preliminary experiments showed that bombardment of fresh leaves yielded more transformed cells than bombarding callus. The highest amount of blue spots expressed in a single alfalfa leaf was 172 at 1550 psi. Similarly, green spots were observed using UV blue light in leaf tissue bombarded with GFP gene. To identify the optimum level of antibiotic to be used in the selection medium, we have tested streptomycin and spectinomycin at 0, 25, 50, 75, 150, and 300mg/L. Efficient selection of transgenic tissue would lead to the recovery of putatively transformed alfalfa tissue expressing the vaccine gene.

P-2046

Field Performance and Evaluation of Micropropagated FHIA Hybrid Banana Plants in the Marshall Islands. DILIP NANDWANI, Arwan Soson, and Diane Myazoe. Agriculture Experiment Station, Cooperative Research and Extension, College of the Marshall Islands, PO Box 1258, Majuro, Republic of the Marshall Islands MH 96960. E-mail: dilipn2@hotmail.com

Banana (*Musa* sp.) is one of the most important and popular fruits grown in the Marshall Islands mainly as subsistence food crop. Infertile coralline soils and exposure of narrow islands to high wind and salt spray, the production techniques for atolls are unique, however, production of atoll foods has declined sharply due to lack of cultivation and country's dependency on imported food. The introduction of exogenous technologies such as Plant Tissue Culture and Biotechnology are the methods undertaken by the Government to know-how and access to production inputs and technology for atoll food crops. Eight varieties of both cooking (Plantain) and dessert banana are recorded in the Marshall Islands. Geographic position, limited production, and smallness of the economy resulted in shortage of quality planting material. Tissue cultures of various varieties of banana with superior characters were obtained from the Regional Germplasm Center through Secretariat of Pacific Community, Fiji in 2001 and 2002. Murashige and Skoog's medium containing benzyl adenine (1.5 mg/l), adenine sulphate (160 mg/l), sucrose (3%) and agar (0.8%) was found optimal for the growth and multiplication of shoots. Field trials and evaluation of tissue culture raised plants of various FHIA cultivars conducted at the Agriculture Experiment Station. Results obtained on the morphological characters namely plant height, fruit quality, yield, insect pests and diseases are discussed. Four hybrid cultivars FHIA-3, FHIA-17, FHIA-23 and Saba were successfully produced quality bunches of fruits and adapted to the atoll environment and soil.

P-2047

Development of Efficient Plant Regeneration Systems for Agrobacterium-mediated Transformation of Sour Cherry (*Prunus cerasus* L.) and Blueberry (*Vaccinium corymbosum* L.) Cultivars. GUO-QING SONG and K. C. Sink. Plant Transformation Ctr., Michigan State University, E. Lansing, MI 48824. E-mail: sink@msu.edu

Efforts are underway to develop efficient plant regeneration systems for cultivars of blueberry and sour cherry important to Michigan in order to enable tests of transgenes. Sour cherry cv. Montmorency proliferating shoot cultures are maintained in vitro on Quoirin and Lepoivre (QL) medium + BAP 0.5 mg/l + IBA 0.05 mg/l. Initial results showed that about 30% of diamond-shaped leaf explants produced at least 1 shoot on QL + NAA 0.5 mg/l + BAP 3.0 mg/l. Recent preliminary studies indicate more frequent shoot formation when whole leaf explants with four cuts transverse to the midrib are used. Results will be presented on current studies on the use of TDZ pretreatments in liquid Murashige and Skoog medium to increase shoot regeneration frequency. Studies with blueberry cv. Elliott for shoot regeneration yielded only 1.8 shoots/leaf explant. Recent studies by Cao et al. (HortSci. 37:819,2002) indicate two-step TDZ pre-treatment of blueberry leaf explants stimulates shoot production. Such studies are now underway on blueberry cvs. Aurora, Blue Crop, Brigitta, Duke, Elliott, Legacy, and Liberty. The results of these and related efforts toward shoot regeneration systems to support transformation of these two crop plants will be presented.

P-2048

Dispersal and Filtration of Embryogenic Callus Increases the Frequency of Embryo Maturation and Conversion for Hybrid Tea Roses. K. KAMO[1], B. Jones[1], J. Castillon[1], and F. Smith[2]. [1]U.S. Department of Agriculture, U.S. National Arboretum, Floral and Nursery Plants Research Unit, B-010A Room 238, Beltsville, MD 20705 and [2]Sanford Scientific, Inc., 877 Marshall Road, Waterloo, NY 13165. E-mail: kkamo@ba.ars.usda.gov

The number of plants regenerated from embryogenic cells of two of three Rosa hybrida cultivars studied, Kardinal and Classy, was increased by shaking embryogenic callus in liquid medium for three hours to disperse the callus followed by filtration through a 530-mm screen to isolate globular-stage embryos and cell clusters. Dispersed callus of three cultivars, Kardinal, Classy, and Tineke, produced 61-135 cotyledonary-stage embryos/100 mg fresh weight callus as compared to intact callus that had not been dispersed and produced 0-3 cotyledonary-stage embryos/100 mg fresh weight callus when the callus was cultured on solidified Murashige and Skoog's basal salts medium supplemented with 0.25% activated charcoal. A maximum of 610 cotyledonary-stage embryos/100 mg fresh weight callus developed from cell clusters and globular-stage embryos that were less than 530 mm in diameter after they were cultured on solidified Murashige and Skoog's basal salts medium for two months. Cotyledonary-stage embryos isolated from both dispersed callus and callus that had passed through various screen sizes showed a significantly higher conversion frequency to plants than cotyledonary-stage embryos isolated from intact callus of the cultivar Classy.

P-2049

Effect of Growth Regulators on Callus Induction and Somatic Embryo Formation From Scutella Cultures of Durum Wheat. V. V. SATYAVATHI and Prem P. Jauhar. Department of Plant Science, North Dakota State University, Fargo, ND 58105. E-mail: valluri.satyavathi@ndsu.nodak.edu

Durum wheat (*Triticum turgidum* L.) is an important cereal crop used for human consumption worldwide. Because of its high protein content and gluten strength, it is the choice wheat for producing pasta products. It is an important crop of the Northern Great Plains of the United States. It is grown in several European countries, including Italy, France, Turkey, Romania and Ukraine, and in Canada. Work on improvement of durum wheat using tools of biotechnology is limited. Development of a reliable *in vitro* plant regeneration procedure for a cultivar is a prerequisite for its improvement by biotechnological methods involving direct gene transfer. Here, we report the effect of three growth regulators, 2,4-dichlorophenoxyacteic acid (2,4-D), picloram, and dicamba, on callus induction and plant regeneration from scutella cultures of four commercial durum cultivars: Ben, Maier, Munich, and Lebsock. Callus induction was obtained from isolated scutella cultured on modified Murashige and Skoog basal medium. The effects of four concentrations (0.5, 1.0, 2.0 and 2.5 mg l^{-1}) of 2,4-D, picloram and dicamba were tested for their ability to induce callus. Calli obtained on different media were either compact or soft and watery. After four weeks of callus induction, all the calli were plated on MS basal medium for regeneration. Genotype and callus induction medium played a dominant role in plantlet regeneration. Data were obtained on callus induction rate, regeneration capacity of the callus, and the number of plantlets regenerated. A Logistic Model was used for the statistical analyses of the data. Significant genotypic differences in culture response were observed. Overall, Maier gave the best response in terms of plantlet regeneration, although 2.0 mg l^{-1} (9.05 æM) dicamba and 2.0 mg l^{-1} (9.05 æM) 2,4-D were equally effective in inducing callus in Maier and Lebsock. Dicamba at 2.0 mg l^{-1} concentration was found to be a better growth regulator for inducing compact calli and gave the highest proportion of regenerated plants across the cultivars. All regenerated plantlets were fertile, maintained the tetraploid chromosome number ($2n = 4x = 28$) and showed no apparent somaclonal variation. Dicamba (9.05 æM) can be utilized in experiments involving transformation of calli from scutella of select durum cultivars and we plan to use this protocol for our transformation work with Maier.

P-2050

Induction of Somatic Embryogenesis in O'Henry Cultivar of Peach (*Prunus persica*). N. JOSHEE[1], A. K. Yadav[1], S. Jayasankar[2], and F. A. Hammerschlag[3]. [1]Agricultural Research Station, Fort Valley State University, 1005 State University Drive, Fort Valley, GA 31030-4313; [2]Tree Fruit Genetics and Breeding, Department of Plant Agriculture, University of Guelph, Vineland Station, ONT, L0R 2E0, CANADA; and [3]USDA-ARS, Fruit Laboratory, Bldg. 010A, Room 238, Beltsville, MD 20705. E-mail: josheen@mail.fvsu.edu

The peach industry plays an important role in the agricultural economy of the southeastern United States, annually producing 35% of the American peach crop. Peach tree short life (PTSL) syndrome results in the drastic decline in peach tree population and orchard longevity. It prematurely kills trees mostly under 6-8 years of age in Georgia and 8-10 years throughout the Southeast. Gene transfer strategies could play a very important role in the improvement of peach cultivars to increase tree survival and enhance orchard longevity. Our primary goal has been to develop efficient and reproducible protocols for plant regeneration amenable to gene transfer strategies. Somatic embryogenesis in peach is not well understood since this species is very highly recalcitrant, and, therefore, there are very few reports. The O'Henry is a highly colorful, late ripening, and heavy yielding commercial peach cultivar with showy blooms. We initiated somatic embryogenesis using immature embryos as explants on Murashige and Skoog (MS) medium. Basal medium was supplemented with different concentrations (0.5, 1.0, 2.0, 4.0 mgL^{-1}) of 2,4-D 400 mgL^{-1} glutamine, 3% sucrose, and 0.25% phytagel as a solidifying agent. Cultures were incubated in the dark and first callus response was seen after 5-6 weeks of culturing, subculturing of explants continued in the same medium and incubating in the dark. Best responses for somatic embryo induction were obtained on MS medium supplemented with 2.0 mgL^{-1} 2,4-D and 400 mgL^{-1} glutamine. Somatic embryos at the globular stage were observed after an incubation period of almost 12-13 weeks. Thereafter, further incubation of somatic embryo masses in the hormone-free medium developed into heart-shaped and bipolar structures. In a few cases, elongation of radicle was also evident in the hormone-free medium. At the present time, majority of the cultures are in the maturation medium for further development. We will continue to further refine and standardize somatic embryogenesis to adopt it for the peach improvement program.

P-2051

Effect of Stage II Duration On Rooting and Survival of Sea Oats (*Uniola paniculata* L.) Genotypes. CARMEN VALERO-ARACAMA[1], M. E. Kane[1], S. B. Wilson[2], and N. L. Philman[1]. [1]University of Florida, Environmental Horticulture Department, Gainesville, FL 32611-0670 and [2]University of Florida, Environmental Horticulture Department, Indian River REC, Fort Pierce, FL 34945. E-mail: cvalero@ufl.edu

Uniola paniculata L. (sea oats) is the primary native dune grass used for beach and dune stabilization and restoration in the southeastern United States. Sea oats micropropagation provides an opportunity to select and rapidly produce genotypes with ecologically valuable characteristics. A sea oats micropropagation protocol was developed, however, significant genotypic differences in shoot multiplication, rooting, and particularly *ex vitro* survival were observed. Physiological and anatomical differences between sea oats genotypes *in vitro* appear to be important factors for successful *ex vitro* acclimatization. Changes in *in vitro* culture conditions, such as medium-components concentration occur with time, altering the physiological and anatomical characteristics of plantlets. The effect of Stage II duration on *ex vitro* survivability, after *in vitro* rooting in Stage III, was compared using an easy- (EK 16-3) and difficult-to-acclimatize (EK 11-1) sea oats genotype. While EK 16-3 plants exhibited 100% survival regardless of Stage II duration, survivability of EK 11-1 plants was 6.3, 27.1 and 60.4% for 4, 8 and 12 weeks culture in Stage II, respectively. However, number of harvestable microcuttings of both genotypes decreased with increasing Stage II duration. Rooting in Stage III was 100% regardless of Stage II duration in EK 16-3 plants and decreased from 100 to 91% from 8 to 12 weeks in EK 11-1 plants. These results suggest that for EK- 11-1, there is a negative carry-over effect of Stage II culture on acclimatization and survival *ex vitro*, which is independent of rooting and that extending Stage II culture duration can decrease it. This information will facilitate refinement of a more effective micropropagation protocol for acclimatization of diverse sea-oats genotypes.

P-2052

Micropropagation of *Lavandula angustifolia* L. for Production of Essential Oils*. ANA M. S. VICENTE[a], Paulo S. C. Braga[b], Maria José Vilaça-Silva[b], Gilda Ramos[c], Cecília Araújo[b], and Manuel Fernandes-Ferreira[b]. [a]ERCA/DRAEDM, S. Pedro de Merelim, Braga, Portugal; [b]Departamento de Biologia, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal; [c]BIOROPE, Rua da Igreja, 4485-242 Guilhabreu, Portugal. E-mail: anamvic@hotmail.com

Apical buds excised from branches of in Nature growing (Ancede) *Lavandula angustifolia* L. plants were used in the establishment of *in vitro* cultures of this species on a modified N30K basal medium. Nine hormonal variants of this modified N30K basal medium (MM-1) and five hormonal variants of the MS basal medium (MM-2) were tested as multiplication media. Complete plantlets were obtained with all the hormonal variants from the both basal media tested. The plantlets formation was however, accompanied by formation of calli irrespectively of the medium conditions tested. In the case of MM-1, the lowest calli formation was got in the absence of exogenous growth regulators. However, the highest multiplication rate as well as the highest linear growth of shoots were obtained in the presence of 2-ip. In the case of MM-2, the highest rate of shoot multiplication and the highest linear shoot growth were obtained with BA, as the only PGR. The combination of BA with NAA afforded a shoot multiplication rate a little lower than that obtained with BA alone. However, the linear shoot growth obtained with the first of these two hormonal variants was clearly lower than that obtained with the second one.

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P-2053

Germplasm Preservation of Fruit, Small Fruit, and Grape Cultures in Kazakhstan. I. KOVALCHUK and S. Kushnarenko. Laboratory of Biotechnology, Kazakh Research Institute of Horticulture and Viticulture, 480060, Almaty, Kazakhstan. E-mail: kovalchuk_i_u@mail.ru

Gene bank materials of fruit crops of Kazakhstan are preserved in the field in pomological gardens, by cold storage of in vitro cultures (3–4° C), and through cryopreservation of meristems in liquid nitrogen (−196° C). Germplasm storage through in vitro cultures and field collections is considered mutually complementary. In the Kazakh Research Institute of Horticulture and Viticulture, in vitro preservation of genetic resources is based on the pomological garden with more than 4000 cultivated varieties and a collection of the wild forms of apple. The collections of fruit plants represent the different climatic zones of Kazakhstan. In vitro cold storage is carried in growth chambers at +3C, with a 10-hr photoperiod (500-1000 lux). Cold storage studies include the influence of sugar content, light intensity, and hormone composition of growth medium on culture preservation. Investigations on preservation in cryogenic conditions (−196° C) were conducted using vitrification and encapsulation-dehydration techniques for cryopreservation of meristems of *Ribes* in liquid nitrogen.

P-2054

In Vitro Regeneration of the Himalayan Medicinal Plant *Lilium nepalense* (D. Don) Via Shoot Organogenesis. PRAKASH MALLA and Sajani Malla. Department of Microbiology, Tri-Chandra Campus, Department of Botany, Amrit CAMPUS, Tu, Kathmandu, NEPAL. E-mail: Prakash_MALLA@HOTMAIL.COM

Lilium nepalense (Liliaceae), a perennial plant commonly known as "Khiraule", has both medicinal and ornamental uses. It grows widely in the east central and western Himalayas of Nepal (1). Its bulbs are used in folk medicine against rheumatism; in addition, they possess antibacterial and antifungal properties. Due to indiscriminate collection and over-exploitation of the natural resources *L. nepalense* is rapidly disappearing and is therefore listed under the endangered species of Nepal (2,3). In view of this there is an urgent need for the conservation of this valuable species, and the use of micropropagation techniques might be a promising approach. A protocol for rapid and large-scale propagation system of *Lilium nepalense* via organogenesis has been developed. Axillary shoots were regenerated from twin scale segment prepared from mature bulbs. Shoots multiplication was carried out on MS medium containing 20 μM Zeatin using longitudinally split shoot halves. Up to 17 shoots were obtained from one explants in a four-week culture period. After rooting the clone plantlets were successfully hardened to ex vitro conditions. Micro propagated plants established in field were uniform and identical to donor plants with respect to growth characteristics and vegetative morphology. This technique is useful for multiplication and preservation of a genotype.

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P-2055

Rooting in Cultures of Two Endangered Florida Pawpaws, *Asimina tetramera* and *Deeringothamnus rugelii*. S. M. CHARLS, J. R. Clark, and V. C. Pence. Center for the Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. E-mail: susicharls@aol.com

Tissue culture methods were applied to the propagation of two endangered Florida pawpaws, *Asimina tetramera* and *Deeringothamnus rugelii* (Annonaceae), as part of the Endangered Plant Propagation Program at CREW, in collaboration with the Center for Plant Conservation (St. Louis, MO) and Historic Bok Sanctuary (Lake Wales, FL). *A. tetramera* cultures were established from shoot tips taken from greenhouse seedlings. Later, *D. rugelii* cultures were initiated from shoot tips and leaf explants taken from wild plants in Florida. Shoot cultures for both species were maintained using MS medium with 1 mg/l BAP. Root initiation was unsuccessful with *A. tetramera* using standard procedures; however, the use of WP medium with 0.5 mg/l IBA supplemented with 100 m*M* silver thiosulfate (STS) yielded roots in 13% of cultures. Further studies indicated that higher concentrations of STS (200 m*M*) resulted in atypical morphology and stunted shoot growth. The highest rooting percentages (23%) were obtained with 50 m*M* STS. Similarly, no rooting occurred in cultures of *D. rugelii* without STS. However, unlike *A. tetramera*, rooting did not appear to be affected by the concentrations of STS tested. Rooted plants have been acclimated and returned to collaborators in Florida. Research funded in part by the Institute of Museum and Library Services, Grants No. IC-00034-00 and IC-03-02-0130-02.

P-2056

Regeneration of *Aloe arborescence* Via Somatic Organogenesis from Young Inflorescences. M. VELCHEVA*, Z. Faltin, A. Vardi, Y. Eshdat, and A. Perl. The Department of Fruit Tree Sciences, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet-Dagan, 50250 Israel and *Department of Genetics, Agricultural University, Plovdiv, Bulgaria. E-mail: perlx@int.gov.il

A system for *in vitro* regeneration of *Aloe arborescence* was developed. Young inflorescences were used as the initial explants in all experiments. Shoot induction was promoted on MS medium supplemented with various concentrations of BA or TDZ. The efficiency of regeneration was increased when ancymidol was added to BA-containing medium. The process of plantlets regeneration and subsequent shoot elongation was strongly affected by the initial medium for shoot induction. Optimal shoot elongation was obtained following shoot initiation on MS medium supplemented with 5 mg/l BA and 5 mg/l ancymidol. Elongation was observed after transfer on ancymidol-free MS medium supplemented with only 1 mg/l BA. Rooting was obtained on MS medium lacking growth regulators. Histological studies indicated that the origin of regenerating shoots was from the receptacle tissue surrounding the residual vascular tissue of the flower buds.

P-2057

Induction of Somatic Embryogenesis in Ovaries of *Vitis vinifera* L.: Effect of the Developmental Stage and Growth Regulators. H. CARDOSO, A. Peixe, and M. S. Pais. Instituto de Ciência Aplicada e Tecnologia (ICAT) - Laboratório de Biotecnologia Vegetal. Campo Grande, 1749-016 Lisboa (Portugal). E-mail: heliacardoso@hotmail.com

The *V. vinifera* cv. 'Aragonez' present a recalcitrant behaviour on embryogenesis induction from stamens (data not published). In different *V. vinifera* cultivars, embryogenesis from ovaries was slightly higher than from anthers and leaf explants (Nakano *et al.*, 1997). These results suggested the possibility to use ovaries for induction of somatic embryogenesis in 'Aragonez' cultivar. To achieve this goal, the phenological stage evaluated as the floral bud length and diameter, as carried out on vineyard-growing 'Aragonez' plants. The floral bud length was determinated as a macroscopic parameter to indicate the ovary developmental stage. Five groups were established ($P \leq 0.05$). The ovaries were induced on Nitsch & Nitsch (1969) medium supplemented with different concentrations of 2,4-D and BAP in the proportion 15:1, 10:1, 5:1, 15:5, 10:5, 5:5 μM. 60 ovaries were used per treatment. These cultures were incubated at $28 \pm 1°$ C in the dark during 30 days. The explants which formed *calli* were transferred to fresh medium with the same composition, without growth regulators or supplemented with 2,4-D or NAA, and BAP, and incubated at $25 \pm 1°$ C at 16h photoperiod. Embryogenic *calli* and embryos formation were obtained for all the ovaries developmental stages. However, the younger stage of ovary development presented the highest embryogenic rate (7%), statistically different from all the others ($P \leq 0.05$). Embryo formation occurred at the excision zone and not from the ovaries. Similar results were described by Nakano *et al.* (2000) which pointed out for the origin of the embryogenic *calli* the connection of the ovaries with the receptacle. The supplementation of the induction medium with low concentrations of 2,4-D was determinant for the formation of embryogenic *calli*. However, this growth regulator presented an inhibitory effect on the somatic embryos formation, while media devoid of growth regulators or supplemented with NAA promoted embryo formation.

P-2058

High Frequency Somatic Embryogenesis from Leaf and Floral Explants of 'Chancellor' Grape. R. M. MULWA, M. A. Norton, and R. M. Skirvin. University of Illinois - Urbana-Champaign, 258 ERML, 1201 W. Gregory Dr.,, Urbana, IL 61801. E-mail: Mulwa@uiuc.edu

Grapes (*Vitis spp.*) exhibit high sensitivity to drift levels of 2,4-D, a common herbicide used to control broad leaf weeds. Although isolated instances of tolerance have been cited in some cultivars, breeding to incorporate these traits into commercial cultivars will be a long and expensive effort as the mechanisms of these tolerances are unknown. Transforming grapes with 2,4-D degradative genes could help resolve the problem. Our objective is to develop efficient somatic embryogenesis systems for grape cultivars to be used for genetic transformation and production of 2,4-D tolerant plants. Experiments on somatic embryogenesis of the cultivar, 'Chancellor', were carried out with leaf explants from the field and young ovaries from greenhouse forced cuttings. Embryogenic callus was obtained after 8-12 weeks of continuous dark culture on solid NN salts medium with 17 micromolar ISAP (indole-3 acetyl-L-aspartic acid) + 9 micromolar 2,4-D (2,4-dichlorophenoxyacetic acid) + 1 micromolar BA (6-benzyladenine) at frequencies of 26.25% and 55% for leaf and ovary explants, respectively. Long term embryogenic callus maintenance was achieved on NN medium containing 4 micromolar ISAP + 2 micromolar 2,4-D + 0.2 micromolar TDZ (thidiazuron). Highly synchronous somatic embryo (SE) development and maturation was obtained from embryogenic callus on solid NN medium supplemented with 10 micromolar ISAP + 8 micromolar NOA (beta-naphthoxyacetic acid) + 1 micromolar TDZ + 1 micromolar ABA (abscisic acid). An 85% germination rate of mature SEs and a 65% conversion rate into plantlets was achieved in vitro on NN salts medium with Staba vitamins and 0.5 micromolar NAA (naphthalene acetic acid) + 0.4 micromolar BA. Further germination and conversion experiments are underway to test the effect of lower NAA and BA levels and sucrose concentrations on plantlet conversion. This protocol is being used in transformation studies to incorporate 2,4-D resistance into grapes.

P-2059

In Vitro Propagation and Phytochemical Production of Kava (*Piper methysticum*). H. KOBAYASHI, M. C. Gawienowski, M. A. Lila, and D. P. Briskin. Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana-Champaign, IL 61801. E-mail: hkobayas@uiuc.edu

Kava (*Piper methysticum*) is a sterile perennial shrub whose roots have been used for treatments of insomnia, agitation, and tension. Diseases, pests, and slow maturity potentially threaten a stable supply and the germplasm of kava. The objectives of the study were to establish an efficient *in vitro* culture system for propagation, and to investigate the tissue specificity and the ontogenic effect of *in vitro* and *in vivo* kavapyrone production. Young leaves from greenhouse kava plants (cv. Makea) were originally introduced to modified ½ strength Murashige and Skoog (MS) medium containing Plant Preservative Mixture (2.0 ml L^{-1}), indole-3-acetic acid (IAA, 1.0 mg L^{-1}) and N^6-benzyladenine (BA, 1.0 mg L^{-1}). Subsequent shoot regeneration from callus was observed on modified ½ MS medium with the following plant growth regulators, in mg L^{-1}, IAA (2.0), BA (1.0) and N^6-furfuryladenine (kinetin, 1.0). With axenic shoot cultures obtained, the optimum conditions for *in vitro* callus and shoot regeneration were further investigated by subjecting *in vitro* foliar pieces to callus initiation medium (½ MS) with the following auxins at 0.5 or 1.0 mg L^{-1}, 2,4-dichlorophenoxyacetic acid, IAA, or α-naphthaleneacetic acid, and with BA (0.5 or 1.0 mg L^{-1}), the sole source of cytokinins. Calli were further subjected to medium treatments to optimize shoot primordium and microshoot formation with following cytokinis, BA, kinetin, and N^6-(3-methylbut-2-enyl)adenine at 0.5 or 1.0 mg L^{-1}. While the formation of shoot primordia was observed in all treatments, the frequency of shoot formation along with the incidence of necrosis differed among treatments. The established kava tissue culture system was further utilized to investigate the tissue specificity and ontogenic effects of kavapyrone accumulation, by comparing kavapyrone contents of *in vitro* cultures with those of the greenhouse grown and acclimatized kava plants. Distinct patterns of kavapyrones represented by different tissue types were observed, suggesting tissue specific accumulation of kavapyrones in respective tissues. A general increase in kavapyrone content was observed as plant tissue matured although a decrease in the total kavapyrone content in senescing tissue was also found. Utilization of an *in vitro* kava culture system under controlled environments enabled elucidation of kavapyrone production at an early stage of organ regeneration and subsequent development.

P-2060

In Vitro Shoot Induction of *Pinus maximartinezii*. E. CÁRDENAS[1], M. C. Ojeda[2], and T. E. Torres[1]. Universidad Autónoma de Nuevo Leõn, Torre de Rectoria 9° piso, San Nicolás de los Garza, N. L., C. P. 66451, MEXICO and ITNL Ave. Eloy Cavazos No. 2001 Guadalupe, N. L., C. P. 67170, MEXICO. E-mail: macardenas@r.uanl.mx

Pinus maximartinezii is an endemic species from Mexico. The success of tissue culture techniques for germplasm preservation depends on regeneration of cultures. A micropropagation protocol using mature zygotic embryos has been developed for *Pinus* species. The objective of this study was to achieve *in vitro* shoot induction of *Pinus maximartinezii.* Mature seeds were surface sterilized in 6% H_2O_2 v/v. Isolated zygotic embryos were cultured on agar solidified shoot induction media. DCR and GD media were supplemented with 3.0 mgl^{-1} BAP and vitamin solution. After 8 weeks of culture, the number of adventitious buds was counted. The composition of culture medium greatly influenced the rate of response for shoot formation.

P-2061

Approaches to Increase Embryogenic Culture Initiation and Cell Line Capture in Loblolly Pine. J. J. CLARK, M. R. Becwar, M. K. Chowdhury, N. S. Nehra, M. R. Rutter, M. J. Cook, J. M. Victor, T. J. Stout, A. M. Perry, P. J. Wade, and M. A. Hinchee. ArborGen, PO Box 840001, Summerville, SC 29484. E-mail: jjclark@arborgen.com

Efficient initiation, establishment and capture of cell lines from genetically diverse families is required for successful application of somatic embryogenesis (SE) to clonal and transgenic plant production of loblolly pine. ArborGen is developing ways to address the limitations imposed by recalcitrant families—those that are difficult to capture in the SE process. Here we describe two of these approaches. The first is based on the fact that there typically is a pronounced family by medium interaction during the initiation and pre-cryo culture establishment phases of the SE process. That is, medium modifications often improve initiation in specific families. By using a "battery" (or series) of initiation and establishment media, we have been able to take advantage of the family by medium interaction and capture more culture genotypes from immature seed explants (zygotic embryos) of different families. Using this approach, culture establishment was significantly increased in 5 of 7 recalcitrant families compared to a control medium on at least one of three medium modifications. The best medium modification varied with family. This approach increased capture by 73% overall, and by as much as 190 and 740% among different family-medium combinations compared to the control. The second approach is based on the fact that there are reciprocal effects on initiation and culture establishment. Therefore, how a particular cross is made (A x B or B x A) in terms of female x male parentage very significantly affects culture initiation and establishment. The degree of the reciprocal effect varies among families. Initiation was improved from 50 to 690% just by using a particular parental combination. These two approaches are being combined to further increase embryogenic culture initiation and capture in loblolly pine. This enables ArborGen to access a broader germplasm base for clients, including genetic families that have been considered recalcitrant to the SE process.

P-2062

Plant Regeneration of *Arundo donax* L. Through Somatic Embryogenesis. M. SINGH, D. Moore, K. Knowles, and S. K. Dhir. Center for Biotechnology, Fort Valley State University, Fort Valley, GA 31030-4313. E-mail: singhm@fvsu.edu

Giant reed (*Arundo donax* L) is a tall, erect, non-food woody perennial crop plant. This plant is valuable as a source of cellulose for rayon and paper pulp. Recently this plant has been considered for its use in phytoremediation, in particular, its phosphorus uptake potential. With this objective in mind, we initiated *in-vitro* regeneration of *Arundo donax*, so as to study genetic transformation and its wide application for phytoremediation and energy source. Embryogenic callus was observed on Murashige and Skoog (MS) media supplemented with 2.0 mg/L of 2, 4-dichlorophenoxyacetic acid (2, 4-D) from inflorescence of selected mature field grown plants. After four weeks of incubation at 26° C in dark, embryogenic callus proliferation was observed on MS medium with 2.0 mg/L of 2,4-D. Two types of embryogenic calli were observed, yellow and hard nodular (embryogenic) and friable (non-embryogenic). On regular sub culturing on MS media with various concentrations of 2,4-D (0.5- 2.5 mg/L), 3% sucrose and 0.4% gelrite, different developmental stages of embryos including torpedo and cotyledonary stages were observed. Mature somatic embryos were transferred onto MS media without any phytohormone and incubated at 26° C at 16/8 hour light/dark photoperiod. Within two weeks of incubation, somatic embryos germinated with appearance of both shoots and roots. The plants were transferred to pots containing soil and peat mixture (3:1) and kept under high humidity environment for further growth. The developed procedure will be used for large scale micro-propagation of selected clones and for studies on genetic manipulations.

P-2063

Development of a Thin Cell Layer Culture System for Rapid and Direct Regeneration of Sugarcane and Other Monocot Species. P. LAKSHMANAN[1], R. J. Geijskes[1], A. R. Elliot[2], L. F. Wang[1], M. G. McKeon[1], R. S. Swain[3], Z. Borg[1], N. Berding[3], C. P. L. Grof[2], and G. R. Smith[1]. [1]David North Plant Research Centre, Bureau of Sugar Experiment Stations, 50 Meiers Road, Indooroopilly 4068, Australia; [2]CSIRO-Plant Industry, Long Pocket Laboratories, Indooroopilly 4068, Australia; and [3]Meringa Sugar Experiment Station, BSES, Bruce Highway, Gordonvale 4865, Australia. E-mail: plakshmanan@bses.org.au

Sugarcane (*Saccahrum* spp. interspecific hybrids), a graminaceous crop, accounts for nearly 70% of sugar production worldwide. The potential of tissue culture to enhance conventional sugarcane breeding and molecular crop improvement has received considerable recent attention, but its use has been limited by the high incidence of somaclonal variation in plants regenerated using the current callus-based tissue culture systems. In an effort to minimise somaclonal variation we have developed a rapid and genotype-independent direct shoot regeneration system in sugarcane using a thin cell layer culture approach. In this system, 1-2 mm transverse sections of young leaves or inflorescence were cultured on MS medium enriched with various concentrations of α-naphthaleneacetic acid (5-20 μM), chlorophenoxyacetic acid (5-10 μM) and 6-benzyladenine (1-5 μM), singly or in combinations. Plantlets regenerate directly from the explant in 8-10 weeks. Developmental polarity and morphogenic competence of explant were the key determinants regulating shoot regeneration. Up to 2000 plantlets can be produced from a single sugarcane leaf roll. Plants have been successfully regenerated from 20 sugarcane genotypes and two genotypes each of wheat and sorghum. Field trials indicate no significant variation in stool weight and sugar yield between tissue culture-derived and traditionally propagated plants. This culture system has also proved to be a rapid method for virus elimination, and is the underlying technology for the most efficient sugarcane transformation system described to date.

P-2064

Somatic Embryogenesis from Immature Embryos of *Phaseolus aureus*. M. MUMINOVA, M. Nasretdinova,S. Djataev, and A. Abdukarimov. Department of Plant Biotechnology, Institute of G&PEB, Tashkent, Uzbekistan. E-mail: mmuminova@yahoo.com

Somatic embryogenesis is an efficient process for production transgenic plants because regeneration takes place either from individual proembryonic cells or from groups. The cotyledons of immature seeds of *Phaseolus aureus* were used to establish embryo culture in vitro. The influence of BAP and NAA in MS medium contain 20 g/l sucrose, 6 g/l agar on induction of embryogenic callus has been assessed. Somatic embryo developed in *Phaseolus aureus* was studied using histology. Our investigations show that the formation of meristematic cells in somatic embryo occurs as early as globular stage of development. Histological observation revealed that the meristematic cells are rich in protein.

P-2066

Somatic Embryogenesis from Callus Cultures of Teak (*Tectona grandis* L.f.) Derived from Leaf Explants. L. YUNAINI and S. N. Widiyanto. Department of Biology, Institut Teknologi Bandung, Bandung - 40132, Indonesia. E-mail: srinanan@bi.itb.ac.id

Research was carried out to evaluate development of somatic embryos regenerated from teak callus tissues derived from leaf explants. We examined the influences of 6-benzyl-amino purine (BA), thidiazuron (TDZ), and gibberellic acid-3 (GA_3) to induce teak somatic embryogenesis. Compact callus with nodular structures were formed on leaf explants cultured in liquid medium consisted of woody plant medium with the addition of 0.1-1.0 μ*M* TDZ in combination with 0.01-1.0 μ*M* indole butyric acid. We observed that early stages of somatic embryos, the globular and heart shaped embryos, were obtained in embryogenesis induction media containing 8.0 μ*M* BA or 1.0 μ*M* TDZ. Clusters of globular and heart shaped embryos developed on the surface of compact callus cultures after 7-14 days. The addition of GA_3 at 2.0 μ*M* induced positive responses if it was added in combination with BA or TDZ. Torpedo and cotyledonary stages were observed after 4-6 weeks in culture medium containing 3%-4.5% sucrose without growth regulator. Further development of this procedure has been in progress and considered to be important for regeneration of selected, transformed tissue cultures of teak.

P-2067

Alternative Media for In Vitro Propagation of Fe'i Bananas. F. B. JAVIER. College of Micronesia-Federated States of Micronesia, Cooperative Research and Extension, Agriculture Experiment Station, Land Grant Program, Kolonia, Pohnpei FM 96941, MICRONESIA. E-mail: fbjavier@mail.fm

In vitro propagation protocol for Fe'i bananas rich in vitamin A was determined. These are the rare bananas in the Federated States of Micronesia particularly in Pohnpei. The bananas are locally called 'Karat' belonging to *Musa troglodytarum Linn.* Two distinguished groups were collected from the upland locally called 'Karat Pako', *M. troglodytarum* (var. *typica) characterized with* prolonged male axis withlarge imbricate, obtuse bracts on the bud; and the group locally called 'Karat Kole', *M. troglodytarum* (var. *acutaebracteata*) having short with rapidly degenerating male axis and less markedly imbricate bracts with acute tips. Developing the in vitro propagation protocol with media modification and substitution was aimed at using the tissue culture technique to propagate endangered food crop with lesser cost and sourcing of media components facilitated specially in small island state with high cost of commodity transport and limited resources capability. Propagation in mass for shorter period could readily enhance household plantings in the entire island and atolls with greater accessibility and availability of the needed vitamin A rich food and conservation of the rare bananas. The Federated States of Micronesia populace was found to be deficient in Vitamin A with 51% of young children suffering from the disorder. Double sterilization of explants (4-5 cm cube excised shoot tissue) using household bleach for 15 minutes and field sampling during the rainy periods decreased significantly percentage of contaminated cultures. Single sterilization of explants during dry periods was comparable to double sterilization results on contamination. Alternative media and media modification were attempted for in vitro culture initiation and multiplication stages. Organic additive or supplement and media components substitution were conducted. Different concentrations of coconut water in combination with BAP were tested for tissue response on regeneration and plantlet multiplication. Substituted sucrose as energy source with available in-the-counter refined white sugar and gelling material were also evaluated. Results showed best initiation media was MS + 2.5 BAP + 1.0 mg/l thiamine + 10% coconut water; and multiplication media with basal MS + 2.5 BAP + 1.0 mg/l thiamine + 15% coconut water. Multiplication response was faster with more number of shoots produced. 'Karat Pako' and 'Karat Kole' exhibited similar response. Substituted sucrose with refined white sugar was shown an effective energy source compared with the analytical grade sucrose (Sigma and Univar). White gelatin bar at 6% was evaluated effective compared with agar-agar (Sigma) and agar (Univar). The cultures were grown at relatively low light intensity (less than 1000 lux). It took 3-4 months for Stage 1; 2-3 months for Stage 2 in all treatments. Increased light intensity (1500-1800 lux) shortened the cycle period to 2 - 3 months for Stage 1; 3 - 4 weeks to 5 - 6 weeks for Stage 2. Tissue response of 'Karat Pako' was faster than 'Karat Kole' in all treatments. Roots developed on the multiplication media after shoot development. Growth room temperature was 22-29 degrees Centigrade.

P-2068

Saffron Micropropagation by Somatic Embryogenesis in a Temporary Immersion System. SILVIA BLÁZQUEZ[1], Abel Piqueras, and José Antonio Fernández[1]. [1]Instituto de Desarrollo Regional, Sección de Biotecnología, Campus Universitario s/n, Albacete 02071, Spain and [2]Centro de Edafología y Biología Aplicada del Segura, Consejo Superior de Investigaciones Cientificas (CEBAS-CSIC), Campus de Espinardo, Murcia 30080, Spain. E-mail: jafernandez@idr-ab.uclm.es

The object of this work has been to improve the yield and quality of the embryogenic cultures of saffron started and subcultured in our laboratory since 1997. The cultures were initiated from meristems of saffrom corms and generated embryogenic calli able to produce somatic embryos on solid medium with low regenerative frequency (less than 8% of the embryos developed into plants) and subculture periods of 6-8 weeks. In an attempt to acelerate the production of embryogenic tissue and to increase the regenerative efficiency of the system, embryogenic calli were cultured in a temporary immersion system and compared to the conventional cultures on solid medium using the same culture medium without agar. After three subcultures of identical duration the results on embryogenic calli production in fresh weight for each individual inoculated calli and for the complete culture were evaluated. In both cases the temporary immersion system showed better results compared to the conventional system, individual embryogenic calli grew 15% more than the cultures on solid medium. A single TIS also produced a remarkable increase in fresh weight equivalent to the production of 40 Petri dishes with solid medium. To prevent tissue browning and phenolic excretion, several antioxidant compounds were tested and PVP (5 g/l) was found to be the most effective and incorporated to the culture medium in the TIS. Hyperhydricity of the cultures in TIS was reduced from 80 to 10% with the addition of 1 mg/l paclobutrazol to the culture medium.To study the effect of jasmonic acid (JA) on embro maturation and development, the embryogenic cultures produced in the TIS were inoculated on solid medium with different concentrations of JA, under these conditons, the induction of mature somatic embryos rise to 25%, almost three times more than control (without JA) conditions. JA addition favoured the growth of both cormogenic and shoot parts in the somatic embryos. A increased sucrose concentration in the culture medium from 3 to 4,5% showed a sinergic effect with JA.

P-2069

Characterisation of a Rab-related Putative Small GTP-binding Protein, *LeRab6* in Tomato (*Lycopersicon esculentum*, Miller). H. A. KHOJA, J. Leclercq, A. Latché, J. C. Pech, and M. Bouzayen. *UMR 990 INP-ENSAT/INRA* "Génomique et Biotechnologie des Fruits", Av de l'Agrobiopole, BP-107, Auzeville-Tolosane, 31326 Castanet-Tolosan, Cedex FRANCE. E-mail: haskhoja@hotmail.com

Considering that fruit ripening as all other developmental processes, involve in protein trafficking, specially transfer to the cell wall enzymes capable of degrading cell wall polymers, we have isolated a gene *LeRab6* encoding the rab-related putative small GTP-binding protein from a cDNA library of tomato fruit (*Lycopersicon esculentum*, Mill). The expression patterns were obtained by Northern blot analysis. A low abundance of *LeRab6* mRNA was found during fruit development in tomato (var. Micro-Tom) as compared to other organs of plants. Protein targeting studies were carried out using LeRab6::GFP (Green Fluorescent Protein) gene fusion construct and transient expression in tobacco protoplasts. Confocal microscope images revealed specific cytoplasmic localisation of this protein, in accordance with their putative function as vesicle trafficking. Tomato plants, were genetically transformed via *Agrobacterium tumefaciens* with an antisense construct for this gene. The transgenic plants underexpressing *LeRab6* exhibited reduced apical dominance, increased tillering, dwarfism, determinate growth, compact inflorescence and abnormal branches. Such phenotypes suggested that the hormonal balance involved in the regulation of plant growth and development was strongly affected. *In vitro* biological assays with different (auxin and cytokinin) hormonal ratio auxin (αANA)/cytokinin (BAP): 0.0/0.0; 0.0/0.1; 0.0/0.5; 0.1/0.0; 0.1/0.1; 0.1/0.5; 0.5/0.0; 0.5/0.1; 0.5/0.5 mg.L^{-1}) respectively have indeed showed that antisense plants carrying *LeRab6* gene exhibited reduced sensitivity to auxin in terms of rhizogenesis.

P-2070

Establishing Cryopreservation Methods for Conserving European Plant Germplasm. ERICA E. BENSON[1], B. Panis, J. Geuns, R. Swennen, K. Harding, D. Bremner, P. Lynch, A. Hargreaves, P. Bonner, S. Dussert, N. Chabrillange, C. Damiano, C. Forni, S. Beninati, L. Alessandro, P. Bruno, H. M. Schumacher, E. Heine-Dobbernack, H. Takagi, L. Maggioni, and F. Engelmann. [1]/Conservation and Environmental Chemistry Centre, Plant Conservation Group, University of Abertay Dundee, Bell Street, Dundee, Scotland DD1 1HG and [2]consortium partnership address contact b.panis@agr.kulueven.ac.be. E-mail: e.e.benson@abertay.ac.uk

Cryopreservation is the method of choice for conserving vegetatively propagated plant germplasm. Whilst many plant species have now been successfully cryopreserved there are still certain species (or genotypes within species) that remain recalcitrant to cryogenic storage. This European cryopreservation consortium is funded by the EU (CRYMCEPT Project QLRT-2002-01279) and comprises a cooperative project with a mandate to apply fundamental research to elucidate those factors that contribute to cryopreservation tolerance, sensitivity and injury. This will be achieved by assessing the physical and biochemical changes that occur in cryopreserved germplasm selected from tropical and temperate species (e.g. *Allium, Musa, Ribes, Solanum, Oleacae, Prunus, Coffea*). These have also been selected because they represent diverse taxonomic groups originating from different centres of origin. Fundamental research will focus on assessing the thermal behaviour of cryogenic treatments and investigating the involvement of oxidative stress, DNA methylation, polyamines, sugars, the cytoskeleton and membranes in cryopreservation tolerance. Protein profiles will also be constructed with a view to elucidating the role of stress, heat shock and cold regulated proteins following exposure to desiccation and low temperatures. The longer-term aim is to apply this fundamental research to improve cryogenic storage methodologies in international, plant genebanks. To achieve this end, the International Plant Genetic Resources Institute will coordinate the technology transfer component of the project.

P-2071

Cellular Location of Oxidative Stress and Antioxidant Enzymatic Response in Hyperhydrated Carnation Plantlets. S. Saher, A. PIQUERAS, E. Hellín, and E. Olmos. CEBAS (CSIC) PO Box 4195, Murcia E-30080, SPAIN. E-mail: piqueras@cebas.csic.es

The physiology of hyperhydricity in relation to oxidative stress, mineral nutrient content and antioxidant enzymes has been studied in three micropropagated carnation cultivars under experimentally induced hyperhydricity in vitro and normal (control) conditions. A signifficant increase in Fe content compared to control tissues was observed in the hyperhydrated tissues from the three cultivars. The levels of ethylene, solute leakage and malondialdehide content measured were also higher in the hypehydrated tissues. In relation to the time course of H_2O_2 production (biochemical indicator of oxidative stress) measured by fluorescence quenching, a similar trend could be observed for the three cultivars with a marked increase in the generation of H_2O_2 in hyperhydrated tissues in relatio to controls.The activities of all the antioxidant enzymes studied except lipooxigenase were higher in the hyperhydrated shoots. Using cytochemical staining, a different location of peroxidase activity bertween control and hyperhydrated leaves could be observed. These results provide for the first time direct evidences for H_2O_2 generation in hyperhydrated tissues, characterize the response of the antioxidant system to the existence of an oxidative stress process during hyperhydricity and point to the accumulation of toxic activated forms of oxygen as the inductors of the observed physiological abnormalities.

P-2072

Cryopreservation Studies in *Carica papaya*—Effect of Some Cryoprotectants on Regrowth and Somatic Embryogenesis in Sunrise Solo papaya. S. A. DHEKNEY[1], R. E. Litz[1], N. Joshee[2], and A. K. Yadav[2]. [1]University of Florida, Tropical Research and Education Center, Homestead, FL 33031 and [2]Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030. Email: sadanand@mail.ifas.ufl.edu

Cryopreservation is an efficient technique for long term storage of embryogenic cultures, and as such can be utilized to back-up plant genetic resources and for storing materials for somatic cell genetic studies. Transformation of papaya involves the use of embryogenic cultures, and cryopreservation provides an ideal means for long term storage of materials for transformation. Therefore, a cryopreservation protocol was developed for papaya to ensure a continuous supply of certain embryogenic lines. The response of embryogenic papaya cultures to different cryoprotectant treatments was determined following their recovery and regrowth after storage in liquid nitrogen (LN). Cultures were induced from hypocotyls of greenhouse-grown papaya seedlings. Four different cryoprotectant combinations were tested: 1) glycerol + DMSO (5% each); 2) glycerol + DMSO (10% each); 3) polyethyleneglycol (10%) + glucose (10%) + DMSO (10%); and 4) Plant Vitrification Solution 2 [glycerol (30%) + ethylene glycol (15%) + DMSO (15%)]. Embryogenic cultures (200mg) were incubated briefly in cryoprotectant in 1.5 ml cryo-vials. The cryo-vials were cooled to&minus1;80° C using a Mr. Frosty (1° C min^{-1}), and then plunged into LN while the cultures treated with PVS-2 were directly plunged into LN. Viability and regrowth of cultures were recorded following storage in LN for 48 h. Cryoprotectant glycerol + DMSO (10% each) resulted in the highest rate of survival immediately after thawing (100%) followed by the vitrification treatment (75%). Two months after recovery from LN, the vitrification treatment (4) resulted in the maximum viability followed by treatment (3). Somatic embryo development occurred on induction medium before transfer to maturation medium and occurred earliest in the vitrification treatment.

VT-2000

An Animal Origin Free Trypsin Alternative to Harvest Cells. L. L. NESTLER, E. K. Evege, J. A. McLaughlin, D. G. Munroe, T. C. Tan, K. E. Wagner, and B. Stiles. Cell Culture Research & Development, GIBCO Invitrogen Corporation, 3175 Staley Road, Grand Island, NY 14072. E-mail: Lori.Nestler@invitrogen.com.

Numerous problems plague the use of cell culture reagents containing components of animal origin. Not the least of these is the difficulty in proving that products produced in cell culture are free of adventitious mammalian viruses such as BVD (Bovine Virus Diarrhea) and prions such as BSE (Bovine Spongiform Encephalopathy). Regulatory pressures have increased to ensure that nutrient media and other cell culture reagents are free of these contaminants. One solution would be to use media and reagents completely free of any animal-derived componenets and processes.
rProtease is a non-animal, recombinant trypsin-like enzyme used for the dissociation of attachment-dependent mammalian cell lines. This highly purified enzyme is certified to be animal-origin free and is readily available at any scale. HPLC analysis indicates a single peak corresponding to the active form of the recombinant enzyme as opposed to the multiple peaks seen with animal (porcine pancreatic) trypsin. The cell removal kinetics are comparable to porcine trypsin and rProtease maintains cell viability and plating efficiency. Cell replating, proliferation kinetics and long-term maintenance were comparable to cells harvested using animal trypsin. It has been tested on multiple cell lines, including strongly adherent lines, with comparable removal times to that of porcine-derived trypsin.

VT-2001

A Filter for Trapping Metastatic Breast Cancer Cells. J. MOORE, P. Elias, C. Davitt, A. Bandyopadhyay, S. Bose, S. Kalita, and H. Hosick. School of Biological Sciences, College of Pharmacy, Electron Microscopy Center, School of Mechanical & Materials Engineering, School of Molecular Biosciences, Washington State University, Pullman, WA 99164. E-mail: moore@mail.wsu.edu.

106 to 109 cells can enter the circulation from a metastasizing tumor each day (2). Some of these cells then colonize different organs, some organs frequently and others rarely (5). Breast cancer patients are the most likely to suffer from metastasis to bone (Figs. 1,2); up to 80% of these women will develop bone lesions (6). Such lesions are characterized by bone destruction, hypercalcemia, and considerable pain (9). Bone provides an unusual microenvironment that facilitates such damage (1). Breast (and prostate) cancer cells appear to be attracted to bone chemotactically (3,7,4). The ensuing cell interactions have been described as a "vicious cycle" in which tumor cells secrete substances that stimulate bone destruction; but this process releases molecules that in turn stimulate tumor cells to be more destructive (1,8). Most current therapies for bone metastasis are designed to minimize damage after cancer cells have already traversed blood vessel walls, invaded bone marrow, and interacted with bone matrix. It would be preferable to prevent breast cancer cells and their products from encountering bone at all. We propose a simple and direct strategy—to filter potentially metastatic cells out of circulation using a surrogate bone-like matrix as a target to which such cells will adhere instead of invading "real" bone (7). We have developed porous ceramic scaffolds and ceramic/polymer scaffolds intended for spinal reconstruction and other bone graft applications. Composition, coatings, porosity, shape, dimensions, and other features of scaffolds can be precisely varied, and do influence bone cell attachment and behavior. Tumor cells adhere avidly to these structures (unpublished obs.). Our aim is thus to design ceramic structures and polymer/ceramic composites to remove metastatic cancer cells directly from circulation.

VT-2002

Induction of a Zone of Cell Death in Multi-well Plates by Refeeding. H. Raabe, G. Moyer, G. Mun, M. Clear, and J. W. HARBELL. Institute for In Vitro Sciences, Inc. Gaithersburg, MD 20878. E-mail: hraabe@iivs.org

Multi-well plates provide an efficient format for cell-based bioassays. As the number of wells increases per plate, the surface area and number of cells per well decreases. Use of the small-well format can present some problems. We have observed cell cultures in 96-well plates where significant populations of cells begin to die shortly after refeeding the cell cultures (dumping the spent medium and adding fresh medium). The zones or areas of cell death appear to occur within a predictable ring around the edges of the multi-well plate wells, coinciding with the formation of a meniscus formed by the residual medium in the well following removal of the spent culture medium. This effect appears in larger well (e.g., 24-well plates) but has more impact in smaller well formats where the ratio of wall circumference to cell area is greater. The impact of these effects tend to be more pronounced when cultures are refed at relatively low confluence, resulting in areas devoid of cells. The zone is also more evident with cell types where cell migration is limited. Cells cultured in the presence or absence of serum show qualitatively similar results. When uniform, 30% confluent lawns of human keratinocytes in 96-well plates were refed, the cells within the zone rapidly lost the ability to take up neutral red and showed nuclear condensation. After 48 hours, the neutral red uptake (OD550) was significantly reduced in the refed wells (0.830 ± 0.058 mean $\pm$ standard deviation $n=6$ assays) compared to wells that were not refed (1.066 ± 0.034 where $p < 0.0001$). These results were confirmed in 3T3 cells cultured in serum-containing medium. These observations show the impact the medium change in the 96-well plate format, and suggest that protocols should be designed to minimize this cell loss.

VT-2003

Comparative Cytotoxicity of Three In Vitro Cell Viability Assays. ANN M. WRIGHT and Mary Mowery-McKee. CIBA Vision Corporation, A Novartis Company, Duluth, GA 30097. E-mail: amy.wright@cibavision.novartis.com

PURPOSE: To determine the cytotoxicity potential of N, N-Dimethylacrlamide (DMA), a polymer component extractable, and benzalkonium chloride (BAK), an ophthalmic preservative, by comparing two colorimetric *in vitro* assays and a direct cell counting assay using an immortalized human corneal epithelial cell line (HCE-T). Cells exposed to media only were used as the negative control. These indirect colorimetric and direct cellular assays are useful for determining the quantitative cytotoxicity within an exposure period of 24 to 72 hours. METHODS: DMA was diluted with growth media to yield a 1000 and 2000 ppm solution. BAK was used as a cytotoxic control at 2.5 and 5 ppm. Tests used were the cell viability assay using MTS/PES (MTS/PES), cell membrane integrity assay using neutral red uptake release (NRUR), and the cell growth inhibition assay (CGI). The endpoint for the MTS/PES and NRUR assays was determined spectroscopically as optical density. using a microplate reader. The endpoint for the CGI assay was a direct cellular count using a Beckman-Coulter cell counter. The MTS/PES and NRUR data were expressed as a percentage of the negative control optical density. The CGI data were expressed as the percent cell growth inhibition compared to the negative control. The percentage values were compared using ANOVA/Tukey HSD test for statistical significance. RESULTS: Test samples exhibiting less than 50% values (ET50) for the MTS/PES and NRUR assays are deemed to be cytotoxic. For the CGI assay test values greater than 30% are considered cytotoxic. Cytotoxicity discrepancies and statistical significance were found between the colorimetric and the direct cell count systems for both DMA concentrations. MTS/PES and NRUR viability percentages with HCE-T cells were similar for DMA at 1000 and 2000 ppm; both values were above the ET 50 for cytotoxicity at 24 hours, not significantly different from the negative control and noncytotoxic. CGI percent inhibition values for 1000 and 2000 ppm DMA at 24 hours were greater than 30% and thus cytotoxic; they were signifiantly different than the negative control. BAK at 5 ppm and 24 hours exposure was cytotoxic for the MTS/PES, NRUR and CC assays. BAK at 2.5 ppm and 24 hours exposure was considered noncytotoxic for all three assays. DISCUSSION: Multiple assays can yield valuable information concerning in vitro cellular toxicity. Acceptable metabolic activities from a colorimetric assay may not identify cellular morphological changes or decreased cellular proliferation or vice versa as demonstrated by DMA.

VT-2004

Mycoplasma Testing Experience of an Academic Support Facility. MARGARET L. SMITH, Lovella Cacho, and Phan Tu. Core Cell Culture Facility, University of California, San Diego, CA 92093-0605. E-mail: m2smith@ucsd.edu

The UCSD Core Cell Culture Facility, an on-campus recharge laboratory, has offered testing for mycoplasma contamination since 1990, and has accumulated data on over 5,750 samples submitted from both academic and industry laboratories. Despite constant education concerning the dangers of mycoplasma contamination, about 17% of cultures continue to test positive, confirming published reports. Analysis of patterns of contamination and interaction with labs submitting samples suggest that there are certain situations that are especially prone to mycoplasma contamination. These include the increasing use of cultures in gene transfer experiments, where continued antibiotic selection is often required. Selection antibiotics do not kill mycoplasma, but they do stop the growth of other bacteria which would normally turn the media turbid. This masks breaches in sterility, and leads to unsuspected mycoplasma contamination. We currently use the Gen-Probe MTC-NI assay, a solution-based hybridization test with a single-stranded, chemiluminescent labeled DNA probe complementary to a highly conserved region of bacterial ribosomal RNA. Therefore this assay can detect both mycoplasma and other bacteria, but does not detect yeast, fungi, or other eukaryotic rRNA. When we last summarized our results at SIVB in 1999, the Gen-Probe mycoplasma assays were among the only ones commercially available. Now however several mycoplasma specific PCR-based testing kits are available, as well as the classic culture assays. Data is presented on the MTC-NI assay's sensitivity, specificity and ease of use in comparison with other mycoplasma testing methods. We hope to provide a focus for discussion of mycoplasma contamination in both animal and plant cell cultures and to exchange ideas for educating the broader scientific community about this problem.

VT-2005

Use of a Self-assembling Pore for the Introduction of Impermeant Molecules Through Mammalian Cell Membranes. L. H. CAMPBELL, K. Sarver, K. Ratcliff, M. J. Taylor, J. Walsh, and K. G. M. Brockbank. Organ Recovery Systems, Inc. Charleston, SC. E-mail: lcampbell@organ-recovery.com

A number of organisms, such as the brine shrimp, take advantage of the protective effects of sugars to survive extreme environmental conditions by dessication. More specifically, disaccharides, trehalose for example, provide protection to the organism by stabilizing the cell membrane during these extreme conditions. These sugars may also be used as a non-toxic cryoprotective agent to partially or completely replace more conventional cryoprotectants, such as dimethyl sulfoxide. However, mammalian cells are impermeable to disaccharides. In an effort, to use sugars for mammalian cell cryopreservation we have developed a method for the introduction of sugars into adherent mammalian cells using a genetically modified pore-forming protein (H5) derived from the α-hemolysin protein of *Staphylococcus aureus*. This protein self-assembles on cell membranes to form a pore containing a switch that can be opened and closed by the introduction of Zn+. Several parameters had to be optimized to develop a relatively non-cytotoxic method of cell poration using H5. These parameters included cell density, H5 treatment (concentration, time and temperature of exposure) the time and concentration required for the trehalose to enter the cell and equilibrate with the outside environment, Zn treatment (concentration required to close the pores, pH), and Zn removal (time and concentration of EDTA required to chelate the Zn) when the pore needed to be opened.. The results demonstrated that it is feasible to use the H5 protein to form pores on the surface of mammalian cells with excellent cell survival. Furthermore, introduction of trehalose is possible using the H5-derived pore with retention of ~50-75% cell viability. Future experiments will examine cell survival after freezing and/or drying.

VT-2006

Enhanced Effectiveness of Non-viral Gene Transfer Using Electroporation. RICHARD HELLER, Richard Gilbert, Kathleen Merkler, and Loree Heller. Department of Surgery, College of Medicine; College of Engineering; Center for Molecular Delivery, University of South Florida, Tampa, FL 33612. E-mail: rheller@hsc.usf.edu

A key issue in the development of effective gene transfer protocols is the development of appropriate delivery methods. Typically, the goal is to target gene delivery to a particular type of cell or to cells within a specific tissue. The delivery of genes that code for biologically active compounds is envisioned as a treatment for many diseases including cancers and metabolic disorders. Our group has initiated several studies to investigate the use of electroporation for plasmid DNA delivery in a variety of tissues. Protocols were developed to allow delivery of the plasmid directly to tumor, normal skin, and normal liver or normal muscle. Initial work was performed using plasmids coding for either the reporter gene luciferase or beta-galactosidase. Different electric pulse conditions were needed to obtain peak expression at each of the various sites. More recently, we have tested the therapeutic potential of this approach. Various plasmids encoding different cytokines were delivered to tumors, muscle or skin either alone or in combination. Long term complete regressions of established tumors have been obtained with various treatment protocols in a murine melanoma model. In addition, we have demonstrated that serum levels of the cytokines are increased using his delivery approach. This work is being continued and expanded to explore the use of this approach for the delivery of DNA vaccines for both cancer and for causative agents of infectious disease. Procedures are being optimized for various tissue sites as well as comparing electroporation delivery with other delivery approaches. (Supported by research grants from the NIH - R21 DK055588 and RO1 CA76181 also supported by the Center for Molecular Delivery, Univ. of South Florida).

VT-2007

Confocal Imaging of Epidermal Growth Factor Peptide Binding Along the Stem Cell Compartment of Mammalian Colonic Crypt. BERTRAND KAEFFER[1], Lissia Pardini[1 & 2], and Alain Trubuil[2]. [1]Institut National Recherche Agronomique, CRNH de Nantes, Unité Fonctions Digestives et Nutrition Humaine and [2]Laboratoire de Biométrie, INRA, Jouy-en-Josas, FRANCE. E-mail: kaeffer@nantes.inra.fr

Context: Mammalian intestinal crypt organization arises from a single stem cell located in a cellular ring near the base of the crypt wherein multiple stem cells are present and replaced through periodic symmetric divisions. Method: We have investigated the distribution of epidermal growth factor and Hoechst bindings along distal colonic crypt of rat by fluorescent laser scanning confocal microscopy with a quantitative multidimensional analysis software (Quant3D, Linux/Unix). Results: Data obtained along the bottom of the crypt showed highly polarized EGF receptors with a basal trend which can be altered by some diet composition. Growth factor signal distribution at the stem cell level by confocal imaging was mapped across crypt closed end in order to visualize cellular activities of active and inactive stem cells. The analytic system was amenable to semi automation by allowing a rapid extraction and labeling of crypt cells from mammal biopsies to enumerate and localize cell nuclei and the corresponding intensity of growth factor labeling. Perspectives: A microscopic multidimensional analytic system to record the expression profiles of biomarkers along intestinal tissues should pave the way of identifying any recruitment factor of inactive stem cells and improve the use of primary cultures of colonocytes for in vitro testing of new food products.

VT-2008

Design and Phase Ia Results of a Validation Study to Evaluate In Vitro Cytotoxicity Assays for Predicting Rodent and Human Acute Systemic Toxicity. M. W. PARIS[1,3], J. A. Strickland[1,3], W. S. Stokes[1], S. Casati[4], A. P. Worth[9], H. Raabe[5], C. Cao[6], R. Clothier[7], J. Harbell[5], R. Curren[5], J. Haseman[2], R. R. Tice[1,3], M. L. Wenk[8], M. K. Vallant[2], G. Mun[5], M. Clear[5], G. O. Moyer[5], J. Madren-Whalley[6], C. Krishna[6], M. Owen[7], and N. Bourne[7]. [1]NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), RTP, NC 27709; [2]National Institute of Environmental Health Sciences (NIEHS), RTP, NC; [3]ILS, Inc., RTP, NC; [4]European Centre for the Validation of Alternative Methods (ECVAM), JRC, Ispra, Italy; [5]Institute for In Vitro Sciences, Gaithersburg, MD; [6]U.S. Army Edgewood Chemical Biological Center, APG, MD; [7]Univ. of Nottingham, Nottingham, UK; [8]BioReliance Corp., Rockville, MD; and [9]European Chemicals Bureau, JRC, Ispra, Italy. E-mail: paris@niehs.nih.gov

Upon the recommendation of an international expert workshop convened by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM in October 2000, NICEATM and ECVAM initiated a three-phase multi-laboratory validation study to evaluate the usefulness of two in vitro basal cytotoxicity assays for predicting rodent and human acute toxicity. Seventy-two coded chemicals (12 from each of six hazard classification categories) will be tested in the mouse 3T3 fibroblast cell line and in normal human epidermal keratinocytes (NHK) using neutral red uptake assays to assess cytotoxicity. Phase Ia established the historical databases for the positive control chemical, sodium laurel sulfate (SLS), for each of the three participating laboratories. In Phase Ia, the average SLS IC50 (inhibitory concentration) values for the three laboratories were 42.3, 38.3, and 40.9 mg/ml for the 3T3 assay, and 6.2, 4.0, and 3.7 mg/ml for the NHK assay. Intra-laboratory IC_{50} coefficients of variation were 8-20% for the 3T3 cells and 15-33% for the NHK cells. Three chemicals will be tested in Phase Ib and another nine in Phase II; the purpose of these phases is to ensure that the protocol (revised in Phase Ia) is as robust as possible and to further minimize intra- and inter-laboratory variation. Sixty chemicals will then be tested in Phase III using the optimized protocol. Rodent oral LD_{50} (lethal dose) values will be estimated using prediction models based on Registry of Cytotoxicity data and Phase I/II results. Human toxicity will be estimated using a prediction model based on data from human poisoning reports and the Multicentre Evaluation of In Vitro Cytotoxicity (MEIC). This study will characterize the usefulness of these cytotoxicity tests for predicting acute systemic toxicity and the extent that they may reduce or replace animal use. Lessons learned in initiating and conducting a validation study will be presented. Supported by: NIEHS contracts N01-ES-85424 and N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission contract No. 19416-2002-04 F2ED ISP GB.

VT-2009

The EpiOcular Prediction Model: In Vivo Versus In Vitro Draize Scores for Consumer Products. M. Klausner [1], M. Osborn[1], K. Bellavance[1], B. Breyfogle[1], J. Kubilus[1], D. R. Cerven[2], and G. L. DEGEORGE[2]. [1]MatTek Corporation, Ashland, MA and [2]MB Research Laboratories, Spinnerstown, PA. E-mail: info@mbresearch.com

EpiOcular™ (OCL-200) is an organotypic tissue model of the human corneal epithelium (HCE) cultured from normal human keratinocytes using serum free medium. Histological cross-sections show that the structure of EpiOcular closely parallels that of the HCE. Previously, the prediction equation for eye irritation, Draize score (MMAS) -4.74 + 101.7/(ET-50)^0.5, was developed by correlating the *in vitro* ET-50 with Draize rabbit eye scores for 19 water-soluble chemicals from the ECETOC database and 41 cosmetic or personal care products/ingredients (ET-50 refers to the time of exposure which reduces the tissue viability to 50%, as determined by the MTT assay, in minutes). The current study reports *in vitro* results for an additional 24 consumer products, including shampoos, hand soaps, laundry detergents, dishwashing liquids, and skin lotions. A plot of the *in vivo* and calculated *in vitro* Draize scores, when correlated to the line *In Vivo* Draize (MMAS) - *In Vitro* Calculated Draize, gave the correlation coefficient, r=0.85; when a single outlier was excluded, r=0.93. The useful range of the EpiOcular test was evaluated by testing surfacatants at concentrations at which the Draize test is insensitive (MMAS scores < 2.0). EpiOcular was able to distinguish between surfactants at concentrations 3-10 fold below this point. Thus, the EpiOcular tissue model appears to be sensitive, accurate *in vitro* means of predicting *in vivo* ocular irritancy for a range of consumer products and raw materials.

VT-2010

Enhanced Phototoxicity Assay in Reconstituted Skin (EPARS). A. C. Gilotti[1], T. L. Ripper[1], T. L. Fox[1], L. Wagner[1], L. Lewis[1], S. H. Young[1], C. A. Kirk[1], M. K. Reeder[1], B. Jones[2], and G. L. DEGEORGE[1]. [1]MB Research Laboratories, Spinnerstown, PA 18968 and [2] Avon Products, Inc., Suffern, NY 10901. E-mail: info@mbresearch.com

We have conceived a high-throughput in vitro screening test for phototoxicity designated the Enhanced Phototoxicity Assay in Reconstituted Skin (EPARS). The phototoxic potential of chemicals, cosmetics, dietary supplements and pharmaceuticals are a growing concern in the consumer products industry. Animal models of phototoxicity are expensive, slow, subjective, and not amendable to high throughput. Currently in the US, there are no regulatory agency-accepted alternatives or *in vitro* phototoxicity tests. The 3T3 NRU Test is under consideration by ICCVAM after having been pre-validated by ECVAM. The EPARS test overcomes many of the limitations of the 3T3 NRU test: 1) EPARS employs multi-layer tissues that closely parallel human skin morphology, instead of a fibroblast monolayer; 2) formulations of test articles can be topically applied vs. the necessary solubilization into culture media; 3) the human primary keratinocyte-based tissues are more relevant than a mouse tumor cell line. In EPARS, the test substance is applied topically to the reconstituted human skin models, with and without UV irradiation. We tested the accuracy of the assay for correctly identifying phototoxins, irritants, and non-irritants. Phototoxic effects are determined by comparing the viability of irradiated vs. non-irradiated tissues by MTT uptake. To increase the sensitivity and specificity of the test, we measured the release of cytokines into the culture media via ELISA. PGE_2 release was shown to be an early predictor of the toxic effects demonstrated in the viability assay. Release of IL-1 alpha, IL-1ra, IL-8 & TNF-alpha supported the results of the cell viability. The effects of irradiation +/- chlorpromazine were further characterized by microarray analysis.

VT-2011

Serum Alternatives to the Use of Fetal Bovine Serum in Cell Culture. B. THOMPSON, B. Fujimoto, and B. Barnett. HyClone Laboratories, Research and Product Development, 925 West 1800 South, Logan, UT 84321. E-mail: bobbie.thompson@perbio.com

Serum is a necessary part of cell culture in many cases. Fetal bovine serum (FBS) has long been researchers' first choice in serum. Although FBS performs very well in cell culture, there are aspects wherein FBS replacements might offer advantages. For instance, cost of sera, less variability in supply, lot to lot variability in composition, and performance for specific cell types. This study examines seven serum alternatives to FBS. The six cell lines used were MRC-5, VERO, BHK-21, CHO-K1, AIF and NS0. All cultures were grown in media supplemented with 10% serum. Results were cell-line dependent. There were definite synergies or preferences with certain FBS replacements proving to be more or, sometimes, less suitable for specific cell lines. In nearly all instances, cell growth in at least one of the FBS replacements was equal to or greater than cell growth in FBS. This data suggests researchers have viable serum replacements for FBS in their cell cultures.

VT-2012

Toxic Effects of Organic and Inorganic Forms of Selenium in Murine Erythroleukemia and Human Prostate Cancer Cells. S. K. MAJUMDAR, S. A. Satuh, J. H. Tchaicha-Pavlic, and E. A. Lucisano. Department of Biology, Lafayette College, Easton, PA 18042. E-mail: majumdas@lafayette.edu

Selenium is an antioxidant more recently noted for its chemopreventive ability to inhibit various types of cancers, such as colon, lung and prostate. It is suggested that selenium activates apoptosis, otherwise known as programmed cell death. This study evaluated organic (seleno-l-methionine) and inorganic (sodium selenate) forms of selenium to analyze their effects on murine erythroleukemic cells (GM-86) and human prostate cancer cells (CRL-10995). The action of selenium was measured by observing cell proliferation, cell surface ultrastructure, disruption of mitochondrial membrane potential, cell organelle integrity and DNA strand breakage. Both forms of selenium resulted in a decrease in cell proliferation and an increase in the formation of blebs and apoptotic bodies on the cell surface as well as an enhancement in mitochondrial membrane disruption in both cancer cell types. Light microscopy revealed an increase in nuclear disruption in both cancer cell lines. DNA fragmentation assay revealed that the organic form had differential effects on the two cell lines, while the inorganic form showed DNA strand breakage in both cancer cell types. In general, the GM-86 cells were found to be more sensitive to the agent and the inorganic form was found to be more potent.

VT-2013

Metalloprotease Inhibitors, Non-microbial Chemically Modified Tetracyclines and Ilomastat, Block Anthrax Lethal Factor Activity In Vitro. S. S. KOCER and S. R. Simon. Department of Biochemistry, Program in Cellular and Molecular Biology, and Institute for Cell and Developmental Biology, State University of New York at Stony Brook, Stony Brook, NY 11794. E-mail: sskocer@hotmail.com

Anthrax lethal toxin, produced by the bacterium *Bacillus anthracis*, is the major cause of death in animals infected with anthrax. One component of this toxin, lethal factor (LF) is a zinc metalloproteinase, which proteolytically cleaves members of the mitogen activated protein kinase kinase (MAPKK or MEK) family through proteolysis of their NH2-termini. Although the relationship between MEK cleavage and the pathology of anthrax remains correlative, it appears that the proteolytic activity of LF is responsible for the activation of macrophages at lower doses and for macrophage cytotoxicity at higher doses. We showed that zinc metalloproteinase inhibitors CMT-3, CMT-308 and Ilomastat inhibit LF-mediated cleavage of the N-terminal domain of MEKs, using lysates of human monocyte cell line (MonoMac-6) as sources of the MAPKKs, separation by SDS-PAGE, and detection by Western Blotting. Clinical trials of the CMTs and Ilomastat on human patients have already been initiated. Here we show that we could combat effects of the lethal toxin of *Bacillus anthracis in vitro*, based on the anti-proteolytic activity actions of CMTs and Ilomastat.

VT-2014

The Effects of Glutamine and Ammonia Concentrations on CHO Cells in Serum-free Media. P. DECARIA, J. Camire, and B. Barnett. HyClone Laboratories, Inc., Logan, UT 84321. E-mail: Paula.Decaria@perbio.com

Glutamine is a vital component of animal cell culture. In addition to its function as an amino acid in protein synthesis, cells convert glutamine into intermediates used in the citric acid cycle, ultimately producing energy. But as it is consumed and converted into these intermediates, ammonia is produced. This study shows performance trends from two Chinese Hamster Ovarian cell lines in different concentrations of glutamine with results showing the best performance for these cells in media with 8 mmol/L L-glutamine, and dropping off at higher concentrations. Another concern with glutamine is that it spontaneously breaks down in culture media, also causing build up of ammonia. This is important because too much ammonia in the system is toxic to the cells. This study also looks at the effects of different concentrations of an ammonium ion on these two cell lines with toxic effects appearing in concentrations of 8 mmol/L and higher. Because of this glutamine decomposition, much debate has risen as to when glutamine should be added to media and how much of it should be added without causing damage to the cells later. To address this, this study also shows the stability of glutamine at different temperatures over time in a serum-free media and how cells perform in each situation, demonstrating how glutamine and ammonia can skew study results if media age is not taken into account. Overall, the results of this study show the importance of limiting glutamine in media, but still adding enough to achieve optimal cell performance, and always taking into account the age of glutamine-containing media before using it.

VT-2015

Protein Kinase C Disrupts the Formation of VA Gene Transcription Initiation Complex. CALVIN B. L. JAMES. Department of Biomedical Sciences, Ohio University, Athens, OH 45701. E-mail: jamesca@ohio.edu and Timothy E. Shannon, Department of Biology, Francis Marion University, Florence, SC 29501. E-mail: tshannon@fmarion.edu

Activation of protein kinase c (PKC) severely reduces transcription from the polymerase III (pol III) transcribed adenovirus VA gene, but the exact mechanism remained unexplained. Successful transcription of a pol III transcribed gene requires the recruitment of the polymerizing enzyme to the transcription start site to permit synthesis of the new transcripts and involves three discrete steps: initiation (the proper assembly of the transcription factors to form transcription complex), elongation and termination. Studies reported here support a conclusion that upon PKC activation a failure to form transcription competent complexes, and not elongation, is responsible for impaired transcription of the VA gene. In these studies, plasmids, either VA or VA/EL (the VA gene with a linker to distinguish its transcription product from that of the VA gene) were used in in vitro assays. In vitro analysis shows that pre-incubation of VA template for a minimum of ten minutes before the activation of PKC did not result in PKC-induced repression of transcription. In contrast, under the same conditions, the addition of a second template, VA/EL, at the time of PKC activation, resulted in efficient transcription from the pre-incubated VA template but not from VA/EL. Simultaneous pre-incubation of both VA and VA/EL resulted in efficient transcription from both templates. Subsequent analysis shows successful elongation of isolated VA transcription complexes formed in the absence of activated PKC, but not for those complexes formed in the presence of the active enzyme. In rescue experiments, a partially purified pol III transcription factor, TFIIIB, successfully rescues VA transcription from PKC-induced repression, suggesting that PKC modified a target within this crude fraction. Further studies confirmed that TATA box binding protein (TBP), a constituent of TFIIIB, could substitute for the crude preparation of TFIIIB.

VT-2016

A Non-animal Alternative Carcinogenicity Assay Using Fertilized Avian Eggs: The In Ovo Carcinogenicity Assay (IOCA). G. L. DEGEORGE[1], D. R. Cerven[1], M. J. Iatropoulos[2], G. M. Williams[2], C. Perrone[2], and H. Enzmann[3]. [1] MB Research Laboratories, Spinnerstown, PA and [2] New York Medical College, Valhalla, NY; and [3] Institute of Toxicology, Bayer AG, Wuppertal, DE. E-mail: info@mbresearch.com

Carcinogenicity testing of substances is currently assessed via costly bioassays utilizing rodents in two year studies. We have developed an alternative assay which is performed on avian embryos as a screening test for carcinogenicity. The in ovo carcinogenicity assay (IOCA), is conducted in fertilized turkey, chicken, or quail eggs, and addresses the three Rs (reduction, refinement and replacement) of alternatives to animal testing. In ovo administration of known carcinogens on day 0 to 4 embryonic development for up to 26 days resulted in the induction of pre-neoplastic lesions in the avian liver. Liver effects included changes in liver weight, enlarged nuclei, foci of altered hepatocytes (FAH), hepatocellular reassignment from a trabecular to tubular pattern, as well as damage and breakage of mitochondrial DNA. Diethylnitrosamine, N-nitrosomorpholine, dimethylnitrosamine, and aflatoxin B, were all positive carcinogens in this assay. The sensitivity of this assay was similar to rodent tests; A single dose of 1.0 - 5.0 mg of DEN per 100 g egg weight induced dose-dependent liver and mtDNA changes whereas 0.5 mg and below did not. Thioacetamide and all dosing vehicles were negative. The IOCA has numerous advantages over the rodent carcinogenicity bioassay, including 10 it is a rapid test, requiring a maximum of 24 days to perform; 2) testing does not require use of any mammals; 3) testing is performed on eggs, which are kept in incubators, requiring less space and equipment than animal facilities; 4) dose amounts are small, which reduces exposure of humans to potential carcinogens; and 5) cost of analysis is greatly reduced when compared to traditional carcinogenicity testing.

VT-2017

Development of an Alternative Test for Photoirritants: The In Ovo Phototoxicity Assay (IOPA). D. R. Cerven, T. L. Ripper, T. L. Fox, S. H. Young, G. L. DEGEORGE. MB Research Laboratories, Spinnerstown, PA 18968. E-mail: info@mbresearch.com

Phototoxicity of pharmaceuticals, consumer products and cosmetics are of increasing concern to regulatory agencies and the public. Preclinical phototoxicity studies are typically performed using large numbers of experimental animals. The 3T3 fibroblast phototoxicity assay has been validated by the EU, but this AND other *in vitro* tests lask complexity, metabolic capability and predictivity of animal models. We have devised an *In Ovo* Phototoxicity Assay based on the well-characterized chorioallantoic membrane (CAM) vascular assay, which uses fertilized chicken eggs as a test system. Groups of eggs were administered increasing concentrations of known irritants, non-irritants, and phototoxins, +/-5 J/cm^2 UVA irradiation. Eggs displaying vascular hemorrhage of the CAM, altered cell proliferation, or death of the embryo were scored as positive responses, and the percent positive eggs was plotted to determine the ECand EC_{50+UV} from the respective log-dose response curves. The Photo-Irritancy Factor (PIF = EC_{50-UV} / EC_{50+UV}) was calculated and a PIF > 5 indicated a positive phototoxic response. Using this assay, the prototypical phototoxins 8-methoxypsoralen, promethazine, chlorpromazine, and 6-methylcoumarim were identified as phototoxic (8-MOP EC_{50+UV} =0.0003 mg/ml > PMZ; 0.0017 mg/ml > CPZ; 0.082 mg/ml > 6-MC 0.44 mg/ml), whereas SLS, dinitrochlorobenzene, benzalkonium chloride, ethanol and mineral oil were negative. In addition, the IOPA correctly identified the metabolically-activated phototoxins nabumetone an 5-aminolevulinic acid, which are phototoxic in vivo (due to hepatic metabolism) but not when assessed in vitro. In these cases, up to 18 hour pre-treatment time prior to UVA irradiation was necessary to allow for distribution and conversion to the phototoxic metabolite. In conclusion, we have developed an alternative phototoxicity assay that allows rapid, inexpensive and sensitive screening of chemicals, while retaining many advantages of animal models.

VT-2018

In Vitro Models of Full-thickness Human Skin (EpiDerm-FT) and Airway Epithelium (EpiAirway-FT) for Toxicology and Drug Development Applications. P. J. HAYDEN, M. Klausner, J. Kubilus, B. Burnham, and G. R. Jackson. MatTek Corporation, Ashland, MA 01721. E-mail: phayden@mattek.com

In Vitro models of human skin and airway epithelia have potential applications in toxicology studies involving chemical warfare agents, environmental chemicals or consumer products, as well as in development of therapeutics delivered via dermal or inhalation routes. However the response of epithelial cells to exogenous chemical exposure is believed to be strongly influenced by paracrine signaling from fibroblasts (FB) residing in the subepithelial stromal tissue. Thus, "full-thickness" models containing an epithelial cell layer as well as an underlying FB-containing stromal matrix are desirable to more fully reproduce the in vivo situation. To enable in vitro study of phenomena in which FB-epithelial cell interactions are important, highly differentiated full thickness models composed of FB-containing collagen matrix and keratinocyte (KC) or airway epithelial cells (AEC) were therefore developed. Histologic examination of the resultant skin equivalent shows a collagen dermis populated by numerous viable FB and an epidermis consisting of stratified KC including basal, spinous, granular and stratum corneum components. The airway equivalent also possesses a collagen matrix populated by numerous viable FB, but with pseudostratified mucociliary morphology typical of the in vivo tracheal/bronchial epithelium. An important aspect for reproducing in vivo-like function of full-thickness epithelial tissues is development of an appropriate basement membrane at the junction between the epithelium and the underlying FB-containing matrix. Therefore the ultrastructure of the basement membrane was examined by transmission electron microscopy. A well-developed basement membrane was evident in both models. Hemidesmosomes were observed at the basal membranes of the epithelial cells, with associated tonofilaments extending into the cytoplasm. Well-defined, continuous lamina lucida and lamina densa and fine anchoring filaments were present beneath the basal epithelial cells. Anchoring fibrils with characteristic striated structure connected the lamina densa to the underlying collagen matrix. EpiDerm-FT and EpiAirway-FT overcome shortcomings of previous models in terms of providing epithelial cell/FB interactions as well as appropriate in vivo-like morphology and basement membrane development. These attributes will enable more realistic in vitro toxicological studies of epithelial phenomena.

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2003 Congress on In Vitro Biology

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Brady Corporation
Milwaukee, WI

CABI Publishing
Wallingford, Oxon, UK

Caisson Laboratories, Inc.
Sugar City, ID

Cascade Biologics, Inc.
Portland, OR

Conviron
Hendersonville, NC

Ecological Chambers, Inc.
Winnipeg, MB, CANADA

Environmental Growth Chambers
Chagrin Falls, OH

Hoffman Manufacturing, Inc.
Albany, OR

Invitrogen Corporation
Grand Island, NY

Jackson ImmunoResearch Labratories, Inc.
West Grove, PA

MB Research Laboratories
Spinnerstown, PA

Olympus America, Inc.
Melville, NY

One Cell Systems, Inc.
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Phytotechnology Laboratories
Overland Park, KS

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Portland, Oregon
May 31 - June 4, 2003

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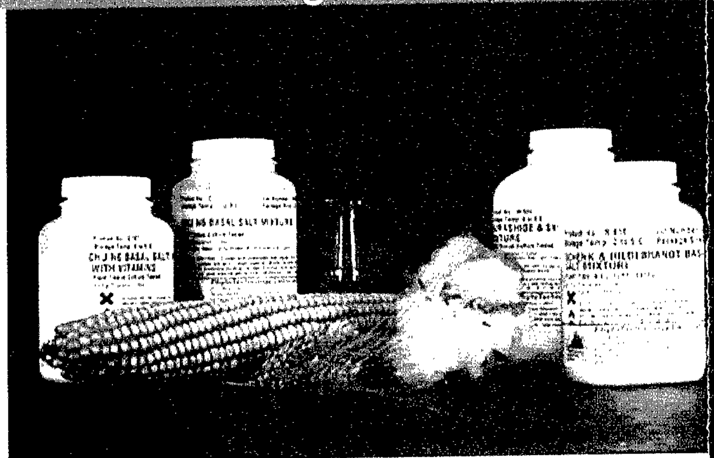

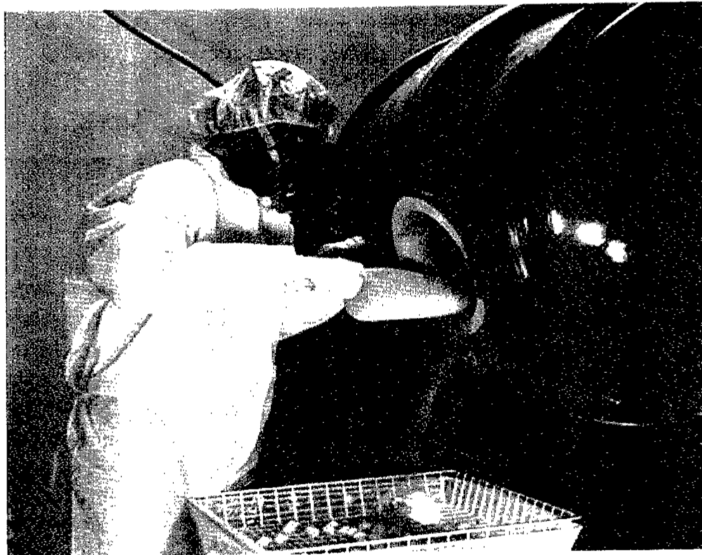

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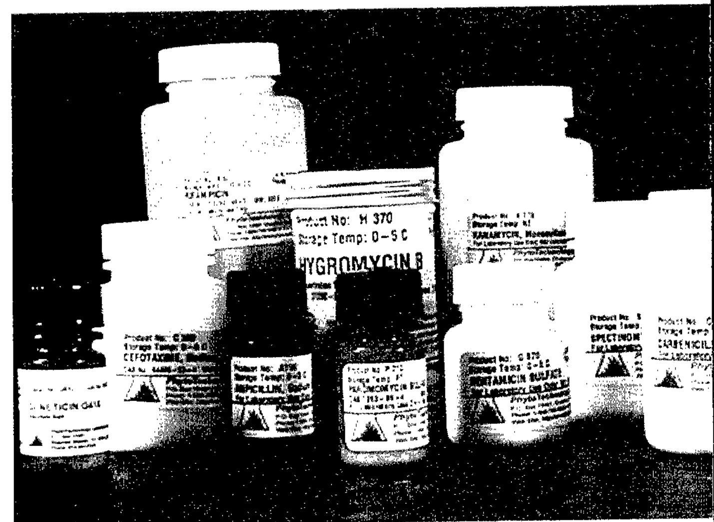

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The 2003 Congress On In Vitro Biology

Final Program

Portland, Oregon
May 31 - June 4, 2003

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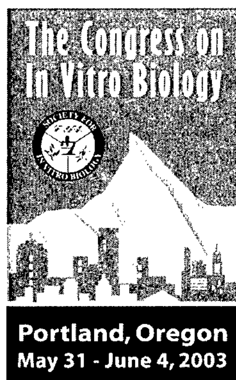

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TIME	TYPE OF FUNCTION	ROOM
FRIDAY, MAY 30		
5:00 pm – 8:00 pm	SIVB Board of Directors Meeting	Crown Zellerbach
SATURDAY, MAY 31		
7:00 am – 7:00 pm	Registration	Grand Ballroom Foyer
8:00 am – 12:00 pm	SIVB Board of Directors Meeting	Crown Zellerbach
11:00 am – 5:00 pm	The Scotts Company Tour	The Scotts Company
12:30 pm – 1:30 pm	2003 Program Planning Committee Meeting	Weyerhaeuser
3:00 pm – 6:00 pm	Poster Set-up	Grand Ballroom
5:00 pm – 6:00 pm	History Society Meeting	Presidential Suite
6:00 pm – 7:00 pm	Student Reception/Poster Session	Grand Ballroom
7:00 pm – 9:00 pm	Opening Reception	Grand Ballroom
7:00 pm – 9:00 pm	City of Roses Silent Auction Kickoff	Grand Ballroom
SUNDAY, JUNE 1		
7:00 am – 6:00 pm	Registration	Grand Ballroom Foyer
7:00 am – 8:00 am	SIVB/CABI/IAPTC&B Business Meeting	Pettygrove Room
7:00 am – 8:00 am	Education Committee Meeting	Glisan Room
7:00 am – 8:00 am	Plant Program Committee Meeting	Overton Room
10:00 am – 3:00 pm	**Exhibits and Posters**	Grand Ballroom
10:00 am – 10:30 am	Coffee Break	Grand Ballroom
12:30 pm – 1:30 pm	In Vitro – Plant Editorial Board Meeting	Pettygrove Room
12:30 pm – 1:30 pm	Lunch	Grand Ballroom
5:00 pm – 9:00 pm	Plenary Reception	Pittock Mansion and the World Forestry Center
MONDAY, JUNE 2		
7:00 am – 6:00 pm	Registration	Grand Ballroom Foyer

Time	Function	Location
7:00 am – 8:00 am	Student Affairs Breakfast	Pettygrove Room
7:00 am – 8:00 am	Publications Committee Meeting	Overton Room
10:00 am – 3:00 pm	**Exhibits and Posters**	Grand Ballroom
10:00 am – 10:30 am	Coffee Break	Grand Ballroom
12:30 pm – 1:30 pm	Lunch	Grand Ballroom
6:00 pm – 9:00 pm	Plant Business Meeting and Social	Clackamas/Multomah
6:00 pm – 9:00 pm	Vertebrate/Toxicology Business Meeting and Social	Washington
6:00 pm – 9:00 pm	Invertebrate Business Meeting and Social	OFF PROPERTY

TUESDAY, JUNE 3

Time	Function	Location
6:00 am – 7:30 am	Fun Run/Walk	Grand Ballroom Foyer
7:00 am – 5:30 pm	Registration	Grand Ballroom Foyer
7:00 am – 8:00 am	Development Committee Meeting	Pettygrove Room
7:00 am – 8:00 am	Membership Committee Meeting	Overton Room
10:00 am – 2:00 pm	**Exhibits and Posters**	Grand Ballroom
10:00 am – 10:30 am	Coffee Break	Grand Ballroom
12:30 pm – 1:30 pm	2004 Program Planning Committee Meeting	Pettygrove Room
12:30 pm – 1:30 pm	Lunch	Grand Ballroom
2:00 pm – 3:00 pm	Poster Breakdown and Removal	Grand Ballroom
2:00 pm	City of Roses Silent Auction Final Bidding	Grand Ballroom
5:00 pm – 6:00 pm	SIVB Business Meeting	Multnomah
7:00 pm – 8:00 pm	Reception & Announcement of City of Roses Silent Auction Winners	Grand Ballroom Foyer
8:00 pm – 10:00 pm	Closing Banquet	Grand Ballroom

WEDNESDAY, JUNE 4

Time	Function	Location
7:00 am – 5:00 pm	Registration	Grand Ballroom Foyer
7:00 am – 8:00 am	Long-Range Planning Committee Meeting	Pettygrove Room
10:00 am – 10:30 am	Coffee Break	Mount St. Helen's Foyer

THURSDAY, JUNE 5

Time	Function	Location
9:30 am – 4:00 pm	Greyhorse Winery Tour	McMinville, OR

Congress Information

ABOUT THE PROGRAM

The Congress program will include symposia, workshops, contributed paper and poster sessions, continuing education programs, exhibits, and other events that reflect state-of-the-art in vitro biology and biotechnology. A program has been developed that will provide you with the latest research in the diverse areas of vertebrate, cellular toxicology, invertebrate, and plant cell and tissue culture. Whether your interest is reproductive health, insect hormones, artificial chromosomes, regulatory affairs, habitat restoration, micropropagation, or tropical plant transformation, there is something for you at the Congress. The Congress is also an opportunity to meet and share science with colleagues from around the world in a comfortable, intimate setting.

AWARDS

INVERTBRATE FELLOW AWARD

The Society for In Vitro Biology – Invertebrate Section will be honoring Marcia Loeb, PhD, with the first Invertebrate Fellow Award. This award recognizes members who have made outstanding contributions to invertebrate science research, teaching, or administration. The fellow is presented to SIVB members who have been active for at least ten years and who have made contributions to the Society. The award ceremony will take place at the Invertebrate Section Social following the Business Meeting on Monday, June 2, at 6:00 pm. Please take time at the meeting to congratulate Dr. Loeb.

PLANT FELLOW AWARD

The Society for In Vitro Biology – Plant Section will be honoring Mary Ann Lila, PhD, and Barbara M. Reed, PhD, with the Plant Fellow Award. This award recognizes members who have made outstanding contributions to plant science research, teaching, or administration. The fellow is presented to SIVB members who have been active for at least ten years and who have made contributions to the Society. The award ceremony will take place at the Plant Section Social following the Business Meeting on Monday, June 2, at 6:00 pm. Please take time at the meeting to congratulate our awardees.

STUDENT AWARDS

The SIVB Student Award Program provides recognition and financial support for students who have contributed and made outstanding achievements in the field of in vitro biology. This year we will be awarding the Cellular Toxicology Award to Salih S. Kocer, State University of New York at Stony Brook; the Joseph E. Morgan and Student Travel Awards to Vivian Dayeh, University of Waterloo; Hope E. Hopps and Student Travel Awards to Tristan F. Kraft Burns, University of Illinois; Honor B. Fell and Student Travel Awards to Shintaro Goto, Kobe University; Student Travel Award to Sinee Pauline Kopsombut, University of Illinois; John S. Song Award to Li Chen, Salem International University; the Student Travel Award to Sadanand Dhekney, University of Florida; Wilton R. Earle and Student Travel Awards to Hyun-Kyung Kim, University of California – Berkeley ; the Student Travel Award to Serena B. McCoy, Kansas State University; and the Student Travel Award to Giang Thi Thanh Dam, Kagawa University. Certificates will be given out at the Closing Banquet, Tuesday, June 3, 2003.

CITY OF ROSES SILENT AUCTION

By popular demand, we will hold our third silent auction fundraiser event at the 2003 Congress. Like previous years, this auction promises items available for the graduate student to the biotech CEO's budget. The City of Roses Silent

Auction Kickoff will coincide with the Opening Reception. Most items will be available for your bidding in the Exhibit Hall. Additionally, a number of the exhibitors have donated items, which will be on display at their booths during the Exhibition. Bidding will conclude at 2:00 pm on Tuesday, June 3. The winners will be announced at the reception prior to the Closing Banquet and can be paid for at that time. Items not retrieved by 7:45 pm that evening will be forfeit to the next highest bidder.

CLASSIFIEDS
Attendees are encouraged to check the bulletin board in the registration area for current listings of the SIVB's classified section of the *In Vitro Report* and website. The SIVB provides an opportunity for individual non-members and members to place classified ads for positions wanted, and for companies to advertise position openings in the pages of *In Vitro Report* and on the website. This service is provided free of charge to SIVB individual members and sustaining (company) members. Non-member rates are as follows: $10.00 per line - government agencies, academic, and non-profit organizations (10 line minimum) and $20.00 per line - for-profit companies (10 line minimum).

CME CREDIT
This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of The University of Vermont and (name of non-accredited provider). The University of Vermont is accredited by the ACCME to provide continuing medical education for physicians. The University of Vermont College of Medicine designates this educational activity for a maximum of 25.5 hour(s) in Category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity. Congress participants can request continuing medical education evaluation forms at the registration desk.

COFFEE BREAKS
Complimentary coffee will be provided during scheduled morning breaks (Sunday, Monday, Tuesday) in the Grand Ballroom with the exhibitors. All registrants are invited to visit the exhibits throughout the conference.

CONGRESS INFORMATION OFFICE
The Congress Information Office is located in the Pendleton Room of the Doubletree Hotel – Jantzen Beach. This office will be open and available to attendees for questions and information throughout the meeting.

DISABILITIES ACT
The Society for In Vitro Biology will make all reasonable efforts to accommodate persons with disabilities at the meeting. The Doubletree Hotels Complex is in compliance with the Americans with Disabilities Act.

EVALUATIONS
In an effort to improve the quality of the Annual Congress Meeting, the Program Planning Committee is requesting that meeting attendees take a moment to complete a short evaluation located on the back of your banquet voucher. This voucher will also be used for the Grand Door Prize drawing. By completing the short survey, you will assist the SIVB in planning the most practical and effective Congresses possible. Thank you in advance for your cooperation.

Congress Information

EXHIBITS

Plan to visit the Exhibits in the Grand Ballroom of the Doubletree Hotel – Jantzen Beach. The Exhibit Hall will be open Saturday evening from 7:00 pm – 9:00 pm, Sunday and Monday from 10:00 am – 3:00 pm, and Tuesday from 10:00 am – 2:00 pm. Stop by each booth and review, at your leisure, the latest products and equipment in cell science research and tissue culture.

INTERACTIVE POSTER SESSIONS

A number of posters have been selected to participate in interactive poster sessions in addition to the regular poster presentations. These sessions are scheduled on Sunday and Monday, 1:30 – 2:30 pm in the Exhibit Hall. Each Interactive Poster Presenter has been informed of their abstract number, date, and time of presentation. Each session will have a moderator and it will begin with each poster presenter giving a short (5-minute maximum) description of their poster. After the brief introduction, the floor will be open for discussion. Please check the program schedule to see which posters are scheduled on each day.

INTERNATIONAL GUESTS

The DoubleTree Hotel – Jantzen Beach will not accept or exchange international currency; however, currency exchange is available at Travelex at the Portland International Airport (PDX). Located in the Ticket Lobby of the airport, Travelex also offers Notary Services, Western Union, and Travel Insurance.

The DoubleTree Hotel currently does not offer a full service business center. However, faxing or copying can be accommodated at the front desk of the hotel. If you are a guest staying at the hotel, your charges can be billed to your room; otherwise, please be prepared to pay.

Payments to the 2003 Congress on In Vitro Biology MUST be made in USA dollars: U.S. denomination travelers checks; checks drawn on a USA bank; or VISA, MasterCard, American Express, or Discover credit cards. The Congress cannot accept any other type of credit card. Please arrange for payments as indicated above.

LUNCH

For your convenience, concessions will be available in the Exhibit Hall from 12:30 pm – 2:30 pm on Sunday, Monday, and Tuesday for Congress registrants and their guests. Please make sure that your guests receive a guest badge for the Exhibit Hall on the days they wish to partake of the amenities.

OPENING RECEPTION

The Opening Reception is the official kick-off for the 2003 Congress on In Vitro Biology. Join us from 7:00 - 9:00 pm on Saturday, May 31 for hors d'oeuvres and refreshments in the Exhibit Hall of the Doubletree Hotel – Jantzen Beach.

PLENARY RECEPTION

On June 1, the Plenary Reception will be held in the evening at the Pittock Mansion and the World Forestry Center for Congress participants and guests. In an idyllic setting high above the city, the Pittock's built their magnificent family home, completed in 1914. Visitors marvel at the impressive view of mountains and city, the beauty of the carefully crafted details and the innovative features that made it a splendid home, far ahead of its time. Henry Pittock, who became owner and publisher of The Oregonian, was an astute business leader, mountaineer, and family man. His wife, Georgiana, was known for her lifelong devotion to many charitable causes and her love for roses,

which led to the establishment of the annual Rose Festival, now a Portland tradition. The mansion is an architectural treasure and the sweeping views from the mansion and grounds overlook Portland's downtown and Willamette River, leading the eye to Oregon's Mt. Hood and other snowcapped Cascade Range peaks. Artfully crafted of native woods, the Forestry Center museum was opened in 1971 by famed Oregon governor Tom McCall. You can enjoy the ambiance of the over 35,000-square foot museum and delight in the seclusion of the Memorial Fountain with the gentle bubbling of its rock waterfall. The museum now features world-class permanent and traveling exhibits.

The evening, co-sponsored by Monsanto Company and Weyerhaeuser Company, will begin with a private wine and cheese reception at the Pittock Mansion at 6:00 pm on Sunday, June 1. Next, attendees will depart the Pittock Mansion to have a special dinner prepared at the Forestry Center beginning at 7:30 pm. After the meal, you will be able to explore the quiet sylvan setting with your fellow attendees. The admission price of $40.00 will include transportation, admission to the Mansion and Forestry Center, and all additional amenities of the evening. Sit back and enjoy this exploration of the City of Roses. Buses will depart from the hotel lobby at 5:00 pm.

POSTER SESSIONS
All poster presenters have been notified of their final abstract number (even or odd) and times to be present to discuss their presentations.

POSTER VIEWING HOURS

Saturday May 31	Sunday June 1	Monday June 2	Tuesday June 3
7:00 pm - 9:00 pm	10:00 am - 3:00 pm	10:00 am - 3:00 pm	10:00 am - 2:00 pm

Posters mounted Saturday, May 31, 3:00 pm - 6:00 pm.
Posters must be removed from Grand Ballroom by 3:00 pm, June 3

Authors will need to be present at their posters the following days and times

Saturday May 31	Sunday June 1	Monday June 2	Tuesday June 3
7:30 pm - 8:30 pm	1:30 pm - 2:30 pm	1:30 pm - 2:30 pm	1:30 pm - 2:00 pm
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present

Poster viewing hours are as follow:

Saturday May 31	Sunday June 1	Monday June 2	Tuesday June 3
7:00 pm - 9:00 pm	8:00 am - 10:00 pm	8:00 am - 10:00 pm	8:00 am - 2:00 pm

PRESENTATION OF PAPERS
Each invited speaker has been notified by his/her convener of the length and time for his/her presentation. The time allotted for each Contributed Paper will be shown in the meeting program, but is normally 15 minutes and includes audience discussion. All papers will be presented at the stated time. In the event of cancellation, the allotted time will, at the discretion of the convener, be used for discussion or recess.

Congress Information

PROGRAM CHANGES AND NOTICES

Changes to the program usually occur immediately prior to the printing of the program or prior to the actual meeting. In an effort to alert participants of these changes, a bulletin board will be located in the registration area with posted changes as submitted by presenters and meeting participants. Please reference "Program Changes" in the Program Booklet contents for scheduling changes to functions and events.

PUBLISHERS' BOOK DISPLAY

This year's Publishers' Book Display promises to provide a wide range of sample books and journals for display. Some are available to meeting registrants at special discount prices for attendees. Visit the Publishers' Book Display, located in the Exhibit Hall, during normal exhibition hours.

REFRESHMENTS CO-SPONSORED BY EXHIBITORS AND SIVB

On Monday, June 2, at 12:30 pm, you are invited to a special refreshment break co-sponsored by many of our Congress Exhibitors and the Society for In Vitro Biology. Refreshments will be located at the participating Exhibitor booths. The following Exhibitors will be participating in this event:

CABI Publishing
Cascade Biologics, Inc.
Ecological Chambers, Inc.
Hoffman Manufacturing, Inc.
Invitrogen Corporation
Jackson ImmunoResearch
Labratories, Inc.
MB Research Laboratories
Olympus America, Inc.
Percival Scientific
PhytoTechnology Laboratories

REGISTRATION/CHECK-IN HOURS

Saturday May 31	Sunday June 1	Monday June 2	Tuesday June 3	Wednesday June 4
7:00 am - 7:00 pm	7:00 am - 6:00 pm	7:00 am - 6:00 pm	7:00 am - 5:30 pm	7:00 am - 5:00 pm

ROSE FESTIVAL CLOSING AWARDS BANQUET

The 2003 Congress Awards Banquet will be held Tuesday, June 3, 2003 at 8:00 pm following a reception from 7:00 – 8:00 pm. Registrants who plan to attend the banquet MUST exchange their banquet VOUCHER for a banquet TICKET on Monday between 7:00 am and 2:00 pm. Remember, if you wish to join us at the banquet, you must exchange your banquet VOUCHER for a TICKET no later than Monday at 2:00 pm.

SIVB FUN RUN/WALK

Share your generosity and be good to yourself while participating in the First Annual SIVB 5k Fun Run/2k Walk, scheduled for 6:00 am on June 3. You won't want to miss it, so register now. Run with your buddies or walk with a friend. Challenge yourself (just to get up early) and have a good time — which is what it is all about: having fun and doing something good for the Society's Education Fund. Remember $25 of your $30 registration fee goes to a worthy cause. Let's run and see what we can raise (other than trouble) in Portland in 2003. The Education Fund has

provided support for the Outreach Programs that have been conducted at the past Congresses. The Outreach Programs have been a great opportunity for focusing on strategies for teaching and mentoring pre-college students in the field of in vitro biology. We will take our "hats or tee-shirts off to you" —well, that is after you sign up and pick up your gift (and maybe more...). Stop by the SIVB registration desk to pick up your gift and further information. If you are registering on-site, we cannot guarantee a gift will be available, but you'll have fun with your SIVB friends and feel "good" about contributing to a good cause!

SPEAKER SLIDE PREVIEW AREA

To assist in preparing for your presentation, the Jantzen Room will be available, beginning on Saturday, May 31, through Wednesday, June 4. Each room will be equipped with slide preview equipment as well as extra slide trays. All presenters (Symposium, Workshop, and Contributed Paper) MUST load the slide trays themselves before giving the tray to the projectionist.

SPECIAL NOTICE TO STUDENTS

The Student Affairs & Awards Committees has planned a special breakfast meeting on Monday, June 2, at 7:00 am in the Pettygrove Room for the Student Affairs Committee and 2003 Award recipients. You are invited to attend this important meeting to address any issues pertaining to student participation in the Society for In Vitro Biology and to learn how the selection process of the awards is developed. All students are encouraged to attend. Also, the Society will host a Plenary Reception for Dr. Richard Stouffer on Sunday, June 1, at 5:00 pm at the Pittock Mansion and the World Forestry Center. Students are invited to meet Dr. Stouffer and members of the SIVB Board and Program Committees.

TOUR A PREMIER PINOT NOIR VINEYARD AND WINERY

Thursday, June 5, from 9:30 am to 4:00 pm, SIVB welcomes all Congress attendees to become a viticulturist for a day! Plans are underway for a special SIVB event at Greyhorse Vineyard, where world-class Oregon Pinot Noir grapes are grown. This special day-event will be a behind-the-scenes tour, which will offer you the unique opportunity to participate in the full spectrum of vineyard activity. This is not a guided tour, but rather a "hands-on" vineyard experience where you will assist the owners with bringing the crop to harvest. Then, round out the day with a picnic lunch and wine tasting at Chateau Benoit, a premier Oregon Winery offering spectacular views of the Willamette Valley. The North Willamette region of Oregon is home to more than 55 wineries and 200 vineyards and has been recognized as one of the premier Pinot Noir producing areas in the world – rivaling the best in Burgundy. Local wineries also produce Pinot Gris, Pinot Blanc, Chardonnay, Melon, Riesling, Gewurztraminer, sparkling wine, and some Sauvignon Blanc, Cabernet, and Merlot. The buses will leave at 9:30 am from the main entrance of the Doubletree Hotel – Jantzen Beach and depart from the Vineyard at 3:00 pm. The cost for this event is $90.00 and includes the tour, lunch, and all amenities of the trip.

TOUR OF THE SCOTTS COMPANY OREGON RESEARCH FIELD STATION

Saturday, May 31, from 11:00 am to 5:00 pm, a tour of the Scotts Company will be held. The Scotts Company Oregon Research Field Station is located about 35 miles south of Portland, in the heart of turfgrass seed production, Willamette Valley. The Scotts Company is the world's leading supplier of consumer products for lawn and garden care, with a full range of products for professional horticulture as well. The company owns the industry's most recognized brands. In the U.S., the company's Scotts®, Miracle-Gro®, and Ortho®

brands are market leading in their categories, as is the consumer Roundup® brand which is marketed in North America and most of Europe exclusively by Scotts and owned by Monsanto. In the U.K., Scotts' brands include Weedol® and Pathclear®, the top-selling consumer herbicides; Evergreen®, the leading lawn fertilizer line; the Levington® line of lawn and garden products; and Miracle-Gro®. The tour will include a turfgrass seed cleaning plant, a picnic, and tour of the traditional turf plots and Roundup® ready creeping bentgrass plots at Scotts Research Field Station. The buses will leave at 11:00 am from the main entrance of the Doubletree Hotel – Jantzen Beach and depart Scotts at 4 pm. The cost for transportation to this event is $15.00.

TRANSPORTATION
The Doubletree Hotels Complex offers a complimentary shuttle to all hotel guests to the airport.

UPCOMING 2004 EVENTS

2004 WORLD CONGRESS
The World Congress on In Vitro Biology, an international congress held every four years, focuses on issues pertinent to Plant, Vertebrate, Invertebrate, and Cellular Toxicology research and will give participants a unique learning experience on plant and animal cell culture and biotechnology. The fifth World Congress in 2004, co-sponsored by the Japanese Tissue Culture Society (JTCA), Japanese Association for Animal Cell Technology (JAACT), and Japanese Society of Plant Cell & Molecular Biology (JSPCMB) will be held May 22-26, 2004 in San Francisco, California. The World Congress theme, "Emerging Global Technologies," will attract scientific participation from many countries. A list of preliminary World Congress plenary symposiums, educational workshops, and program topics will be available at the 2003 Congress on In Vitro Biology. A pre-registration rate will be offered to members and interested scientists who register for the World Congress before July 4, 2003. Scientists, sponsors, and exhibitors interested in participating in the 2004 World Congress are asked to contact Wayne Parrott, 2004 Program Chair, or the specific Section Program chairs, Alda Vidrich, Vertebrate Program Chair, Gordana Vunjak-Novakovic, Toxicology Program Chair, Amy Wang, Invertebrate Program Chair, or Mark C. Jordan, Plant Program Chair.

The Hyatt Regency San Francisco at Embarcadero Center will be the site of the 2004 World Congress on In Vitro Biology. This downtown luxury hotel on San Francisco Bay in the Financial District across from the Ferry Building, is part of the dynamic 8-block Embarcadero Center, convenient to Fisherman's Wharf, Chinatown, Moscone Convention Center, Ghirardelli Square, North Beach, and Union Square. It is adjacent to all forms of transportation - BART (Bay Area Rapid Transit), Muni intra-city transit, and California Street Cable Car.

San Francisco is a golden dream come true, a place where heart, mind and soul embrace, lost in the simplicity of delightful deliverance. Fog and sun mingle playfully above America's favorite city; the cool, cloudy comfort of early morning slowly dissolving into the peaceful warmth of a gentle afternoon glow. There's little wonder why San Francisco has been named the world's top city twice by readers of *Condé Nast Traveler;* the top U.S. city seven times since 1988.

11TH INTERNATIONAL CONFERENCE ON INVERTEBRATE CELL AND TISSUE CULTURE

The 11th International Congress on Invertebrate Cell & Tissue Culture will meet in conjunction with the World Congress on Saturday, May 22, 2004. Scientists, sponsors, and exhibitors interested in participating in the 11th International Conference on Invertebrate Cell and Tissue Culture are asked to contact the Invertebrate Program Committee. The Invertebrate Program Committee members are: Amy Wang (Chair), Guy Smagghe (Co-chair), Cynthia Goodman, Robert R. Granados, Raziel S. Hakim, Shirley Pomponi, J. Denry Sato, and Dwight Lynn.

2004 EMERGING GLOBAL BIOTECHNOLOGIES EXHIBITION

The SIVB will host an exhibition for industry and academic institutions to showcase their technologies, programs, and products. This is an opportunity for organizations to meet face to face with thousands of researchers and professions from around the world who are working in the fields of plant and animal biotechnology, cell and tissue culture, genetics, genomics, transformation, micropropagation, toxicology, cellular pathology, virology, and tissue engineering. A preliminary Exhibitor's Prospectus will be available at the Congress.

2003 Congress on In Vitro Biology
Exhibitor's List

Brady Corporation
6555 W. Good Hope Road
Milwaukee, WI 53223
Phone: 414-358-6600
Fax: 414-358-6642
Email: sue_blanchard@bradycorp.com
Booth: 9

High performance labels, barcode printers, software, and scanners. Full range of label sizes, materials, and adhesives designed for laboratory applications like vial and plate identification.

CABI Publishing
CAB International
Nosworthy Way
Wallingford, Oxon OXO 8DE
UNITED KINGDOM
Phone: 44-1491-832111
Fax: 44-1491-829198
Email: publishing@cabi.org
Booth: 23

CABI Publishing publishes *In Vitro Biology – Plant*, together with a range of electronic products, books, and journals, which focus on biotechnology.

Caisson Laboratories, Inc.
Attn: Gordon W. Reese
5 West Center
Sugar City, ID 83448
Phone: 208-656-0880
Fax: 208-656-0888
Email: gordon@caissonlabs.com
Booth: 16

Caisson Laboratories, Inc. specializes in plant tissue culture and mammalian cell culture media.

Cascade Biologics, Inc.
Attn: Matt Osborne
1341 SW Custer Dr.
Portland, OR 97219
Phone: 503-292-9521
Fax: 503-292-0566
Email: matto@cascadebio.com
Booth: 28

Cascade Biologics offers innovative cell culture products for cutaneous, ocular, and cardiovascular biology research. Includes a variety of human cell types and corresponding specialized media.

Conviron
Attn: Joseph C. Hildebrand
2741 Miller Lane
Hendersonville, NC 28791-1363
Phone: 828-693-6227
Fax: 828-693-4799
Email: joe@conviron.com
Booth: 8

Conviron will display information on their extensive line of plant growth chambers, incubators, germinators, and tissue culture chambers for the precise control of environmental conditions.

Ecological Chambers, Inc.
Attn: William S. Porter
477 Jarvis Avenue
Winnipeg, MANITOBA R2W 3A8
CANADA
Phone: 204-589-8900
Fax: 204-582-1024
Email: rpauls@enconair.com
Booth: 13

Enconair will exhibit one of their modern chambers. Be sure and visit to pick up information on their popular new 'Bigfoot' Tissue Culture Chambers.

Environmental Growth Chambers
510 East Washington Street
Chagrin Falls, OH 44022
Phone: 800-321-6854
Fax: 440-247-8710
Email: sales@egc.com
Booth: 6

EGC manufactures the largest selection of plant growth chambers worldwide. Also, produced are tissue culture chambers, controlled environment rooms, incubators, and other specialty chambers.

Hoffman Manufacturing, Inc.
PO Box 547
Albany, OR 97321
Phone: 541-926-2920
Fax: 541-926-3949
Email: sales@hoffmanmfg.com
Booth: 7

Manufacturer of custom environmental chambers. You select the components desired and we build it for you at an affordable price. Distributor for Mantis viewing system and Nova microscopes.

Exhibitors

Invitrogen Corporation
Attn: Linda Majewski
3175 Staley Road
Grand Island, NY 14072
Phone: 716-774-6735
Fax: 716-774-6760
Email: linda@majewski@invitrogen.com
Booth: 15

Invitrogen, under the GIBCO™ brand, is the leading supplier of cell culture products, services, and technologies offering catalog and custom media, sera, and reagents for research and biopharmaceutical manufacturing.

Jackson ImmunoResearch Laboratories, Inc.
872 West Baltimore Pike
West Grove, PA 19390
Phone: 800-367-5296
Fax: 610-869-0171
Email: cuserv@jacksonimmuno.com
Booth: 1

Affinity-purified secondary antibodies (many absorbed against other species), anti-digoxin, anti-biotin, anti-FITC, streptavidin, and purified immunoglobulins are conjugated with fluorophores, enzymes, Biotin-SP (spacer), colloidal gold, and phycoerythrin.

MB Research Laboratories
Attn: Ed Delacruz
PO Box 178
Spinnerstown, PA 18968
Phone: 215-536-4110
Fax: 215-536-1816
Email: mbinfo@mbresearch.com
Booth: 27

MB Research Laboratories Contract toxicology since 1972 and a leader in the use and development of alternative and in vitro toxicology methods.

Olympus America, Inc.
Attn: Patricia Pascarella
2 Corporate Center Dr.
Melville, NY 11747
Phone: 800-446-5967
Fax: 516-844-5112
Email: micro@olympus.com
Booth: 2

IX71 inverted microscope is designed for live cell applications. Its modular frame and optical design provides 9 access ports for multiple input or output devices.

One Cell Systems, Inc.
100 Inman Street
Cambridge, MA 02139
Phone: 617-868-2399 Ext. 309
Fax: 617-492-7921
Email: keri.devon@onecell.com
Booth: Table Top

The Gel Microdrop Secretion Assay permits isolation of individual cells based on level of secreted protein, antigen specificity, isotype, surface marker, or multiple parameters simultaneously.

Percival Scientific
Attn: H. Donald Fronc
505 Research Drive
Perry, IA 50220
Phone: 515-465-9363
Fax: 515-465-9464
Email: dfronc@percival-scientific.com
Booth: 22

Percival Scientific continues to set the standard for the environmental control industry. We invited you to visit our booth to discuss your requirements.

PhytoTechnology Laboratories, LLC
Attn: Kenneth Torres
7895 Mastin Drive
Overland Park, KS 66204
Phone: 913-341-5343
Fax: 913-341-5442
Email: info@phytotechlab.com
Booth: 21

PhytoTechnology Laboratories offers a complete line of products for the plant tissue culture and plant biotechnology markets.

Thermo Forma
401 Millcreek Road
Marietta, OH 45750
Phone: 800-848-3080
Fax: 740-374-1817
Email: marketing@thermoforma.com
Booth: 14

Stop by our booth for information on cell culture incubators, ULT freezers, biological safety cabinets, cryopreservation equipment, and orbital shakers.

SIVB/Exhibitors Refreshment Break

Monday, June 2 - 12:30 pm

Co-sponsored by

CABI Publishing
Wallingford, Oxon, UK

Cascade Biologics, Inc.
Portland, OR

Ecological Chambers, Inc.
Winnipeg, MB, CANADA

Hoffman Manufacturing, Inc.
Albany, OR

Invitrogen Corporation
Grand Island, NY

Jackson ImmunoResearch Labratories, Inc.
West Grove, PA

MB Research Laboratories
Spinnerstown, PA

Olympus America, Inc.
Melville, NY

Percival Scientific
Perry, IA

PhytoTechnology Laboratories
Overland Park, KS

Registration Bag Advertisers

CABI Publishing
Wallingford, Oxon, UK

Cascade Biologics, Inc.
Portland, OR

ICN Biomedicals
Aurora, OH

Invitrogen Corporation
Grand Island, NY

PUBLISHERS' BOOK DISPLAY

2003 Congress on In Vitro Biology Exhibition
Portland, Oregon
May 31 – June 3, 2003

While at the meeting, be sure to drop by the Publishers' Book Display, located near the SIVB booth in the Exhibit Hall. A number of publishers will have books on display and many are available at a special price just for the meeting. On Tuesday, June 3, beginning at 12:30 pm, all books will be available for sale at 60% off the list price (first come, first served).

The following publishers and titles will be displayed:

Annual Reviews

4139 El Camino Way
PO Box 10139
Palo Alto, CA 94303-0139
Tel: 650-493-4400
Fax: 650-424-0910
Email: service@annualreviews.org

Annual Review of Genetics, Vol. 36
Allan Campbell. ISBN 0-8243-1236-8. December 2002. List price: $67.00 US, $72.00 Int'l (+$3 handling per volume).

Annual Review of Pharmacology and Toxicology, Vol. 43
Arthur K. Cho. ISBN 0-8243-1432-8. December 2001. List price: $65.00 US, $70.00 Int'l (+$3 handling per volume); Discount Price: $52.00 US, $56.00 Int'l (+$3 handling per volume).

Annual Review of Ecology and Systematics, Vol. 32
Daphne Gail Fautin. ISBN 0-8243-0443-8. February 2003. List price: $70.00 US, $75.00 Int'l (+$3 handling per volume).

Annual Review of Microbiology
L. Nicholas Ornston. ISBN 0-8243-1156-6. October 2002. List price: $67.00 US, $72.00 Int'l (+$3 handling per volume).

Annual Review of Genomics and Human Genetics, Vol. 3
Eric Lancer. ISBN 0-8243-3703-4. September 2002. List price: $67.00 US, $72.00 Int'l (+$3 handling per volume).

Annual Review of Immunology, Vol. 20
William E. Paul. ISBN 0-8243-3020-X. April 2002. List price: $72.00 US, $77.00 Int'l (+$3 handling per volume).

Annual Review of Biochemistry, Vol. 71
Charles C. Richardson. ISBN 0-8243-0871-9. July 2002. List price: $77.00 US, $82.00 Int'l (+$3 handling per volume).

Annual Review of Cell and Developmental Biology, Vol. 18
Randy Schekman. ISBN 0-8243-3118-4. November 2002. List price: $72.00 US, $77.00 Int'l (+$3 handling per volume).

Annual Review of Biomedical Engineering, Vol. 4
Martin L. Yarmush. ISBN 0-8243-3504-X. August 2002. List price: $67.00 US, $72.00 Int'l (+$3 handling per volume).

Annual Review of Physiology, Vol. 64
Joseph F. Hoffman. ISBN 0-8243-0364-4. March 2002. List price: $67.00 US, $72.00 Int'l (+$3 handling per volume).

Annual Review of Plant Biology, Vol. 53
Deborah P. Delmer. ISBN 0-8243-0653-8. June 2002. List price: $67.00 US, $72.00 Int'l (+$3 handling per volume).

Annual Review of Phytopathology, Vol. 40
Robert K. Webster. ISBN 0-8243-1340-2. September 2002. List price: $67.00 US, $72.00 Int'l (+$3 handling per volume).

Blackwell Publishing

9600 Garsington Road
Oxford, Oxfordshire OX4 2DQ
UNITED KINGDOM
Tel: 44-1865-476270
Fax: 44-1865-471270
Email: mjohnstone@blackwellpublishers.co.uk

Plant Biotechnology Journal, Vol. 1
Keith Edwards, ed. ISSN 1467-7644. 2003. List price: $84.00, personal print and online subscription.

Traffic, Vol. 4
Frances M. Brodsky, Mark C.P. Marsh and Sandra L. Schmid, eds. ISSN 1398-9219. September 2002. List price: $182.00, personal print and online subscription.

Molecular Plant Pathology, Vol. 4
Gary D. Foster, ed. ISSN 1464-6722. 2003. List price: $117.00, personal print and online subscription.

Genes to Cells, Vol. 8
Jun-ichi Tomizawa, ed. ISSN 1356-9597. 2003. List price: $176.00, personal print and online subscription.

EJB: European Journal of Biochemistry, Vol. 270
Richard Perham, ed. ISSN 0014-2956. September 2002. List price: No personal rate.

Animal Genetics, Vol. 34
A.L. Archibald and Noelle E. Cockett, eds. ISSN 0268-9146. 2003. List price: $25 personal online subscription only.

Cambridge University Press

40 West 20th Street
New York, NY 10011
Tel: 212-924-3900
Fax: 212-691-3239
Email: achan@cup.org

Fungi in Bioremediation
G. M. Gadd. ISBN: 0-521-78119-1. December 2001. List price: $120.00; Discount price: $96.00.

Flexible Pattern Matching in Strings
Gonzalo Navarro and Mathieu Raffinot. ISBN: 0-521-81307-7. May 2002. List price: $50.00; Discount price: $50.00

An Introduction to Genetic Engineering, Second Edition
Desmond S. T. Nicholl. ISBN: 0-521-00471-3. February 2002. List price: $25.00; Discount price: $20.00.

Reshaping Life: Key Issues in Genetic Engineering
G. J. V. Nossal and Ross L. Coppel. ISBN: 0-521-52423-7. August, 2002. List price: $22.00; Discount price: $17.60.

Basic Biotechnology, Second Edition
Colin Ratledge and Bjørn Kristiansen, eds. ISBN: 0-521-77917-0. April 2001. List price: $45.00; Discount price: $36.00.

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H. W. Denker, A. W. English (Eds.) ISSN: 1422-6405
List price: $186.00 per year personal subscription rate.

Society for In Vitro Biology
SIVB Publication Sales

In Vitro Cellular & Developmental Biology – Plant
Greg Phillips and Trevor A. Thorpe, Eds. ISSN: 1054-5476 Volumes available from 1998 through 2002. List price per volume $45.00, Discount Price per volume $22.50. List price per issue $15.00. Discount price per issue $7.50.

In Vitro Cellular & Developmental Biology – Animal
Wallace McKeehan, Ed. ISSN: 1071-2690. Volumes available from 1998 through 2002. List price per volume $60.00; Discount Price per volume $30.00. List price per issue $15.00; Discount price per issue $7.50.

Invertebrate Cell Culture: Looking Toward the Twenty-First Century
Proceedings of the IX International Conference on Invertebrate Cell Culture (1996)
1996. List Price: $60.00; Discount price: $30.00.

Proceedings of the Eighth International Conference on Invertebrate and Fish Tissue Culture (1991)
ISBN: 0-931767-02-4. 1991. List price: $60.00; Discount price: $30.00.

Monograph #3: Interferon
1974. List price: $25.00; Discount price: $12.50.

Monograph #5: Uses and Standardization of Vertebrate Cell Cultures
ISBN: 0-931767-00-8. 1984. List price: $30.00; Discount price: $15.00.

Monograph #6: Abnormal Cells, New Products and Risk
ISBN: 0-931767-01-6. 1985. List price: $40.00; Discount price: $20.00.

Time	Saturday, May 31	Location
7:00 am - 7:00 pm	Registration	Grand Ballroom Foyer
8:00 am - 12:00 pm	SIVB Board of Directors Meeting	Crown Zellerbach
11:00 am - 5:00 pm	The Scotts Company Tour	The Scotts Company
12:30 pm - 1:30 pm	2003 Program Committee Meeting	Weyerhaeuser
3:00 pm- 6:00 pm	Poster Set up	Grand Ballroom
5:00 pm - 6:00 pm	History Society Meeting	Presidential Suite
6:00 pm - 7:00 pm	Student Reception/Poster Session	Grand Ballroom
7:00 pm - 9:00 pm	2003 Congress Opening Reception	Grand Ballroom
	City of Roses Silent Auction Kickoff	Grand Ballroom
7:30 pm - 8:30 pm	All Poster Authors will be present	Grand Ballroom

SATURDAY, MAY 31

7:00 am – 7:00 pm Registration .... Grand Ballroom Foyer

8:00 am – 12:00 pm
SIVB BOARD OF DIRECTORS MEETING .... Crown Zellerbach

7:00 pm – 9:00 pm
2003 CONGRESS OPENING RECEPTION .... Grand Ballroom

7:00 pm – 9:00 pm
CITY OF ROSES SILENT AUCTION KICKOFF .... Grand Ballroom

7:30 pm – 8:30 pm
All Poster Authors will be present .... Grand Ballroom

Sunday, June 1

Time	Sunday, June 1	Location
7:00 am - 6:00 pm	Registration	Grand Ballroom Foyer
7:00 am - 8:00 am	Plant Program Committee Meeting	Overton Room
	CABI/IAPTC&B/SIVB Meeting	Pettygrove Room
	Education Committee Meeting	Glisan Room
8:00 am -10:00 am	Cryopreservation Joint Symposium	Washington/Clark
10:00 am -10:30 am	Coffee Break	Grand Ballroom
10:00 am - 3:00 pm	Exhibits and Posters	Grand Ballroom
10:30 am - 12:30 pm	Creating Connections Between Scientists and Educators: Bringing Biotech to the Classroom Education Symposium	Timberline
	The Future of Serum: A Roundtable Discussion Animal Symposium	Multnomah
	High Throughput Methods Plant Symposium	Washington/Clark
	In Vitro Tools Plant Contributed Paper Session	Clackamas
12:30 pm - 1:30 pm	Plant Editorial Board Meeting	Pettygrove
1:30 pm - 2:30 pm	INTERACTIVE POSTER SESSIONS: In Vitro Tools and Techniques *Vertebrate/Toxicology* Insect Cell Lines for Biocontrol *Invertebrate* Stress Biology *Plant*	Grand Ballroom
3:00 pm - 4:30 pm	Plenary Session "ARTistic Use of Nonhuman Primates: IVF to Cloning and Beyond"	Multnomah/ Clackamas
5:00 pm - 9:00 pm	Plenary Reception and Dinner	Pittock Mansion and the World Forestry Center

SUNDAY, JUNE 1

7:00 pm – 6:00 pm Registration .... Grand Ballroom Foyer

CRYOPRESERVATION

Conveners: Barbara M. Reed, USDA-ARS
Valerie C. Pence, Cincinnati Zoo and Botanical Garden
Lia H. Campbell, Organ Recovery Systems, Inc.

8:00 am – 10:00 am Joint Symposium .... Washington/Clark

Techniques for storing living materials in liquid nitrogen have become important tools in the areas of agriculture, medicine, and conservation, and in vitro systems are often particularly suited for long-term storage at freezing temperatures. Research into cryoprotective and recovery mechanisms, while dealing with specific taxa, can provide guidance for the application of cryopreservation to a variety of systems. This session will provide an update on recent work on oxidative stress and desiccation tolerance in cryobiological systems as well as important principles needed for successful cryopreservation.

8:00 Introduction (B. Reed, V. Pence, and L. Campbell)
8:15 J-1 The Role of Small Amphiphilic Solutes in Desiccation Tolerance
***Ann E. Oliver**, University of California – Davis*
8:45 J-2 Cryopreservation Principles for Mammalian Tissues: The Basis of Success or Failure
***Michael J. Taylor**, Organ Recovery Systems, Inc.*
9:15 J-3 A Free Radical View of Cryopreservation
***David H. Bremner** and **Erica E. Benson**, University of Abertay Dundee*

10:00 am – 10:30 am Coffee Break .... Grand Ballroom

10:00 am – 3:00 pm Exhibits and Posters .... Grand Ballroom

CREATING CONNECTIONS BETWEEN SCIENTISTS AND EDUCATORS: BRINGING BIOTECH TO THE CLASSROOM

Convener: Carol M. Stiff, Kitchen Culture Kits, Inc.

10:30 am – 12:30 pm Education Symposium .... Timberline

Incredible advances are being made in the fields of plant, vertebrate, and invertebrate in vitro biology. It is our duty as scientists to insure that instructional resources, conceptual background information, and hands-on laboratory experiences can be incorporated into classrooms. This serves to both educate the public and foster development of our future scientists and educators. The session will discuss the various ways this has been accomplished and will provide web resources for continued networking.

10:30		Introduction (C. Stiff)
10:45	E-1	Connecting with Teachers Through Hands-on Workshops, Online Courses and Listservs, and User-friendly Kits ***Carol M. Stiff,*** *Kitchen Culture Kits, Inc.*
11:10	E-2	LIGASE Loaners: Bringing Scientific Expertise to the High School Classroom ***Patricia E. Bossert,*** *Northport/East Northport School District*
11:35	E-3	Creating a Viable Biotechnology Program in High School ***Judi Heitz,*** *San Diego High School*
12:00	E-4	Bio-Link: Promoting Biotechnology Education in Community Colleges ***Beth Pitonzo,*** *Mount Hood Community College*

THE FUTURE OF SERUM: A ROUNDTABLE DISCUSSION

Convener: William J. Smith, U.S. Army Medical Research Institute of Chemical Defense

10:30 am – 12:30 pm Animal Symposium .... Multnomah

Serum has been a critical supplement to cell culture media for many years. Developments in serum collection and preparation have refined the quality of the product, but questions persist as to the standardization and purity of serum in critical cell culture studies. Many efforts have been made to produce serum-free or defined media. A number of successes have resulted for a limited span of cell types. The panel, consisting of representatives of three major cell culture media and reagent suppliers, will help us understand the problems and benefits associated with the use of serum in culture systems. They will also give us insights into what the future holds in the formulation of media and the selection of supplements.

Panelists:
David Jayme, *Invitrogen Corporation*
Bill B. Barnett, *HyClone Laboratories*
Gary Shipley, *Cascade Biologics, Inc.*

HIGH THROUGHPUT METHODS

Conveners:
Allan R. Wenck, Syngenta
Ebrahim Firoozabady, Del Monte Fresh Produce

10:30 am – 12:30 pm Plant Symposium .... Washington/Clark

We have entered an era where high quality sequence is being made available to researchers throughout the world. The rice, Arabidopsis, and soon other sequences will be known. As methods improve, more and more information will flow through the scientific databases. How do we deal with this sequence information? How can science make biological sense out of the genetic code and identify important areas of interest? How are we to identify and validate genes with potential impact on such things as yield, stress tolerance and pest resistance? Computer programs are in place to help sift

out potentially important sequences from the billions of base pairs into thousands or tens of thousands of genes. This subset of genes needs to be expressed in a high throughput manner within biological systems generating thousands or tens of thousands of events for analysis. Both molecular and other characterization data must be collected in a high throughput manner in order to validate the predicted functions of these chosen sequences. This session will provide examples of how we can identify potential genes of interest, transform them in a high throughput manner, and characterize them in an equally high throughput manner.

10:30 Introduction (A. Wenck and E. Firoozabady)

10:45 P-1 High-throughput Methods for Determining Transgene Copy Number and Expression in Plants
Wen-Jin Yu, *Syngenta Biotechnology, Inc.*

11:15 P-2 A Maize "Whiskers" Transformation System
W. Paul Bullock, *Garst Seed Company*

11:45 P-3 High Throughput Plant Gene Function Analysis
Keith Davis, *Paradigm Genetics, Inc.*

IN VITRO TOOLS

Moderators: Bernadette Lourdes Plair, Cincinnati Zoo and Botanical Garden
Maureen M. M. Fitch, USDA/ARS

10:30 am – 12:30 pm
Plant Contributed Paper Session .... Clackamas

10:30 P-1000 Development of an In Vitro Tuberization System for Sweet Potato Micro-storage Root Formation
Latrice Crawford, *Tuskegee University, Marceline Egnin, and Anne Sama*

10:45 P-1001 Cryopreservation of Shoot Tips of the Endangered *Asimina tetramera* by Encapsulation-vitrification
Bernadette Lourdes Plair, *Cincinnati Zoo and Botanical Garden, and V. C. Pence*

11:00 P-1002 In Vitro Collecting and Establishment of Tissue Culture Lines of Three Endangered Florida Pawpaws
Valerie C. Pence, *Cincinnati Zoo and Botanical Garden, and S. M. Charls*

11:15 P-1003 Control of Bacterial Contamination in Large Scale Papaya Micropropagation
Maureen M. M. Fitch, *USDA/ARS, T. Leong, N. Saito, G. Yamamoto, A. Dela Cruz, A. Yeh, S. White, S. Maeda, S. Ferreira, and P. Moore*

11:30 P-1004 Factors Affecting In Vitro Establishment of Cocoyam (*Xanthosoma sagittifolium* L Schott)
Anne Eyango Sama, *Tuskegee University, M. Egnin, and S. Zok*

11:45 P-1005 Somaclonal Variation Detection and Chimerism in Somatic Embryo-derived Cocoa
Carlos Marcelino Rodriguez Lopez, *Reading University, M. J. Wilkinson, and A. C. Wetten*

12:00	P-1006	Encapsulation of Orchid-Phalaenopsis Shoot Tips for Storage and Exchange of Germplasm ***W. T. P. S. K. Senarath,*** *Chonbuk National University, Kui Jae Lee, and S. Rehman*
12:15	P-1007	A Novel Disposable Film Culture Vessel for Photoautotrophic Micropropagation of *Epidendrum* Orchid ***Giang Thi Thanh Dam,*** *Kagawa University, H. Watanabe, M. Ujike, Y. Kume, and M. Tanaka*

1:30 pm – 2:30 pm
Non-interactive Even Poster Authors will be present .... Grand Ballroom

Interactive Poster Authors listed below will be present during their sessions

IN VITRO TOOLS AND TECHNIQUES

Moderator: John W. Harbell, Institute for In Vitro Sciences, Inc.

1:30 pm – 2:30 pm
Joint Interactive Vertebrate/Toxicology Poster Session ...... Grand Ballroom

VT-2000	An Animal Origin Free Trypsin Alternative to Harvest Cells ***Lori L. Nestler***, *Invitrogen Corporation, E. K. Evege, J. A. McLaughlin, D. G. Munroe, T. C. Tan, K. E. Wagner, and B. Stiles*
VT-2001	A Filter for Trapping Metastatic Breast Cancer Cells ***Jessica L. Moore,*** *Washington State University, P. Elias, C. Davitt, A. Bandyopadhyay, S. Bose, S. Kalita, and H. Hosick*
VT-2002	Induction of a Zone of Cell Death in Multi-well Plates by Refeeding ***John W. Harbell,*** *Institute for In Vitro Sciences, Inc., H. Raabe, G. Moyer, G. Mun, and M. Clear*
VT-2003	Comparative Cytotoxcity of Three In Vitro Cell Viability Assays ***Ann M. Wright,*** *CiBA Vision/Novartis Company, and Mary Mowery-McKee*
VT-2004	Mycoplasma Testing Experience of an Academic Support Facility ***Margaret L. Smith,*** *Riggs Consulting, Lovella Cacho, and Phan Tu*
VT-2005	Use of a Self-assembling Pore for the Indtroduction of Impermeant Molecules through Mammalian Cell Membranes ***Lia H. Campbell,*** *Organ Recovery Systems, K. Sarver, K. Ratcliff, M. J. Taylor, J. Walsh, and K. G. M. Brockbank*
VT-2006	Enhanced Effectiveness of Non-viral Gene Transfer Using Electroporation ***Richard Heller,*** *University of South Florida, Richard Gilbert, Kathleen Merkler, and Loree Heller*

VT-2007 Confocal Imaging of Epidermal Growth Factor Peptide Binding Along the Stem Cell Compartment of Mammalian Colonic Crypt
***Bertrand A. Kaeffer**, Institut National Recherche, Lissia Pardini, and Alain Trubuil*

VT-2015 Protein Kinase C Disrupts the Formation of VA Gene Transcription Initiation Complex
***Calvin B. James**, Ohio University*

INSECT MIDGUT STEM CELL LINES AND INSECT CELL LINES FOR BIOCONTROL

Moderator: Guy Smagghe, Ghent University

1:30 pm – 2:30 pm
Interactive Invertebrate Poster Session .... Grand Ballroom

I-2001 Effect of Bt Proteins on the Viability of Selected Insect Cell Lines
***Cynthia L. Goodman**, USDA/ARS/BCIRL, H. Nabli, J. Baum, T. Malvar, B. Isaac, Y.-J. Lee, A. H. McIntosh, and S. J. Phipps*

I-2002 Insulin-like Peptides Stimulate Midgut Stem Cell Proliferation of Lepidopteran Larvae *In Vitro*
***Shintaro Goto,** Kobe University, Marcia J. Loeb, and Makio Takeda*

I-2003 Effects of Insect Hormone Actions, 20E and JH, on Midgut Stem Cells of Lepidoptera
***Guy Smagghe,** Ghent University, W. Vanhassel, C. Moeremans, K. Elsen, and M. Loeb*

I-2004 Who Controls Midgut Stem Cell Differentiation – the Stem Cell or the Environment?
***Raziel S. Hakim,** Howard University, M. Loeb, and J. Young*

STRESS BIOLOGY

Moderator: Lynn S. Dahleen, USDA/ARS

1:30 pm – 2:30 pm Interactive Plant Poster Session .... Grand Ballroom

P-2000 Transformation of Barley with Two Antifungal Genes
***Lynn S. Dahleen**, USDA/ARS, and M. Manoharan*

P-2001 Regeneration and Genetic Transformation of Durum Wheat
***M. Manoharan,** USDA/ARS, L. S. Dahleen, and P. B. Jauhar*

P-2002 Effects of UV-B on the Development of In Vitro Propagated African Violet Chimera (*S. ionantha*)
***Wai-Yei Leung,** Champlain Regional College Saint Lambert, P. Castillo-Ruiz, E. Belanger, and S. Taylor*

P-2003 Overexpression Antioxidant Gene in Tomato Increases Tolerance to Heat and Chilling Stress
Yueju Wang, *Oregon State University, Michael Wisniewski, Lailiang Cheng, Richard Meilan, Minggang Cui, and Leslie Fuchigami*

P-2004 Expression of the *Arabidopsis* CBF1 Gene in Poplar Confers Elevated Freezing Tolerance
Yongjian Chang, *Oregon State University, Jeffery S. Skinner, and Tony H.H. Chen*

PLENARY SESSION

Convener: William J. Smith, US Army Medical Research Institute of Chemical Defense

3:00 pm – 4:30 pm Plenary Session .... Multnomah/Clackamas

ARTistic Use of Nonhuman Primates: IVF to Cloning and Beyond

3:00 Introduction: William J. Smith, 2003 Congress Program Chair

Opening Remarks: Sandra L. Schneider, Research and Clinical Systems and President, Society for In Vitro Biology

PS-1 Plenary Speaker: ***Richard Stouffer****, Head of the Reproductive Sciences Division, Oregon Regional Primate Research Center*

5:00 pm – 9:00 pm
PLENARY RECEPTION AND DINNER .... Pittock Mansion and The World Forestry Center

Co-sponsored by Monsanto Company and Weyerhaeuser Company

Time	Monday, June 2	Location
7:00 am - 6:00 pm	Registration	Grand Ballroom Foyer
7:00 am - 8:00 am	Publication Committee Meeting	Overton Room
	Student Affairs Breakfast	Pettygrove Room
8:00 am -12:30 am	Growth Factors Animal Symposium	Timberline
8:00 am -10:00 am	Biopharmaceutical Manufacturing Animal Roundtable Session	Multnomah
	Biotechnology of Grapevine Improvement Plant Symposium	Washington/Clark
	Monocot Transformatiom Plant Contributed Paper Session	Clackamas
10:00 am -10:30 am	Coffee Break	Grand Ballroom
10:00 am - 3:00 pm	Exhibits and Posters	Grand Ballroom
10:30 pm - 12:30 pm	Epigenetic Toxicants Animal Symposium	Multnomah
	Forest Biotechnology Plant Symposium	Washington/Clark
1:30 pm - 2:30 pm	INTERACTIVE POSTER SESSIONS: Biotechnology *Plant* In Vitro Tools *Vertebrate Toxicology*	Grand Ballroom
3:00 pm - 5:00 pm	Dicot Transformation Plant Contributed Paper Session	Clackamas
	Growth Regulators Plant Symposium	Washington/Clark
	In Vitro Quantitation of Angiogenesis: A BD Biosciences Workshop Animal Workshop	Timberline
6:00 pm - 9:00 pm	Plant Business Meeting and Social	Clackamas/ Multnomah
	Vertebrate/Toxicology Business Meeting and Social	Washington
	Invertebrate Business Meeting and Social	Off Property

Monday, June 2

7:00 am – 6:00 pm Registration .... Grand Ballroom Foyer

GROWTH FACTORS IN GROWTH, REGENERATION, AND DIFFERENTIATION OF INVERTEBRATE CELLS

Conveners: Marcia J. Loeb, USDA
Raziel S. Hakim, Howard University

8:00 am – 12:30 pm Animal Symposium .... Timberline

Growth factors play a large role in regulating the growth, development, and differentiation of mammalian tissues. It is only recently that the roles of peptidic growth factors in the growth, development, and differentiation of invertebrates have been revealed. There are a very few known factors that are specific to development in insects and molluscs; most of the invertebrate organisms that have been studied in this respect are controlled by the same factors that regulate vertebrates. Their mechanisms of action for those invertebrates, such as Caenorhabditis elegans, that have been extensively characterized, are similar as well. Therefore, invertebrate tissues may serve as models for vertebrate organisms. In addition, invertebrate pest control agents whose mechanisms involve manipulation of growth factors will have to be studied in depth in order to avoid doing harm to the general environment where all animals have to live in harmony with each other.

8:00		Introduction (R. Hakim)
8:15	I-1	Co-culture of *Trypanosoma musculi* with Spleen-derived Adherent Fibroblasts and Macrophages Ensures Survival In Vitro ***Winston A. Anderson,*** *Howard University*
8:45	I-2	Lymnaea EGF, Roles in Neurite Outgrowth In Vitro and In Vivo ***Andrew G. M. Bulloch,*** *University of Calgary*
9:15	I-3	Growth-blocking Peptide Family and Its Multiple Effects on Insect and Vertebrate Cells ***Yoichi Hayakawa,*** *Hokkaido University*
9:45		Discussion

10:00 – 10:30 Invertebrate Session Coffee Break ..... Grand Ballroom

10:30	I-4	Regeneration of Walking Legs in the Fiddler Crab, *Uca pugilator*. The Role of Growth Factors ***Penny M. Hopkins,*** *PhD, University of Oklahoma*
11:00	I-5	Regulation of the Fate of Stem Cells from the Midgut of the Caterpillar, *Heliothis virescens* ***Marcia J. Loeb,*** *USDA*
11:30	I-6	Modulation of Growth Factor Signaling During *C. elegans* Vulva Development ***Nadeem Moghal,*** *California Institute of Technology*

BIOPHARMACEUTICAL MANUFACTURING: ADDRESSING THE NEEDS FOR STAFFING, FACILITIES, AND PROCESSES DEVELOPMENT

Moderators: Dennis A. Laska, Eli Lilly and Company
William J. Smith, U.S. Army Medical Research Institute of Chemical Defense

8:00 am – 10:00 am Animal Roundtable Session .... Mutlnomah

Current bottlenecks and future needs for state of the art biopharmaceutical manufacturing facilities coupled with shortages of highly trained technical and professional staff threaten to slow or even impede development and commercialization of greatly needed bio-molecules, vaccines, and therapeutics. This roundtable session will address in depth the current situation, pose scenarios for remediation, and project future trends in professional and technical training, process improvement and efficiency, as well as quality initiatives.

8:00		Introduction (D. Laska)
8:15	W-1	Academic Institutions Response to the Staffing Needs of the Biopharmaceutical Industry ***Kamal A. Rashid,*** *Biotechnology and Genomics* Research Center
8:45	W-2	Industrial Mammalian and Microbial Cell Culture System for Biopharmaceutical Manufacture of Therapeutics ***Sarad Parekh,*** *Dow AgroSciences, LLC*
9:15	W-3	Ways to Approach Process Development and Improve Production Efficiency in Mammalian Cell Culture ***Julia Cino,*** *New Brunswick Scientific Co., Inc.*
9:45		Questions and Discussion

BIOTECHNOLOGY OF GRAPEVINE IMPROVEMENT

Convener: Cecilia Zapata, Yoder Brothers, Inc.

8:00 am – 10:00 am Plant Symposium .... Washington/Clark

Techniques for genetic modification in grapevine (Vitis spp.) using somatic embryogenesis (SE) include transformation and in vitro selection. All successful examples of genetic transformation in grapevine have utilized embryogenic cells as targets for gene insertion and somatic embryos for recovery of transformed plants. Transgenics have been used to solve problems with disease and stress resistance as well as manipulation of qualitative traits. In vitro selection has also been used as an alternate method to select for disease resistance in grapevine. In the area of grape genomics, incredible advances have been made in the recent past, where an estimate of 44,928 grape (Vitis vinifera) ESTs have been produced. These code for approximately 18,500 grape genes, representing 2/3 of the grape genome. This symposium will present a talk on the uses of somatic embryogenesis and transformation for grapevine improvement. The session will include another presentation on in vitro selection to enhance disease resistance. It also includes a presentation on the grape genome project focusing on gene discovery.

Monday, June 2

8:00		Introduction (C. Zapata)
8:15	P-4	Applications of Somatic Embryogenesis and Transformation in Grapevine Improvement ***Dennis J. Gray***, *University of Florida*
8:45	P-5	Gene Discovery in Grapes: The Grape Genome Project ***Effie Ablett***, *Southern Cross University*
9:15	P-6	In Vitro Selection to Enhance Disease Resistance in Grapevine ***Jayasankar Subramanian***, *University of Guelph*

MONOCOT TRANSFORMATION

Moderators: Jane Vishnevetsky, The Volcani Center
Diaa F. Al-Abed, The University of Toledo

8:00 am – 10:00 am Plant Contributed Paper Session .... Clackamas

8:00	P-1008	Transformation and Regeneration of Wetland Monocot *Juncus accuminatus* Using Different Binary Vectors ***Li Chen***, Salem International University, R. Nandakumar, P. K. Lai, and S. M. D. Rogers
8:15	P-1009	An Efficient System for Biolistic Transformation and Plant Regeneration of Pearl Millet Using Spikelets Shaved from Immature Inflorescences ***Jason James Goldman,*** *University of Georgia, W. W. Hanna, G. Fleming, and P. Ozias-Akins*
8:30	P-1010	Alleviation of Wheat Allergenicity Using the Thioredoxin System ***Hyun-Kyung Kim,*** *University of California-Berkeley, M.-J. Cho, H. R. Jung, Y.-B. Kim, S. Morigasaki, J. H. Wong, P. G. Lemaux, and B. B. Buchanan*
8:45	P-1011	Transgenic *Spirodela*: A Unique, Low-risk, Plant Biotechnology System ***Avihai Perl,*** *Volcani Center, Marvin Edelman, Ron Vunsh, Jihong Li, Uri Hanania, Moshe Flaishman, and Jane Vishnevetsky*
9:00	P-1012	Expression of a Synthetic Avidin Gene in Maize for Control of Corn Rootworm (*Diabrotica* spp.) and Other Insect Pests ***Serena B. McCoy***, *Kansas State University, Mitsuhiro Ueda, Karl J. Kramer, Subbaratnam Muthukrishnan, and Harold N. Trick*
9:15	P-1013	Shoot Meristem: An Ideal Explant for *Zea mays* (L.) Transformation ***Diaa F. Al-Abed,*** *The University of Toledo, R. V. Sairam, and S. L. Goldman*
9:30	P-1014	Enhanced Fungal Tolerance in Transformed Banana (*Musa* spp. AAA cv. 'Grand Nain') Plants Regenerated Through Somatic Embryogenesis ***Jane Vishnevetsky,*** *The Volcani Center, Y. Cohen, M. A. Flaishman, and A. Perl*

10:00 am – 10:30 am Coffee Break .... Grand Ballroom

10:00 am – 3:00 pm Exhibits and Posters .... Grand Ballroom

EPIGENETIC TOXICANTS, ALTERED INTRA-CELLULAR SIGNALING AND MODULATED GAP JUNCTIONAL INTERCELLULAR COMMUNICATION

Convener: Alda Vidrich, University of Virginia Health System

10:30 am – 12:30 pm Animal Symposium .... Multnomah

Many chemicals capable of inducing multiple health risks do so without causing genetic mutations. The mechanisms whereby these chemicals cause disease apart from mutagenesis include cytotoxicity (either necrosis or apoptosis) and epigenetic toxicity. Via epigenetic toxic mechanisms chemicals can alter gene expression at the level of transcription, translation or post-translational events in a stem cell, a precursor cell or a terminally differentiated cell. This session will explore the role of intercellular signaling and communication in the cellular response to epigenetic toxicants as well as the mode by which epigenetic toxicants can alter the channels of intercellular communication.

10:30		Introduction (A. Vidrich)
10:45	VT-1	Gap Junctions, Homeostasis, and Epigenetic Toxicology ***Randall J. Ruch,*** *Medical College of Ohio*
11:15	VT-2	Integrated Signaling Effects of Epigenetic Toxicants ***Brad Upham,*** *Michigan State University*
11:45	VT-3	Stem Cells, Cell-cell Communication and Epigenetic Toxicants: Risk Assessment Implications ***James E. Trosko,*** *Michigan State University*

FOREST BIOTECHNOLOGY: SHOULD, COULD, AND WOOD

Conveners: Todd J. Jones, Weyerhaeuser Technology Center
Maud A. Hinchee, ArborGen

10:30 am – 12:30 pm Plant Symposium .... Washington/Clark

Researchers now have the tools to apply genetic engineering and biotechnology for the improvement of commercially important forest tree species. We are currently on the threshold of major advancements in our understanding of gene function in trees. The Poplar Genome Sequencing Project is the first full-scale sequencing of a forest tree species and it will provide the fundamental basis for functional tree genomics. This project, which is nearing completion, will add thousands of tree genes to the molecular toolbox and provide new insights into what genes contribute to commercially valuable tree phenotypes. For some biochemical pathways, we already know which genes encode many of the important enzymes. One such pathway is the lignin biosynthetic pathway, and efforts to modify the quantity and quality of lignin have already begun. Deployment of transgenic trees will face potential challenges on the regulatory and public acceptance fronts. Trees present unique concerns, such as large scale and widespread pollen dispersal, along with perennial persistence in the environment. For instance, certain plantation tree species are grown in their native

habitat, and in these cases, the potential for gene flow of engineered traits from modified trees into native stands is a real possibility. Several approaches have been taken to mitigate gene flow. This session will present speakers who will address the potential and challenges associated applying biotechnology to tree improvement.

10:30 Introduction (T. Jones and M. Hinchee)

10:45 P-7 Gene Flow Control in Trees: Technology Development in Transgenic Poplars
Steven H. Strauss, *Oregon State University*

11:15 P-8 The *Populus* Genome: Development of the Information Resource
Gerald A. Tuskan, *Oak Ridge National Lab*

11:45 P-9 Genetic Engineering of Wood Formation in Forest Trees
Vincent L. Chiang, *North Carolina State University*

1:30 pm – 2:30 pm
Non-interactive Odd Poster Authors will be present .... Grand Ballroom

Interactive Poster Authors listed below will be present during their sessions

BIOTECHNOLOGY

Moderator: Nancy A. Reichert, Mississippi State University

1:30 pm – 2:30 pm Interactive Plant Poster Session .... Grand Ballroom

P-2005 Kanamycin Resistant Alfalfa Has a Point Mutation in the 16S Plastid rRNA
Pierluigi Barone, *University of Perugia, Italy, D. Rosellini, P. LaFayette, F. Veronesi, and W. A. Parrott*

P-2006 An Approach for Fiber Improvement in Kenaf Using a Gibberellin Oxidase Gene
Margaret M. Young, *Mississippi State University, and N. A. Reichert*

P-2007 A Poplar Promoter Functions in Potato and is Induced by Wounding and Fungal Infection
Dmytro P. Yevtushenko, *University of Victoria, Rafael Romero, William W. Kay, and Santosh Misra*

P-2008 Screening *Arabidopsis thaliana* and *Chlamydomonas reinhardtii* for Their Phenotypic Response to 2,4,6-trinitrotoluene (TNT)
Nrupali Patel, *University of Tennessee at Knoxville, and C. Neal Stewart, Jr.*

P-2009 DNA Fingerprinting of Orchardgrass (*Dactylis glomerata* L.) cv. Persist for Plant Variety Protection
Judith K. McDaniel, *University of Tennessee, and B. V. Conger*

P-2009A Reactivation of a Disabled Virus Vector by the Utilization of an Inducible Recombination System in Tobacco Plants
Arianne Tremblay, *Federal Government of Canada, C. Beauchemin, V. Bougie, A. Séguin, and J.-F. Laliberté*

IN VITRO TOOLS/EVALUATION OF TOXICITY

Moderator: Bobbie L. Thompson, HyClone Laboratories

1:30 pm – 2:30 pm
Joint Interactive Vertebrate/Toxicology Poster Session ...... Grand Ballroom

VT-2008 Design and Phase Ia Results of a Validation Study to Evaluate In Vitro Cytotoxicity Assays for Predicting Rodent and Human Acute Systemic Toxicity
Michael W. Paris, *NIEHS, J. A. Strickland, W. S. Stokes, S. Casati, A. P. Worth, H. Raabe, C. Cao, R. Clothier, J. Harbell, R. Curren, J. Haseman, R. R. Tice, M. L.* Wenk, M. K. Vallant, G. Mun, M. Clear, G. O. Moyer, J. Madren-Whalley, C. Krishna, M. Owen, and N. Bourne

VT-2009 The EpiOcular Prediction Model: In Vivo Versus In Vitro Draize Scores for Consumer Products
George L. DeGeorge, *MB Research Laboratories, M. Klausner, M. Osborn, K. Bellavance, B. Breyfogle, J. Kubilus, and D. R. Cerven*

VT-2010 Enhanced Phototoxicity Assay in Reconstituted Skin (EPARS)
George L. DeGeorge, *MB Research Laboratories, A. C. Gilotti, T. L. Ripper, T. L. Fox, L. Wagner, L. Lewis, S. H. Young, C. A. Kirk, M. K. Reeder, and B. Jones*

VT-2011 Serum Alternatives to the Use of Fetal Bovine Serum in Cell Culture
Bobbie L. Thompson, *HyClone Laboratories, B. Fujimoto, and B. Barnett*

VT-2012 Toxic Effects of Organic and Inorganic Forms of Selenium in Murine Erytholeukemia and Human Prostate Cancer Cells
Shyamal K. Majumdar, *Lafayette College, S. A. Satuh, J. H. Tchaicha-Pavlic, and E. A. Lucisano*

VT-2013 Metalloprotease Inhibitors, Non-microbial Chemically Modified Tetracyclines and Ilomastat, Block Anthrax, Lethal Factor Activity In Vitro
Salih Silay Kocer*, State University of New York at Stony Brook, and Sanford Simon*

VT-2014 The Effects of Glutamine and Ammonia Concentrations on CHO Cells in Serum-free Media
Paula N. Decaria, *HyClone Laboratories, Inc., J. Camire, and B. Barnett*

Monday, June 2

DICOT TRANSFORMATION

Moderator: Y. Judy Zhu, Hawaii Agriculture Research Center
Zhiwu Li, Kansas State University

3:00 pm – 5:00 pm Plant Contributed Paper Session .... Clackamas

3:00 P-1015 Heterogeneity in Terpenoid Gene Expression in Transformed Roots of *Artemisia annua* L. Grown in Bioreactors
Pamela J. Weathers, *Worcester Polytechnic Institute, F. F. Souret, Y. J. Kim, B. E. Wyslouzil, and K. K. Wobbe*

3:15 P-1016 Utilization of RNA Interference to Confer Resistance to the Soybean Cyst Nematode, *Heterodera glycines*
Ryan Matthew Steeves, *Kansas State University, Timothy C. Todd, and Harold N. Trick*

3:30 P-1017 Genetic Transformation and Plant Regeneration in *Stevia rebaudiana* Using Microprojectile Bombardment
Kaye M. Knowles, *Fort Valley State University, Seema Dhir, M. Singh, and Sarwan Dhir*

3:45 P-1018 Shoot Formation of *Agrobacterium* Co-cultivated Tissues of Teak
Sri N. Widiyanto, *Institut Teknologi Bandung, H. Rahmania, and S. Suhandono*

4:00 P-1019 Towards a Mucosal Vaccine Against HIV
Nobuyuki Matoba, *Arizona State University, M. Bomsel, C. J. Arntzen, and T. S. Mor*

4:15 P-1020 Metabolic Engineering Phytoalexins from the *Vitaceae* Improves Antifungal Activity in Tropical Plants
Y. Judy Zhu, *Hawaii Agriculture Research Center, C. S. Tang, and P. H. Moore*

4:30 P-1021 A Novel Protocol for Regeneration of Soybean from Mature and Immature Cotyledon; Suitable for Genetic Transformation
Gregory Franklin, *University of Toledo, E. Davis, S. Ismail, L. Carpenter, A. Hampton, B. Smith, S. Ibeji, J. McDougall, B. Sadia, M. Parani, S. L. Goldman, and R. V. Sairam*

GROWTH REGULATORS

Convener: David D. Songstad, Monsanto Company

3:00 pm – 5:00 pm Plant Symposium .... Washington/Clark

The influence of growth regulators on plant tissue culture and biotechnology is profound. The routine manipulation of cells in vitro was possible only through the use of specific plant growth regulators to promote either undifferentiated cell division or the formation of plants via organogenesis or embryogenesis. In this symposium, the speakers will cover three plant growth regulators, abscisic acid, gibberellins and ethylene, as they relate to in vitro culture and regenerated plants.

3:00		Introduction (D. Songstad)
3:15	P-10	Persistent Abscisic Acid Analogs ***Sue R. Abrams,*** *Plant Biotechnology Institute at National Research Council of Canada*
3:45	P-11	Gibberellins, Their Effects on and Roles in Growth and Differentiation of *In Vitro* Cultured Plant Tissues, Organs, and Somatic Embryos ***Richard Persons Pharis,*** *University of Calgary*
4:15	P-12	Ethylene Based Opportunities in Horticultural Biotechnology ***Franzine D. Smith,*** *The Scotts Company*

IN VITRO QUANTITATION OF ANGIOGENESIS: A BD BIOSCIENCES WORKSHOP

Convener: Steve Ilsely, BD Biosciences Discovery Labware

3:00 pm – 5:00 pm Animal Workshop .... Timberline

Angiogenesis, the formation of new blood vessels, is essential for normal growth and homeostasis. Certain disease states can be exacerbated by the loss of tight control of angiogenesis, which results in either excessive or insufficient blood vessel formation. The modulation of angiogenesis as a therapeutic strategy is a rapidly expanding field for the drug discovery and research scientist. Several *in vitro* assays have been developed to identify potential therapeutic molecules and to understand the mechanisms of angiogenesis. However, because most of these assays are cumbersome, laborious, poorly quantitative, and lack standardization, rapid progress in screening for effective therapeutic agents has been hampered. BD Biosciences Discovery Labware has developed a portfolio of products designed to provide the scientist easy, quick, robust, reproducible, standardized and readily available assay platforms for quantifying the effects of angiogenesis modulating compounds. These assay products recapitulate one or more of the neoangiogenic processes such as endothelial cell migration, invasion, and differentiation into vessels. The use of these products for the *in vitro* quantitation of angiogenesis will be presented.

3:00 Introduction (S. Ilsley)

Panelists:
Stephen Ilsley, *BD Biosciences Discovery Labware*
Min Wu, *BD Biosciences Discovery Labware*
James Maliakal, *BD Biosciences Discovery Labware*

Tuesday, June 3

Time	Tuesday, June 3	Location
6:00 am - 7:30 am	Fun Run/Walk	Grand Ballroom Foyer
7:00 am - 5:30 pm	Registration	Grand Ballroom Foyer
7:00 am - 8:00 am	Development Committee Meeting	Pettygrove Room
	Membership Committee Meeting	Overton Room
8:00 am -10:00 am	Bioreactors and Biopharming Plant Symposium	Washington/Clark
	Countering Chemical and Biological Threats: Current Research Animal Symposium	Multnomah
10:00 am -10:30 am	Coffee Break	Grand Ballroom
10:00 am - 3:00 pm	Exhibits and Posters	Grand Ballroom
10:30 pm - 12:30 pm	Advances in Cancer Modeling and Treatment Animal Symposium	Multnomah
	Disease and Pest: Pathways to Resistance Plant Symposium	Washington/Clark
10:30 pm - 12:45 pm	Plant Tissue Culture and Micropropagation Plant Contributed Paper Session	Clackamas
12:30 pm - 1:30 pm	2004 Program Committee Meeting	Pettygrove Room
1:30 pm - 3:00 pm	Cell Models and Cellular Differentiation Joint Vertebrate/Toxicology Contributed Paper Session	Clackamas
2:00 pm - 3:00 pm	Poster Breakdown and Removal City of Roses Silent Auction Final Bidding	Grand Ballroom Grand Ballroom Foyer
3:00 pm —5:00 pm	Delivery of Genes to Mammalian Cells with Baculoviruses Animal Symposium	Multnomah
	Predictive Toxicology Animal Roundtable Session	Timberline
	Transgenic Cereals Plant Symposium	Washington/Clark
5:00 pm - 6:00 pm	SIVB Business Meeting	Multnomah
7:00 pm - 8:00 pm	Reception Announcement of City of Roses Silent Auction Winners	Grand Ballroom Foyer Grand Ballroom Foyer
8:00 pm - 10:00 pm	Closing Banquet	Grand Ballroom

TUESDAY, JUNE 3

7:00 am – 5:30 pm Registration .... Grand Ballroom Foyer

BIOREACTORS AND BIOPHARMING

Conveners: Marceline Egnin, Tuskeegee University
Mary Ann Lila, University of Illinois

8:00 am – 10:00 am Plant Symposium .... Washington/Clark

High value plant-derived pharmaceutical proteins promise to reduce human and animal suffering, and can be manufactured using a delivery and production system that is safe, cost-efficient, and convenient. Plants can be engineered to produce drugs for disease prevention or therapy, nutraceutical or health-protective compounds, or agents that may combat terrorism's threats. This session will overview the commercial outlook for molecular farming approaches, the use of plants as bioreactors for production of uniform, scaled-up quantities of active compounds, and the prospects for capitalizing on plants to produce agents that can intervene in the event of biological or chemical warfare.

8:00		Introduction (M. Egnin and M. Lila)
8:15	P-13	Molecular Farming: Current Products and Future Prospects ***Michael E. Horn,*** *ProdiGene*
8:45	P-14	Improving Nutraceuticals Through Tissue Culture ***Alison M. R. Ferrie,*** *Plant Biotechnology Institute*
9:15	P-1036	Chemical Warfare Countermeasures: Expression of Human Acetylcholinesterase in Plants ***Samuel Patrick Fletcher,*** *Arizona State University*
9:35	P-1037	Plant-based Production of a Subunit Mucosal Vaccine for Pneumonic Plague ***Bonnie Jean Woffenden,*** *Virginia Tech*

COUNTERING CHEMICAL AND BIOLOGICAL THREATS: CURRENT RESEARCH

Convener: Elizabeth J. Roemer, State University of New York – Stony Brook

8:00 am – 10:00 am Animal Symposium .... Multnomah

Continuing concern about the threat of chemical and biological agents both on the battlefield and as tools of terror has lead to increased research in these arenas. Multiple agents ranging from organisms such as anthrax and small pox; potent toxins including botulinum toxin and aflotoxin, and chemicals such as ricin, sulfur mustard, and phosgene are all considered to pose potential dangers. Research is underway in a variety of venues, both civilian and military, to develop better understanding of the mechanism of action of these and others. Today's session will present three talks on current, ongoing projects in the area of chemical and biological threats.

8:00 Introduction (E. Roemer)

8:15	VT-4	Ex Vivo Utilization of Dendritic Cells to Identify Vaccine Targets for Control of Infectious Biothreat Agents ***Kamal U. Saikh,*** *US Army Medical Research Institute of Infectious Diseases (USAMRIID)*
8:45	VT-5	Evaluating Potential Inhibitors of Anthrax Lethal Factor Protein ***Sanford R. Simon,*** *State University of New York – Stony Brook*
9:15	VT-6	Morphological Expression of Mustard Gas-induced Lesions In Vivo and In Vitro ***John P. Petrali,*** *US Army Medical Research Institute of Chemical Defense (USAMRICD)*

10:00 am – 10:30 am Coffee Break .... Grand Ballroom

10:00 am – 2:00 pm Exhibits and Posters .... Grand Ballroom

ADVANCES IN CANCER MODELING AND TREATMENT

Conveners: Richard Heller, University of South Florida
Paul J. Price, GIBCO Invitrogen

10:30 am – 12:30 pm Animal Symposium .... Multnomah

The first decade of this century may very well be the turning point in our diagnosis and treatment of cancer. Cancer models are allowing us to understand what changes are occurring when a normal cell becomes a cancer cell and how different approaches may signal out the cancer cell for destruction. It is the understanding of unique or up-regulated pathways in the cancer cell that is allowing for targeted therapy. The knowledge of up-regulation of specific genes yielding up-regulated proteins and peptides is allowing for the priming of the cytotoxic "T" to seek and destroy the tumor cells. Cancer immunotherapy is predicted to become a significant adjunct to other areas of cancer treatment. The three speakers will cover these new and exciting approaches to cancer treatment.

10:30		Introduction (R. Heller and P. Price)
10:45	VT-7	STI571: A Tyrosine Kinase Inhibitor for the Treatment of CML - Validating the Promise of Molecularly Targeted Therapy ***Michael W. N. Deininger,*** *Oregon Health and Science University*
11:15	VT-8	Dendritic Cell Immunotherapy of Cancer ***Reiner Laus,*** *Dendreon Corporation*
11:45	VT-9	Identification of Casual Genetic Alterations in Human Breast Cancer Using New Model Cell Lines and Xenografts ***Stephen P. Ethier,*** *University of Michigan Cancer Center*

DISEASE AND PEST: PATHWAYS TO RESISTANCE

Convener: Heidi F. Kaeppler, University of Wisconsin

10:30 am – 12:30 pm Plant Symposium .... Washington/Clark

Pathogens and insects are the major causes of crop quality and yield losses worldwide. Breeding efforts aimed at enhancing resistance to diseases and pests have been successful in many cases. Continued research aimed at improving resistance is needed, however, because current levels of resistance are inadequate, the resistance is of a nondurable form and/or sources of adequate resistance are unavailable within the crop species germplasm. Expression of simple antifungal proteins in transgenic plants has resulted in mixed effects on resistance, and has led to investigation of more complex transgenic strategies for enhancing resistance. Detailed characterization of plant defense genes and regulatory cascades should result in improved design of genetic resistance strategies, both for breeding and transgenic approaches toward enhancing resistance. The speakers in this symposium will discuss findings from investigations of plant defense products and pathways, and how results can be used for enhancing plant resistance to pathogens and pests.

10:30		Introduction (H. Kaeppler)
10:45	P-15	The XA21 Receptor Kinase Mediated Defense Response in Rice ***Pamela C. Ronald,*** *University of California – Davis*
11:15	P-16	Lipid Signaling in Plant Defense ***Jyoti Shah***, *Kansas State University*
11:45	P-17	The Interface Between Bacterial Pathogens and Plants: Virulence Functions and Resistance Responses ***David Mackey,*** *The Ohio State University*

PLANT TISSUE CULTURE AND MICROPROPAGATION

Moderator: Baochun Li, University of Kentucky
M. Manoharan, USDA/ARS

10:30 am – 12:45 pm
Plant Contributed Paper Session .... Clackamas

10:30	P-1022	Shoot Organogenesis in *Nicotiana* Species: Shoot Production per Responsive Leaf Explant Increases Exponentially with Explant Organogenic Potential ***Baochun Li,*** *University of Kentucky, W. (Q. W.) Huwang, and T. Bass*
10:45	P-1023	Micropropagation of *Cordyceps sinensis* (Berk) Sacc., a High Value Medicinal Fungus Wildly Growing in Himalayan Region ***Narendra Kumar,*** *Government of India – Ministry of Defence, and P. S. Negi*

11:00	P-1024	Micropropagation Saves the Endangered *Musa* Germplasm in the FSM ***Hattie Andrew,*** *Micronesia Plant Propagation Research Center, P. C. Josekutty, N. H. Nena, R. A. George, T. N. Kilafwasru, and S. S. Cornelius*
11:15	P-1025	Micropropagation and Field Performance Evaluation of Eight Micronesian Bananas ***Puthiyaparambil Chacko Josekutty,*** *Micronesia Plant Propagation Research Center*
11:30	P-1026	*In Vitro* Initiation of *Artocarpus heterophyllys* Lam. (Jak Fruit) – Effect of the Explant Type and the Season of Explant Collection ***W. T. P. S. K. Senarath,*** *Chonbuk National University, K. A. H. K. Kasturiarachchi, and Kui Jae Lee*
11:45	P-1027	Shoot Tip Culture: A Powerful Model System for In Vitro Flowering and Transformation Studies in Maize (*Zea mays* L.) ***Swati Bhargava,*** *G. B. Pant University of Agriculture and Technology, Alok Shukla, and R. C. Pant*
12:00	P-1028	Micropropagation of *Papuacalia versteegii*, an Important Endemic Plant to Mount Jaya ***Erly Marwani,*** *Institute of Technology Bandung, and W. Sarosa*
12:15	P-1029	Somatic Embryogenesis from Ovules of Kinnow (*Citrus nobilis* X *C. deliciosa*) for Elimination of ICRSV ***Gita Rani,*** *Guru Nanak Dev University, B. Singh, S. Sharma, A. A. Zaidi, V. Hallan, A. Nagpal, and G. S. Virk*
12:30	P-1040	A Comparative Account of the Studies on Embryogenesis and Organogenesis in Various Cultivars of Cotton (*Gossypium spp.*) ***Tanveer Khan,*** *G. B. Pant University of Agriculture and Technology, and R. C. Pant*

CELL MODELS AND CELLULAR DIFFERENTIATION

Moderator: Kim C. O'Connor, Tulane University

1:30 pm – 3:00 pm
Joint Vertebrate/Toxicology Contributed Paper Session .... Clackamas

1:30	VT-1000	Differentiation Kinetics of In Vitro 3T3-L1 Preadipocyte Cultures ***Kim C. O'Connor,*** *Tulane University, H. Song, K. D. Papadopoulos, and D. A. Jansen*
1:45	VT-1001	Characterization of I-11.15, an Immortalized Murine Splenic Macrophage Cell Line ***Aicha Delafoulhouse,*** *California State University at Fullerton, C. Calumpong, and D. B. Drath*
2:00	VT-1002	Improving the Embryonic Stem Cell Test (EST) by Establishing Molecular Endpoints of Tissue-specific Development ***Roland Buesen,*** *Federal Institute for Risk Assessment, A. Seiler, A. Visan, B. Slawik, E. Genschow, and H. Spielmann*

2:15 VT-1003 Growth and avb3 Expression In OPC1 Cells Grown on Thermanox® Plastic and Collagen Substrates
Jessica Lee Moore, *Washington State University, C. Davitt and H. Hosick*

2:30 VT-1004 The Extracellular Matrix Laminin, Fibronectin, and Collagen IV in Green Sea Turtle Gonadal Cell Cultures
Anggraini Barlian, *Institut Teknologi Bandung, S. Sudarwati, L. A. Sutasurya, and H. Hayashi*

1:30 pm – 2:00 pm
All Poster Authors will be present .... Grand Ballroom

2:00 pm – 3:00 pm
Poster Breakdown and Removal .... Grand Ballroom

2:00 pm
City of Roses Silent Auction Final Bidding .... Grand Ballroom Foyer

DELIVERY OF GENES TO MAMMALIAN CELLS WITH BACULOVIRUSES

Convener: Guido F. Caputo, Natural Resources Canada

3:00 pm – 5:00 pm Animal Symposium .... Multnomah

3:00 Introduction (G. Caputo)

3:15 I-7 Isolation and Characterization of Transgenic Insect Cell Lines with Humanized Glycoprotein Processing Pathways
Donald L. Jarvis, *University of Wyoming*

3:45 I-8 Recombinant Baculoviruses as Mammalian Cell Gene-delivery Vectors
Tom A. Kost, *GlaxoSmithKline*

4:15 I-9 Enhancing the Sensitivity of Rainbow Trout Cells in Culture to the Toxicity of Metals
Vivian Rashida Dayeh, *University of Waterloo*

PREDICTIVE TOXICOLOGY

Moderator: John W. Harbell, Institute for In Vitro Science, Inc.

3:00 pm – 5:00 pm Animal Roundtable Session .... Timberline

Traditionally, application of in vitro methods to drug discovery or toxicology often focused on an understanding of the mechanisms underlying the changes in target tissues or organs occurring in vivo. Increasingly though, in vitro methods are being applied prospectively to predict the action of a chemical on the target tissue or whole organism. There are several forces driving the increased emphasis on in vitro cell and tissue based models and accompanying test procedures. One primary force has been the need to support the high through put screens to address the chemical libraries developed from combinatorial chemistry. The large number of potential active compounds and the

small quantity of each chemical have precluded most in vivo assessments. Concerns about extrapolation across species have also increased the focus on human cell systems, especially in organ-specific and metabolism studies. In vivo, absorption, distribution, metabolism and excretion (ADME) are measured to determine the effective dose and duration of exposure to the parent compound and any active metabolites. To predict the potential action of the chemical in vitro, the ADME factors must be addressed along with the action on the target tissue(s). This symposium will focus on the in vitro approaches to assessing intestinal absorption, hepatocyte metabolism (phase 1 bioactivation and phase 2 inactivation) as well as basal and organ-specific toxicity.

3:00		Introduction (J. Harbell)
3:15	W-4	The Caco-2 Assay: An In Vitro Models for Prediction of Intestinal Permeability ***Dennis A. Laska***, *Eli Lilly and Company*
3:45	W-5	Hepatocyte Systems for Predicting First-pass Metabolism and Bioavailability of Chemicals In Vitro ***Edward L. LeCluyse***, *University of North Carolina*
4:15	W-6	Model Systems for Cytotoxicity Screening and Predicting Target Organ Effects ***Charles A. Tyson***, *SRI International*

TRANSGENIC CEREALS

Conveners: Mark C. Jordan, Agriculture & Agri-Food Canada
Harold N. Trick, Kansas State University

3:00 pm – 5:00 pm Plant Symposium .... Washington/Clark

The present large-scale commercial production of transgenic maize is largely dependent on advances in transformation technology leading to high throughput transformation systems. The procedures and practices for selection of an appropriate transformation system in maize will ultimately be applied to other cereals such as wheat. The next bottleneck in cereals is the ability to isolate genes for important traits due to the large and complex genome. In spite of this, wheat is now coming into its own as a tool for functional genomics. Several international programs in wheat genomics have generated a wealth of sequence information and expression data. Transgenic wheat is poised to play a major role in the assignment of function to these sequences and the potential to develop gene tagging strategies in wheat will be discussed. After successful transformation and gene identification strategies are in place for wheat, there are still barriers to commercial production. There is no commercial production of transgenic wheat in the world today; however, there are products in advanced field trials that could be ready for release in 2004. Given potential impacts on international export markets as well as other issues, there has been pressure exerted on governmental agencies to prevent the release of transgenic wheat. Herbicide tolerant transgenic wheat has been bred and tested in the Pacific Northwest and we will hear from a wheat breeder who has worked with the product and can discuss the risks and benefits.

3:00		Introduction (M. Jordan and H. Trick)
3:15	P-17A	Gene Identification in Wheat ***Camille M. Steber***, *USDA-ARS, Washington State University*

3:45 P-1038 Statistical Analysis of Frequency and Quality of Transgenic Maize Production from Three Transformation Methods: *Agrobacterium*, Gunpowder Gun, Electric Gun
David D. Songstad, *Monsanto Company*

4:15 P-18 Risk Assessment of RoundUp® Ready Wheat Production in the Pacific Northwest
Kimberlee K. Kidwell, *Washington State University*

5:00 pm – 6:00 pm
SIVB Business Meeting .... Multnomah
(All Members are Urged to Attend)

7:00 pm – 8:00 pm
Reception .... Grand Ballroom Foyer

7:00 pm – 8:00 pm
Announcement of
City of Roses Silent Auction Winners .... Grand Ballroom Foyer

8:00 pm – 10:00 pm
Closing Banquet .... Grand Ballroom
Seating is Limited. Admittance to Banquet by Advance Ticket Holders Only.

Wednesday, June 4

Time	Wednesday, June 4	Location
7:00 am - 5:00 pm	Registration	Grand Ballroom Foyer
7:00 am - 8:00 am	Long Range Planning Committee Meeting	Pettygrove Room
8:00 am -10:00 am	Biomass Conversion for Fuels Plant Symposium	Washington/Clark
	Biotechnology Plant Contributed Paper Session	Multnomah
10:00 am -10:30 am	Coffee Break	Grand Ballroom
10:30 pm - 12:30 pm	Transgenes Blowing in the Wind? Plant Symposium	Washington/Clark
2:30 pm - 4:30 pm	Gene Silencing: Use for High Throughput Gene Validation and/or Functional Genomics Plant Symposium	Washington/Clark
	New and Developing Technologies for Micropropagation Plant Symposium	Multnomah

WEDNESDAY, JUNE 4

7:00 am – 5:00 pm Registration .... Grand Ballroom Foyer

BIOMASS CONVERSION FOR FUELS

Conveners: Michael E. Horn, Prodigene
Elizabeth E. Hood, Plant Biotechnologist

8:00 am – 10:00 am Plant Symposium .... Washington/Clark

Fossilized hydrocarbon-based energy sources, such as coal, petroleum and natural gas, provide a limited, non-renewable resource pool. Because of the worlds increasing population and increasing dependence on energy sources for electricity and heating, transportation fuels, and manufacturing processes, energy consumption is rising at an accelerating rate. Renewable resources, such as those derived from plants, make economic and environmental sense, and we should investigate and implement models for moving to these new energy sources. The goal is to derive 10% of our liquid fuels from renewable plant biomass by 2020, a 10-fold increase over todays production levels. This session will present current research in several areas affecting our ability to produce fuels from lignocellulosic materials and the impact those issues have on the economics of the process.

8:00		Introduction (M. Horn and E. Hood)
8:15	P-19	Transgenic Plant-produced Cellulases for Biomass Conversion ***Elizabeth E. Hood**, Plant Biotechnologist*
8:45	P-20	The Impact of Feedstock Composition on Biomass Conversion Process Economics ***Steven R. Thomas**, National Renewable Energy Laboratory*
9:15	P-21	Economics and Opportunities for Improvements for Biological Conversion of Cellulosic Biomass to Ethanol ***Charles E. Wyman**, Dartmouth College*

BIOTECHNOLOGY

Moderator: Anton S. Callaway, North Carolina State University
Ryan Matthew Steeves, Kansas State University

8:00 am – 10:00 am Plant Contributed Paper Session .... Multnomah

8:00	P-1030	Interactions Between Post-transcriptional and Transcriptional Silencing Pathways in *Arabidopsis thaliana* ***Anton S Callaway**, North Carolina State University, William F. Thompson, George C. Allen, Lindsay D. Jones, and Dolores, A. Sowinski*
8:15	P-1031	A New Glyphosate Tolerance Strategy in Transgenic Crops ***Michael W. Lassner**, Verdia Inc., Daniel L. Siehl, Rebecca Gorton, Sean Bertain, Hyeon-Je Cho, Donglong Liu, James Wong, Nick Duck, and Linda A. Castle*

8:30 P-1032 Profiling of Differentially Expressed Genes in Yam (*Dioscorea rotundata*) During Post-harvest Storage
Sali Kone-Coulibaly, *Tuskegee University, M. Egnin, and G. He*

8:45 P-1033 Chromatin Structure of T-DNA Integration Sites in *Arabidopsis*
Kirk E. Francis, *North Carolina State University, and S. L. Spiker*

9:00 P-1034 SSR Markers as a Suitable Tool for Checking Recombination Events During Somatic Embryogenesis from Floral Explants in Grapes
Lucia Martinelli, *Instituo Agrario Provinciale, M. S. Grando, J. Zambanini, V. Poletti, E. Maffettone, and R. Marconi*

9:15 P-1035 Transgenic "Sweet Rice" Expressing a Thermostable Amylopullulanase in Seeds Leads to Starch Autohydrolysis and Production of Nutritionally-improved High-protein Flour
Su-May Yu, *Academia Sinica, C.-M. Chiang, F.-S. Yeh, and J.-F. Shaw*

10:00 am – 10:30 am Coffee Break .... Mount St. Helen's Foyer

TRANSGENES BLOWING IN THE WIND?

Convener: Wayne Parrott, University of Georgia

10:30 am – 12:30 pm Plant Symposium .... Washington/Clark

Pollen has been blowing in the wind ever since it first appeared on earth, facilitating gene flow among plants. With the advent of transgenic crops, gene flow is being viewed increasingly not as a natural phenomenon, but as something that must be avoided. Hence, gene flow and its control is playing a larger role in the regulatory approval of transgenic crops. This session will present a talk on measuring gene flow and determining its impact, another on the legal aspects of gene flow, and specific study of gene flow from transgenic grasses.

10:30 Introduction (W. Parrot)

10:45 P-22 Transgenic Turfgrasses and Issues with Gene Flow
Eric K. Nelson, *The Scotts Company*

11:15 P-23 Gene Flow from Transgenic Crops to Wild and Weedy Relatives: When is It a Problem?
Allison A. Snow, *Ohio State University*

11:45 P-24 Liability Issues Related to Transgene Flow in Agriculture
Drew L. Kershen, *University of Oklahoma College of Law*

GENE SILENCING: USE FOR HIGH THROUGHPUT GENE VALIDATION AND/OR FUNCTIONAL GENOMICS

Conveners: Peggy J. Ozias-Akins, University of Georgia
Dwight T. Tomes, Pioneer Hi-Bred International, Inc.
Theodore M. Klein, Pioneer/DuPont Crop Genetics

2:30 pm – 4:30 pm Plant Symposium .... Washington/Clark

Science magazine's "Breakthrough of the Year: New Roles for RNAs" (2002. Science 298:2296) highlights a rapidly emerging realization of the important and varied roles for small RNAs. Independent research in plants and animals has converged to reveal related mechanisms for phenomena variously known as RNA interference, quelling, co-suppression, or post-transcriptional gene silencing. The outcome, RNA-mediated gene silencing, is similar for all of these phenomena, and involves double-stranded RNA that is recognized by the host cell machinery as aberrant and is subsequently cleaved into smaller molecules. These smaller molecules continue to propagate degradation in a sequence-dependent manner. RNA silencing in the form of co-suppression was first recognized in plants, and the silencing of endogenous genes by homologous transgenes remains a useful tool for exploring gene function. More recently, RNA silencing also has been determined to play a role in plant development and defense. The use of RNA silencing as a tool for functional genomics and its participation in natural biological processes will be addressed in this symposium.

2:30		Introduction (P. Ozias-Akins, D. Tomes, and T. Klein)
2:45	P-25	Inhibition of Plant Gene Expression Technologies for Fun and Profit ***Gregory P. Pogue,*** *Large Scale Biology Corporation*
3:15	P-26	Use of Transiently Expressed RNAi to Dissect Signaling Pathways in Plants ***Tuan-Hua David Ho,*** *Washington University*
3:45	P-27	Plant MicroRNA's and Their Targets ***James Carrington,*** *Oregon State University*

NEW AND DEVELOPING TECHNOLOGIES FOR MICROPROPAGATION

Convener: Bruce A. Stermer, PhD

2:30 pm – 4:30 pm Plant Symposium .... Multnomah

Plant micropropagation by conventional technology is labor intensive and requires considerable inputs of space and materials. However, new technologies are being developed that offer significant improvements over conventional micropropagation. These emerging liquid culture systems can reduce the costs of labor and materials, lessen the time required for production and also result in increased product quality. Today's session will present talks on the application of liquid culture systems for increased efficiency and quality in plant product production.

2:30 Introduction (B. Stermer)

Wednesday, June 4

2:40	P-28	Somatic Embryo Development in Liquid Medium for Large-scale Propagation of Conifer Trees ***Pramod K. Gupta,*** *Weyerhaeuser Company*
3:10	P-29	Liquid Systems for Micropropagation, Storage, Shipping, and Acclimatization ***Jeffrey W. Adelberg****, Clemson University*
3:40	P-1039	Gibberellin Synthesis Inhibitors Improve Conifer Embryogenic Tissue Initiation ***Gerald S. Pullman,*** *Institute of Paper Science and Technology*
4:10	P-30	Alternative Media for the Micropropagation of Plants ***Kenneth C. Torres,*** *Phytotechnology Laboratories, L. Williams, N. Philman, and M. Kane*

Education and Invertebrate Posters

Saturday May 31	Sunday June 1	Monday June 2	Tuesday June 3
7:00 pm - 9:00 pm	10:00 am - 3:00 pm	10:00 am - 3:00 pm	10:00 am - 2:00 pm

Posters mounted Saturday, May 31, 3:00 pm - 6:00 pm.
Posters must be removed from Grand Ballroom by 3:00 pm, June 3

Authors will need to be present at their posters the following days and times

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7:30 pm - 8:30 pm	1:30 pm - 2:30 pm	1:30 pm - 2:30 pm	1:30 pm - 2:00 pm
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present

EDUCATION POSTERS

STUDENT POSTERS

E-2000 The Effect of Trace Metals on Bioluminescent Dinoflagellates
***Peter Douglas Clark**, St. Andrew's Episcopal School*

E-2001 Identification of Drug Resistant Loci in Breast Cancer Cells by RAPD-PCR Fingerprinting
***Raymond Mailhot**, The Altamont School, Kamleshwar P. Singh, Satya Narayan, and Deodutta Roy*

E-2002 Some Effects of Plant Growth Regulators on Mammalian Cell
***Kendra C. Cawley,** Portland Community College, R. Aleman, M. Blackledge, C. Campbell, J. Cohen, B. Lindsey, M. Kondapalli, J. Marsh-Haffner, N. Myint, and K. Taylor*

EDUCATOR POSTERS

E-2003 Plant Tissue Culture and Plant Conservation: In Vitro Methods for Introducing Concepts to the Classroom
***Bernadette Lourdes Plair,** Cincinnati Zoo and Botanical Garden, V. C. Pence, and S. M. Charls*

E-2004 Investigating the Use of Oil Degradation Microbes in the Secondary Science Classroom
***Carol Harrison,** Booker T. Washington High School and Tuskegee University*

INVERTEBRATE POSTER

SILENT ABSTRACT

I-2005 Ecological Monitoring of the Ai River (Ural, Russia) Under the Influence of Oil Spills
***Anton Ilyich Vagapov,** Municipal Ecological Lyceum of Chelyabinsk*

Plant Posters

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All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present

BIOTECHNOLOGY

P-2010 Positive Correlation Occurs Between Cytosine Methylation and Adventitious Shoot Induction in Petunia
Prakash P. Kumar, *National University of Singapore, A. P. Prakash, A. Kush, and P. Lakshmanan*

P-2011 Plant Transformation Center
Kenneth C. Sink, *Michigan State University*

P-2012 Aluminum Tolerance in Alfalfa with the Citrate Synthase Gene
Pierluigi Barone, *University of Georgia, D. Rosellini, J. H. Bouton, P. L. LaFayette, M. Sledge, F. Veronesi, and W. A. Parrott*

P-2013 A Non-antibiotic Marker for the Selection of Transformed Plants
Peter LaFayette, *University of Georgia, P. M. Kane, and W. A. Parrott*

P-2014 Co-ordinate Expression of β and δ Zeins in Transgenic Tobacco
Jennifer Randall, *New Mexico State University, Dennis Sutton, Soumitra Ghroshroy, Suman Bagga, and John D. Kemp*

P-2015 Characterizing the Plastid Ribosomal Region Among 25 Diverse Angiosperms: An Early Step Towards Universal Plastid Transformation Vectors for Plant Expression
James R. Wright, *Madigan Army Medical Center, and P. M. McNutt*

P-2016 DNA Methylation and Transgene Silencing in Wheat Transformed with the Wheat Streak Mosaic Virus Coat Protein Gene
Zhiwu Li, *University of Idaho, J. Hanson, R. S. Zemetra, and P. H. Berger*

P-2017 RAPD Analysis Concerning Genetic Variability of *Phytophthora infestans* in Potato
Constantin Botez, *University of Agricultural Sciences and Veterinary Medicine, D. Pamfil, M. Ardelean, R. Sestra, Elena Tăma, Katalin Kovacs, and J. Ekart*

P-2018 Expression of GFP Reporter Gene in *Arundo donax* Following Microprojectile Bombardment
M. Singh, *Fort Valley State University, K. Knowles and S. K. Dhir*

P-2070 Establishing Cryopreservation Methods for Conserving European Plant Germplasm
Erica E. Benson, *University of Abertay Dundee, B. Panis, J. Geuns, R. Swennen, K. Harding, D. Bremmer, P. Lynch A. Hargreaves, P. Bonner, S. Dussert, N. Chabrillange, C. Damiano, C. Forni, S. Beninati, L. Alessandro, P. Bruno, H. M. Schumacher, E. Heine-Dobbernack, H. Takagi, L. Maggioni, and F. Engel-mann*

IN VITRO TOOLS

P-2019 Cryopreserved Storage of Hops (*Humulus* L.) Germplasm
Barbara M. Reed, *USDA/ARS, Carolyn Paynter, Jeanine DeNoma, Jeff D'Achino, and Nese Okut*

P-2020 Elimination of Grapevine Virus A (GVA) by Cryopreservation of *In Vitro*-grown Shoot Tips of *Vitis vinifera* L.
Qiaochun Wang, *The Hebrew University of Jerusa-lem, M. Mawassi, P. Li, R. Gafny, I. Sela, and E. Tanne*

P-2021 *In Vitro* Selection of Somaclones Through Phosphate Starvation in Maize (*Zea mays* L.)
Anshu Miglani, *G. B. Pant University of Agriculture and Technology, Alok Shukla, and R. C. Pant*

P-2022 The Use of Clontech Coral Reef Proteins as Tools in Plant Transformation
Allan R. Wenck, *Syngenta, Celine Pugieux, Mark Turner, Martha Dunn, Cheryl Stacy, Annalisa Tiozzo, Erik Dunder, Emiel Van Grinsven, Rafiqul Kahn, Marina Sigareva, Wen Chung Wang, Janet Reed, Shanaaz Tayab, Paul Drayton, Duncan Oliver, Gaston Legris, Helen Rushton, Hugh Trafford, Karen Launis, Yin-Fu Chang, Dong-Fang Chen, and Leo Melchers*

P-2023 Growth Response of In Vitro Cultured *Campanula* Plantlets to Trophic Phase, Photosynthetic Photon Fluxes, and Temperature
Kim Gyeong Hee, *Gyeongsang National University, Nam Hee Choi, Mi Young Lim, Young Hoe Kim, and Byoung Ryong Jeong*

P-2024 Monitoring Phytohormone-induced Changes in Cotton Ovule Culture Gene Expression Using Real-time, Reverse-transcription Polymerase Chain Reaction
Barbara A. Triplett, *USDA/ARS, and H. J. Kim*

Plant Posters

TRANSFORMATION

P-2025 Accumulation Profiles of Lipid and Phenolic Antioxidant Compounds During a Growth Cycle of Suspended Cells of *Salvia officinalis* L.
Manuel Fernandes-Ferreira, *Universidade Do Minho, Paulo S. C. Braga, Paula C. Santos-Gomes,, Rosa M. Seabra, and Paula B. Andrade*

P-2026 Stable Transformation of *C. annuum* and *C. baccatum* Explants Inoculated with *A. rhizogenes* and *A. tumefaciens*
Luis L. Valera-Montero, New Mexico State University, and Gregory C. Phillips

P-2027 Herbicide Resistant Celery (*Apium graveolens* L.) Plants Produced by *Agrobacterium*-mediated Transformation Using the Bar Gene
Andrey V. Loskutov, *Michigan State University, and K. C. Sink*

P-2028 Transgenic Chrysanthemum (*Dendranthema grandiflora* cv. Subangyuk) with *Ls Like* Gene Expresses Branchlessness Habit
Su Young Lee, *National Horticultural Research Institute, B. H. Han, H. J. Yoo, H. K. Shin, I. G. Mok, E. J. Suh, and Y. P. Lim*

P-2029 Phloem-specific Expression of the β-glucuronidase Reporter Gene in Transgenic Strawberry (*Fragaria vesca* and *F. x ananassa*)
Qingzhong Liu, *USDA/ARS, Y. Zhao, and R. E. Davis*

P-2030 *Agrobacterium*-mediated Transformation and Plant Regeneration from Leaf Segments of Sweet Cherry Dwarf Rootstock 'Gisela 6' (*Prunus cerasus X P. canescens*)
Qingzhong Liu, *USDA/ARS, Y. Zhao, R. W. Hammond, H. Zhao, and R. E. Davis*

P-2031 Transformation of Rice with Bacterial Artificial Chromosomes (BAC) DNA
Bao H. Phan, *University of Georgia, C. Topp, C. Zhong, U. Akoh, J. Jiang, R. K. Dawe, and W. A. Parrott*

P-2032 *Agrobacterium*-mediated Genetic Transformation of Pigeon Pea with Hemagglutinin Nuraminidase (HN) Gene of *Peste des Petits Ruminants Virus* as a Source of Edible Vaccine
Valluri Venkata Satyavathi, *Indian Institute of Science, V. Prasad, K. J. M. Valli, K. Abha, M. S. Shaila, and G. Lakshmi Sita*

P-2033 *Vaccinium angustifolium* Cell Cultures: An Alternative Method of Studying the Anti-cancer Potential of Wild Blueberries
Tristan F. Burns Kraft, *University of Illinois, Chris Knight, Barbara Schmidt, Randy Rogers, David Seigler, and Mary Ann Lila*

P-2034 Differential Accumulation of Flavonoids in Cell Cultures of *Vitis vinifera*: Implications for Pharmacological Research
Sinee Pauline Kopsombut, *University of Illinois, K. A. Marley, R. A. Larson, and M. A. Lila*

P-2035 Transformation of Carnations with Flavonoid Biosynthesis Related Genes
Byung Joon Ahn, *Dankook University, K. H. Hwang, B. H. Min, and H. Y. Joung*

P-2036 Development of a Robust Tissue Culture System: Ideal for *Agrobacterium* Mediated Transformation in Sorghum
Bushra Sadia, *University of Toledo, W. Alaiwi, J. MacDougall. K. Meeker, B. Smith, S. Ibeji, G. Franklin, M. Parani, S. L. Goldman, and R. V. Sairam*

P-2037 Biolistic Transformation and Expression of Fertile Soybean Transgenics for Chitinase and Glucanase
Jung-Hoon Lee, *Kansas State University, William Schapaugh, Subbaratnam Muthukrishnan, and Harold N. Trick*

P-2038 Genetic Transformation of Chilli (*Capsicum annuum* L. var Pusa Jwala and California Wonder) via *Agrobacterium tumefaciens* with Coat Protein of Pepper Vine Binding Virus (PVBV)
K. J. Maragatha Vally, *Texas A&M University, and G. Lakshmi Sita*

P-2039 Using Leaf Disk for Guayule Transformation
Niu Dong, *USDA/ARS, and Katrina Cornish*

P-2040 Increased Transformation and Rooting Efficiencies in Canola (*Brassica napus* L.) Using *Agrobacterium* Mediated Transformation
Vinitha Cardoza, *University of Tennessee, and C. Neal Stewart, Jr.*

P-2041 Utilization of Glufosinate Selection and Cysteine in *Agrobacterium*-mediated Cotyledonary Node Transformation in Twelve Soybean Cultivars
Margie Margarita Mercado Paz, *Iowa State University, Z.-B. Guo, Z.-Y. Zhang, A. K. Banerjee, and K. Wang*

P-2042 Use of a Site Directed Recombination Strategy for Selectable Marker Removal in *Glycine max* (Soybean)
Joanne L. Ekena, *Monsanto Company, Michael Petersen, and Larry Gilbertson*

P-2043 Selection of Transgenic Papaya Seedlings Using Kanamycin and DMSO
Thomas W. Zimmerman, *University of the Virgin Islands, and Nina St. Brice*

P-2044 High-frequency Transformation of Undeveloped Plastids in Tobacco Suspension Cells
Camri L. Langbecker, *Monsanto, Guang-Ning Ye, Peter T. J. Hajdukiewicz, Charles W. Xu, Charles L. Armstrong, and Jeffrey M. Staub*

Plant Posters

P-2045 Somatic Embyrogenesis and Transformation in Alfalfa Using Gene Gun
Kaye Knowles, *Fort Valley State University, Seema Dhir, and S. K. Dhir*

PLANT TISSUE CULTURE AND MICROPROPAGATION

P-2046 Field Performance and Evaluation of Micropropagated FHIA Hybrid Banana Plants in the Marshall Islands
Dilip Nandwani, *College of the Marshall Islands, Arwan Soson, and Diane Myazoe*

P-2047 Development of Efficient Plant Regeneration Systems for *Agrobacterium*-mediated Transformation of Sour Cherry (*Prunus cerasus* L.)
Guo-qing Song, *Michigan State University, and K. C. Sink*

P-2048 Dispersal and Filtration of Embryogenic Callus Increases the Frequency of Embryo Maturation and Conversion for Hybrid Tea Roses
Kathryn K. Kamo, *UDSA, B. Jones, J. Castillon, and F. Smith*

P-2049 Effect of Growth Regulators on Callus Induction and Somatic Embryo Formation from Scutella Cultures of Durum Wheat
Valluri Venkata Satyavathi, *North Dakota State University, and Prem P. Jauhar*

P-2050 Induction of Somatic Embryogenesis in O'Henry Cultivar of Peach (*Prunus persica)*
Nirmal Joshee, *Fort Valley State University, A. K. Yadav, J. Subramanyan, and F. A. Hammerschlag*

P-2051 Effect of Stage II Duration on Rooting and Survival of Sea Oats (*Uniola paniculata* L.) Genotypes
Carmen Valero-Aracama, *University of Florida, M. E. Kane, S. B. Wilson, and N. L. Philman*

P-2052 Micropropagation of *Lavendula angustifolia* L. for Production of Essential Oils
Ana M. S. Vicente, *Universidade Do Minho, Paulo S. C. Braga, Maria José Vilaça-Silva, Gilda Ramos, Cecilia Araújo, and Manuel Fernandes-Ferreira*

P-2053 Germplasm Preservation of Fruit, Small Fruit, and Grape Cultures in Kazakhstan
Irina Y. Kovalchuk, *Kazakh Research Institute of Horticulture and Viticulture, and S. Kushnarenko*

P-2054 In Vitro Regeneration of the Himalayan Medicinal Plant *Lilium nepalense* (D. Don) via Shoot Organogenesis
Prakash Raj Malla, *Tribhuvan University, and Sajani Malla*

P-2055 Rooting in Cultures of Two Endangered Florida Pawpaws, *Asimina tetramera and Deeringothamnus Rugelii*
Susan M. Charls, *Cincinnati Zoo and Botanical Garden, J. R. Clark, and V. C. Pence*

P-2056 Regeneration of *Aloe arborescence* via Somatic Organogenesis from Young Inflorescences
M. Velcheva, *Volcani Center, Z. Faltin, A. Vardi, Y. Eshdat, and A. Perl*

P-2057 Induction of Somatic Embryogenesis in Ovaries of *Vitis vinifera* L.: Effect of the Developmental Stage and Growth Regulators
Hélia Guerra Cardoso, *Instituto de Ciência Aplicada e Tecnologia, A. Peixe, and M. S. Pais*

P-2058 High Frequency Somatic Embryogenesis from Leaf and Floral Explants of 'Chancellor' Grape
Richard Mulwa, *University of Illinois, M. A. Norton, and R. M. Skirvin*

P-2059 *In Vitro* Propagation and Phytochemical Production of Kava (*Piper methysticum*)
Hideka Kobayashi, *University of Illinois, M. C. Gawienowski, M. A. Lila, and D. P. Briskin*

P-2060 In Vitro Shoot Induction of *Pinus maximartinezii*
Elizabeth Cardenas, *Universidad Atónoma de Nuevo Leõn, M. C. Ojeda, and T. E. Torres*

P-2061 Approaches to Increase Embryogenic Culture Initiation and Cell Line Capture in Loblolly Pine
John Joseph Clark, *Arborgen, M. R. Becwar, M. K. Chowdhury, N. S. Nehra, M. R. Rutter, M. J. Cook, J. M Victor, T. J. Stout, A. M. Perry, P. J. Wade, and M. A. Hinchee*

P-2062 Plant Regeneration of *Arundo donax* L. Through Somatic Embryogenesis
M. Singh, *Fort Valley State University, D. Moore, K. Knowles, and S. K. Dhir*

P-2063 Development of Thin Cell Layer Culture System for Rapid and Direct Regeneration of Sugarcane and Other Monocot Species
Prakash Lakshmanan, *David North Plant Research Centre, R. J. Geijskes, A. R. Elliot, L. F. Wang, M. G. McKeon, R. S. Swain, Z. Borg, N. Berding, C. P. L. Grof, and G. R. Smith*

P-2064 Somatic Embryogenesis from Immature Embryos of *Phaseolus aureus*
Magfrat Muminova, *Institute of G&PEB, M. Nasretdinova, S. Djataev, and A. Abdukarimov*

P-2072 Cryopreservation Studies in *Carica papaya* – Effect of Some Cryoprotectants on Regrowth and Somatic Embryogenesis in Sunrise Solo Papaya
Sadanand A. Dhekney, *University of Florida, R. E. Litz, N. Joshee, and A. K. Yadav*

PLANT SILENT ABSTRACTS

P-2066 Somatic Embryogenesis from Callus Cultures of Teak (*Tectona grandis* L.f.) Derived from Leaf Explants
Luluk Yunaini, *Institut Teknologi Bandung, and S. N. Widiyanto*

Plant Posters

P-2067 Alternative Media for In Vitro Propagation of Fe'i Bananas
Flordeliza Briones Javier, *College of Micronesia – FSM*

P-2068 Saffron Micropropagation by Somatic Embryogenesis in a Temporary Immersion System
Silvia Blázquez Martin, *Instituto de Desarrollo Regional, Abel Piqueras, and José Antonio Fernández*

P-2069 Characterization of a Putative Rab-related Small GTP-binding Protein, *LeRab6* in Tomato (*Lycopersicon esculentum, Miller)*
Hyder Ali S. Khoja, *INP/ENSAT, J. Leclercq, A. Latché, J. C. Pech, and M. Bouzayen*

P-2071 Cellular Location of Oxidative Stress and Antioxidant Enzymatic Response in Hyperhydrated Carnation Plantlets
Abel Piqueras, *CEBAS (CSIC), S. Saher, E. Hellin, and E. Olmos*

Vertebrate and Toxicology Posters

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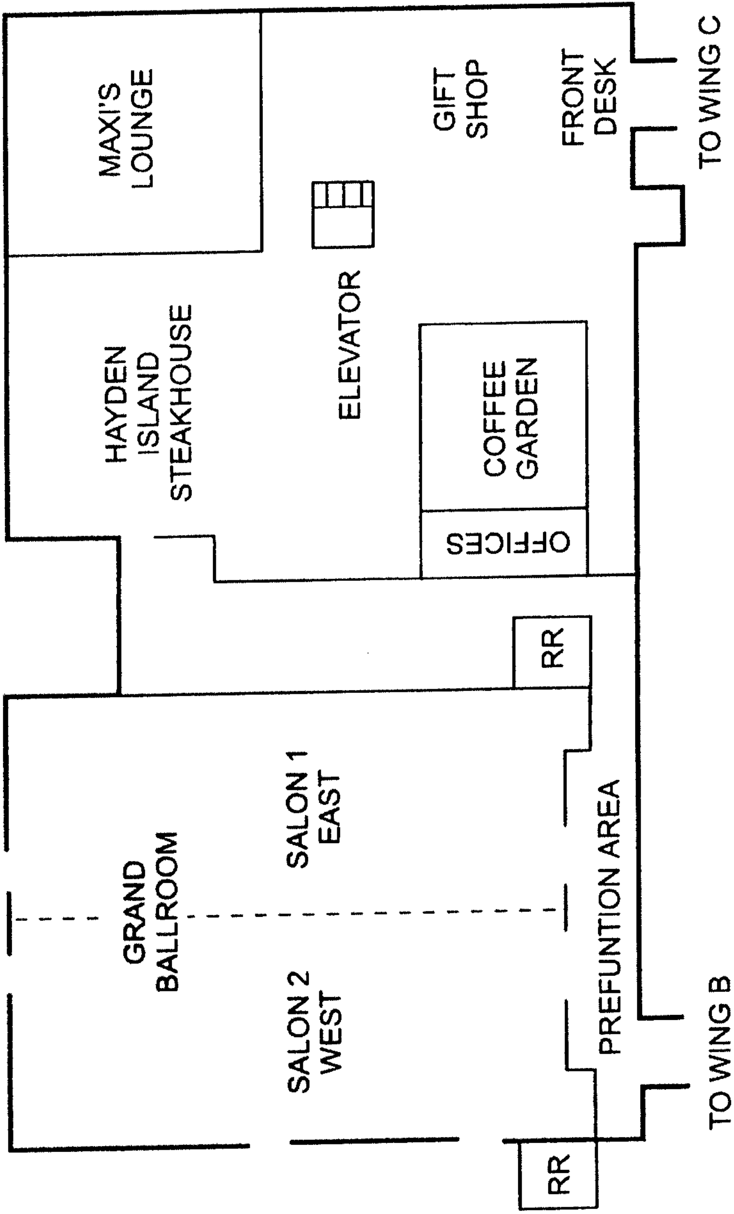
VERTEBRATE/TOXICOLOGY SILENT ABSTRACTS

VT-2016 A Non-animal Alternative Carcinogenicity Assay Using Fertilized Avian Eggs: The In Ovo Carcinogenicity Assay (IOCA)
George L. DeGeorge, *MB Research Laboratories, D. R. Cerven, M.J. Iatropoulos, G. M. Williams, C. Perrone, and H. Enzmann*

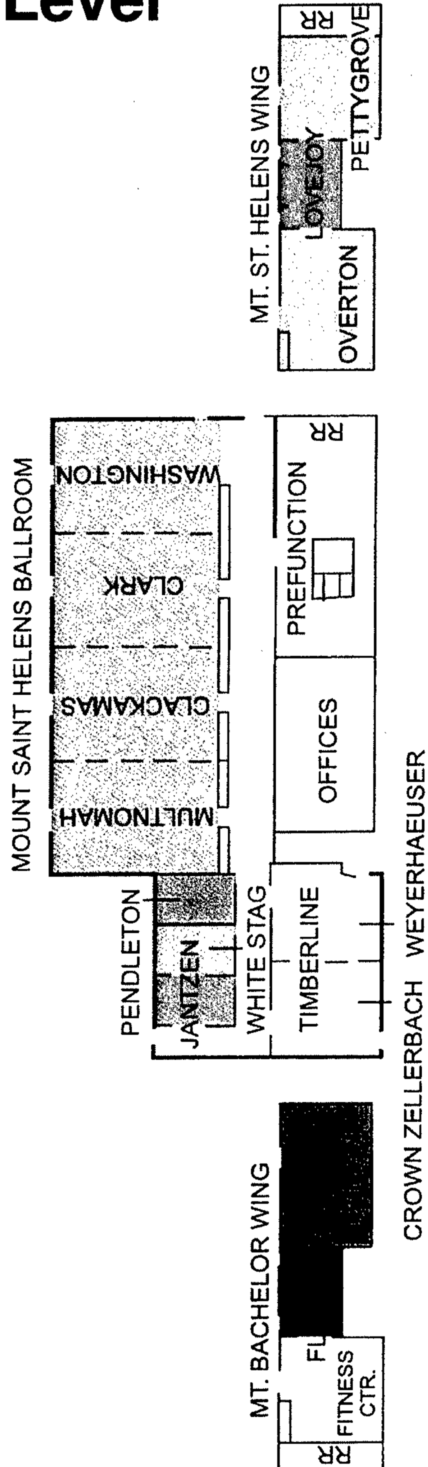
VT-2017 Development of an Alternative Test for Photoirritants: The In Ovo Phototoxicity Assay (IOPA)
George L. DeGeorge, *MB Research Laboratories, D. R. Cerven, T. L. Ripper, T. L. Fox, and S. H. Young*

VT-2018 In Vitro Models of Full-thickness Human Skin (EpiDerm-FT) and Airway Epithelium (EpiAirway-FT) for Toxicology and Drug Development Applications
Patrick J. Hayden, *MatTek Corporation, M. Klausner, J. Kubilus, B. Burnham, and G. R. Jackson*

Main Level



Lower Level



Program Changes and Notices

Changes to the program may occur on-site at the meeting. In an effort to alert attendees of these changes, a bulletin board will be located in the registration area with posted notices and changes as submitted by presenters.

PROGRAM

Tuesday, June 3, Plant Tissue Culture and Micropropagation
Session will run till 12:45 pm

SUPPORTERS

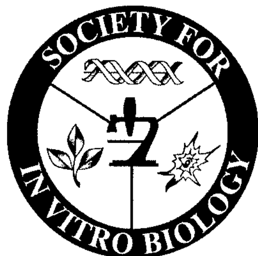
CA Dept. of Education, SSPG is a Congress Contributing Supporter

PLENARY RECEPTION AND DINNER

Monsanto Company and Weyerhaeuser Company are co-sponsoring the Plenary Reception and Dinner.

Don't forget to visit the

Society for In Vitro Biology's Booth Displays



Booth position 00

Society for In Vitro Biology
9315 Largo Drive West, Suite 255
Largo, MD 20774

Phone: (301) 324-50504
Fax (301) 324-5057
Email: sivb@sivb.org

The Society for In Vitro Biology is the successor to the Tissue Culture Association, founded in 1946. Its mission is to foster exchange of knowledge of the in vitro biology of cells, tissues, and organs from both plants and animals (including humans). The focus is on biological research, development, and applications of significance to science and society. The mission is accomplished through the Society's publications; national and local conferences; meetings; and workshops; and through support of teaching initiatives in cooperation with educational institutions.

Once you return home, don't forget to visit the SIVB website to receive special discounted rates on products and services for SIVB members only.

PUBLISHER'S BOOK DISPLAY

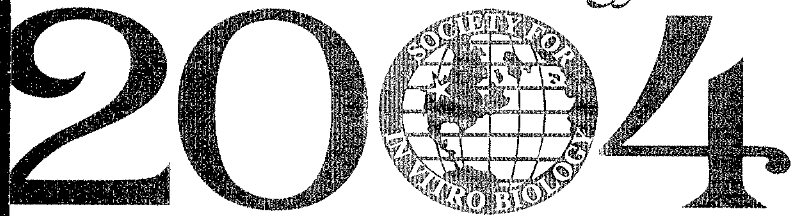
While at the meeting, be sure to drop by the Publishers' Book Display, located near the SIVB booth. A number of publishers will have books on display and many are available at a special price just for the meeting. On Tuesday, June 3, beginning at 12:30 pm, all books will be available for sale at 60% off the list price (first come, first served).

2004 WORLD CONGRESS REGISTRATION DISCOUNTS

The World Congress on In Vitro Biology, an international congress held every four years, focuses on issues pertinent to Plant, Vertebrate, Invertebrate, and Cellular Toxicology research and will give participants a unique learning experience on plant and animal cell culture and biotechnology. The fifth World Congress in 2004, co-sponsored by the Japanese Tissue Culture Society (JTCA), Japanese Association for Animal Cell Technology (JAACT), and Japanese Society of Plant Cell & Molecular Biology (JSPCMB) will be held May 22-26, 2004 in San Francisco, California. The World Congress theme, "Emerging Global Technologies," will attract scientific participation from many countries. A pre-registration rate will be offered to members and interested scientists who register for the World Congress before July 4, 2003. Additional information and registration forms will be available at the SIVB Booth.

Notes

WORLD CONGRESS
on In Vitro Biology



San Francisco, California ◆ May 22-26, 2004

EMERGING GLOBAL BIOTECHNOLOGIES

Don't forget to mark your calendar!

www.sivb.org

for the latest updates

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May 31 - June 4, 2003

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and Materiel Command

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