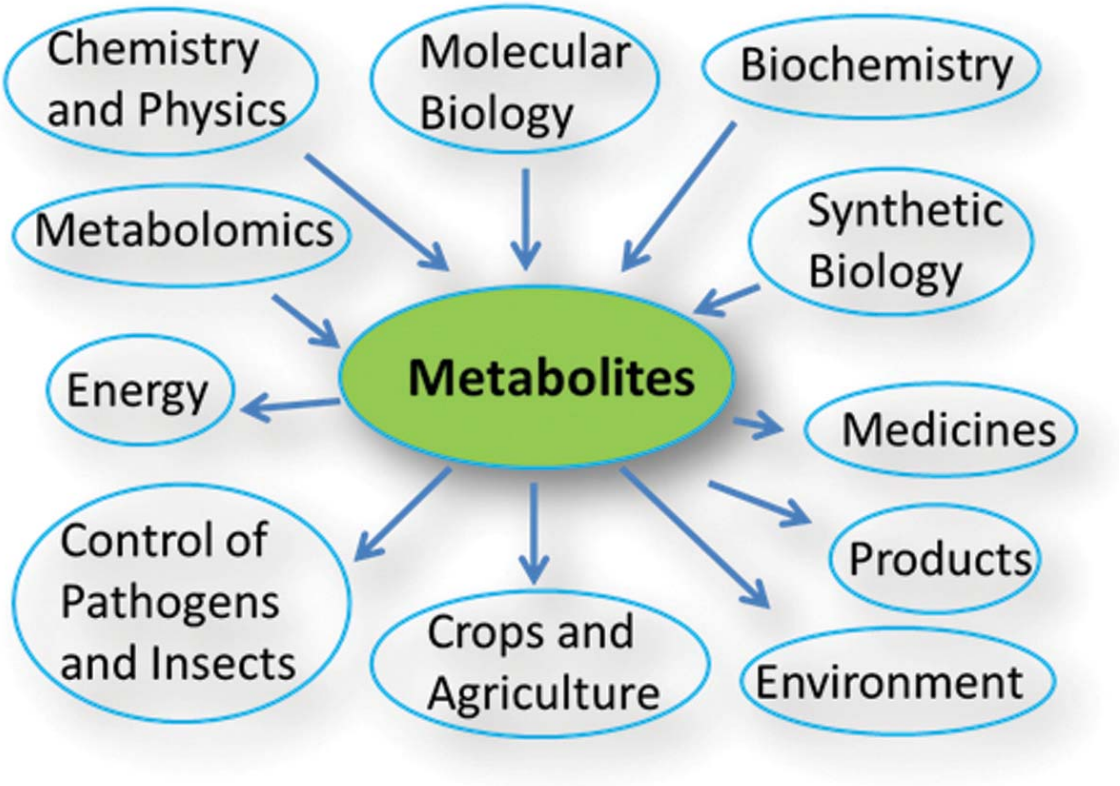
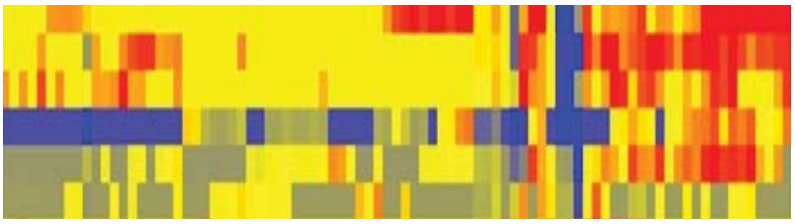
53rd Annual Meeting of the Phytochemical Society of North America

Aug. 9-13, 2014

McKimmon Conference and Training Center
North Carolina State University
Raleigh, NC 27695





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53rd Annual Meeting of the Phytochemical Society of North America

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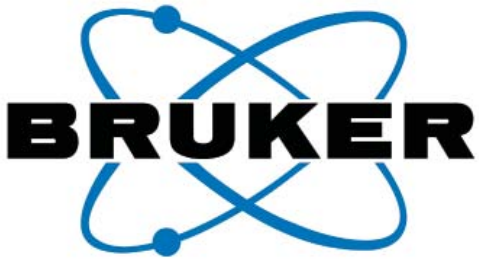

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Table of Contents

PSNA 2014

53rd Annual Meeting of the Phytochemical Society of North America
North Carolina State University, Raleigh, North Carolina
August 9 – 13, 2014

Welcome to the 53rd Annual Meeting of the Phytochemical Society of North America (PSNA)! We are very pleased to have the meeting at the McKimmon Center on the campus of North Carolina State University, Raleigh, NC. As you know, there is nothing without "Phytochemicals". This meeting will gather scientists from all phytochemicals-related disciplines to share their research successes. The PSNA annual meeting is a perfect gathering for you and other scientists - from young students to seniors - in the areas of medicinal chemistry, biosynthesis, metabolic engineering, agriculture, pathogen, metabolomics, genomics, metabolomics, systems biology, synthetic biology, medicinal plants and so on. Your attendance is essential to strengthen the PSNA and research in understanding phytochemicals- the gifts of Mother Nature.

For those who have never before had the opportunity to visit the Raleigh area, North Carolina has much to offer - from beautiful beaches to snow-capped mountains, historic attractions to the Research Triangle. North Carolina's special "barbecue" served with southern hospitality will make this region one of your favorites. Research Triangle Park (RTP), where "Taxol" and "Camptothecin" were discovered, is a dream location for scientists in different disciplines.

Welcome to the 53rd Annual Meeting in Raleigh, North Carolina!

DeYu Xie, Ph.D.
Chair of the 53rd anual meeting of the PSNA

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Agenda of the 53rd Annual Meeting

Saturday, August 9 (Day 1: Arrival)

Time	Meeting activities	Location
12:00 noon-3:00 PM	PSNA Executive Meeting	2212 Gardner Hall, on campus of North Carolina State University
3:00-7:30 PM	Registration Deck Open	McKimmon Center
6:00-8:00 PM	Evening Reception and Setup of Posters	McKimmon Center
7:00-8:00 PM	Scientific organization committee meeting (only for committee members)	McKimmon Center

Sunday, August 10 (Day 2), **McKimmon Center**

Morning

Time	Meeting activities
7:15-8:15 AM	Registration desk open
	Breakfast and poster setup
8:15-8:30 AM	**Welcome opening remarks (Dr. Richard Linton, Dean, College of Agriculture and Life Sciences**
8:30-8:35	**Conference Announcements**
Plenary Symposium: Plant Metabolic Biology **(Chair: Dr. Argelia Lorence)**	
8:35-9:20 AM ***(45 min)***	**P1: Dr. Richard Dixon** (University of North Texas, USA) Title: "Biology Meets Phytochemistry- Lessons from Lignin Biosynthesis"
Symposium I: Biosynthesis of Plant Natural Products **(Chairs: Dr. Sangeeta Dhaubhadel, Dr. Li Tian)**	
9:20-9:55 AM (35 min)	**KS-1: Dr. Vincenzo de Luca** (Brock University, Canada) Title: "Discovery and Metabolic Engineering of Iridoid/Secoiridoid and Monoterpenoid Indole Alkaloid Biosynthesis"
9:55-10:15 AM	**Coffee break**
10:15-10:50 AM (35 min)	**KS-2: Dr. Asaph Aharoni** (Weizmann Institute of Science, Israel) Title: "Deciphering the Steroidal Alkaloids Pathway in Tomato and Other Solanaceous Species through the Integration of Heterogeneous Data from Genetics, Informatics and Metabolomics"
10:50-11:10 AM (20 min)	**OP-1: Dorothea Tholl** Title "Protein-Structure Function Relationships and Evolution of Triterpene-derived Homoterpene Volatile Formation"

11:10-11:30 AM (20 min)	**OP-2: Xu Li** Title: "Exploiting Natural Metabolic Variation to Discover a Novel D-Amino Acid Racemase in *Arabidopsis thaliana*."
11:30-11:45AM (15 min)	**OP-3: Anna Berim** Title: "Delineation of the Lipophilic Flavone Biosynthetic Network in *Ocimum basilicum* Trichomes"
11:45-12:00 ***(15 min)***	**OP-4: Weili Yang** Title: "Quantitative Analysis of Glycerol in Arabidopsis Dicarboxylic Acid-Rich Cutins Provides New Insights into Cutin Structure"

Lunch (12:00-1:30 PM, 90 min)

12:20-1:20 PM ***(60 min)***	**Young members Luncheon** **Chairs: Dr. Jing Xi and Lorenzo Rossi** **Special Speakers: Drs. Richard Dixon and Mansukh C. Wani** **Topic: "Stories of Research-Roads towards a Successful Career"**

Afternoon-Evening, McKimmon Center

Symposium II: Plant Metabolomics **(Chair: Dr. Lining Guo and Reinhard Jetter)**	
1:30-2:05 PM ***(35 min)***	**KS-3: Dr. John Ryals** (Metabolon Inc., USA) Title: "Insights into Plant Water Stress Pathway and Crop Diversity through Global Metabolomics"
2:05-2:40 PM ***(35 min)***	**KS-4: Dr. Lloyd Sumner** (Plant Biology Division, Noble Foundation, USA) Title: "Integrated Metabolomics, Gene Expression, and GWAS Identify New Saponin Biosynthetic Genes in *Medicago truncatula*"
2:40-3:00 PM	**Coffee break**
3:00-3:25 PM ***(25 min)***	**OP-5: Berin Boughton** Title "Spatial Metabolomics: Mapping the Distribution of Metabolites Using Imaging Mass Spectrometry"
3:25-3:45 PM ***(20 min)***	**OP-6: Junhua Wang** Title: "Coupling Ion Chromatography with a New Q Exactive Orbitrap Mass Spectrometer for High Throughput Targeted Metabolomic Analysis"
3:45-4:00 PM ***(15 min)***	**OP-7: Shivakumar P. Devaiah** Title: "Towards Understanding of Glucosyltransferase Specificity in *Citrus paradisi*"
4:00-4:15 PM ***(15 min)***	**OP-8: Stephen Grace** Title: "Haystack, a Web-Based Tool for Metabolomics Research"

4:15-4:30 PM	**OP-9: Aruna Kilaru**
(15 min)	Title: "Discovery of a Mammalian Endocannabinoid Ligand and Its Metabolites in Early Land Plants"
4:30-4:45PM	**OP-10: Massuo J. Kato**
(15 min)	Title: "Secondary Metabolite Richness in Piperaceae Species"
4:45-5:00 PM	**OP-11: Robert Mistrik**
(15 min)	Title: "A Novel Cloud Approach to Solving the Identification Bottleneck in the Untargeted Metabolomics"
5:00-7:30 PM	Dinner on your own
7:30-9:30 PM	Poster section and reception

Monday, August 11 (Day 3)

Morning

Time	Meeting activities
7:15-8:15 AM	Registration desk open
	Breakfast
Plenary Symposium: Plant Synthetic Biology **(Chair: Dr. Deyu Xie)**	
8:15-9:00 AM	**P2: Dr. Christopher Paddon** (Amyris Inc., Emeryville, USA)
(45 min)	Title: "Semi-Synthetic Artemisinin: Using Synthetic Biology to Develop Industrial Production of an Essential Plant Natural Product"
Symposium VII: Phytochemicals, Pathogens and Insects **(Chairs: Dr. Mark Bernards and Dr. Eric Johnson)**	
9:00-9:35 AM	**KS-5: Dr. Reuben Peters** (Iowa State, University, USA)
(35 min)	Title: "Manifold Roles For Rice Diterpenes"
9:35-10:10 AM	**KS-6: Dr. Soren Bak** (University of Copenhagen, Denmark)
(35 min)	Title: "Evolution and Impact of Cyanogenic Glucosides In Zygaena Moths"
10:10-10:30 AM	**Coffee break**
10:30-10:45 AM	**OP-12: Mark A. Bernards**
(15 min)	Title: "Rhizosphere Ginsenosides Affect *Pyhtium irregulare* growth *in vitro* and *in vivo*"
10:45-11:00 AM	**OP-13: Reinhard Jetter**
(15 min)	Title: "Waxes Coating Fern Fronds: Fatty Acid Derivatives and Secondary Metabolites"
11:00-11:15 AM	**OP-14: Jeremy J. Heath**
(15 min)	Title: "Exploring Llant Defense Theory in Tall Goldenrod"

11:15-11:30 AM	**OP-15: Dhirendra Kumar**
(15 min)	Title: "Tobacco SBIP-428: A SIR2 Like Deacetylase, and Its Role in SA Mediated Pathway""
11:30-11:45 AM	**OP-16: Nikolaj Hansen**
(15 min)	Title: "Evolutionary Cues from Functional Switching of Two Closely Related Class II Diterpene Synthases"
11:45-12:00 noon	**OP-17: Christopher S. Jeffrey**
(15 min)	Title: "Amides and Steroids from *Manekia obtusa* (Piperaceae) and their synergistic activity against insect herbivores"

Lunch (12:00 noon-1:00 PM, 60 min)

12:10-12:50 PM	**Contest Activity (12:10-12:50 PM)**
(40 min)	**Topic: "Critical Assessment of Small Molecule Identification (CASMI) Contest"**
	Chair: Dr. Dejan Nikolic

Afternoon-Evening

Symposium III: Plant Systems Biology	
(Chairs: Dr. Rosangela Sozzani and Dr. David Gang)	
1:00-1:35 PM	**KS-7: Dr. Joerg Schwender** (Brookhaven National Laboratory, USA)
(35 min)	Title: "Quantitative Analysis of Metabolism on a Network Scale Unlocks the Complexity of Central Carbon Metabolism"
1:35-2:10 PM	**KS-8: Dr. Adrienne Roeder** (Cornell University, USA)
(35 min)	Title: "A Systems Biology Approach to Understanding the Relationship between Plant Cell Size and Organ Size"
2:10-2:25 PM	**OP-18: David R.Gang**
(15 min)	Title: "From Temporal Systems Biology to Regulatory Networks in the Microalga *Chlamydomonas reinhardtii*""
2:25-2:40 PM	**OP-19: Sangeeta Dhaubhadel**
(15 min)	"Soybean 14-3-3 Proteins and GMMYB176 Interactome: The Key Players Involved in the Regulation of Isoflavonoid Biosynthesis"
2:40-3:00 PM	**Coffee break**
Symposium I: Biosynthesis of Plant Natural Products	
(Chairs: Dr. Monica Borghi and Dr. Xu Li)	
3:00-3:35 PM	**KS-9: Dr. Natalia Dudareva** (Purdue University, USA)
(15 min)	Title: "Aromatic Amino Acid Network: Biosynthesis, Regulation and Transport"
3:35-4:10 PM	**KS-10: Dr. Jose Alonso** (North Carolina State University, USA)
(15 min)	Title: "Auxin Biosynthesis and Its Regulation"

4:10-4:30 PM	**OP-20: Toshiaki Umezawa**
(20 min)	Title: "CAD2 Deficiency Causes Both Brown Midrib and Gold Hull and Internode Phenotypes in *Oryza sativa*""
4:30-4:45 PM	**OP-21: Russell Chedgy**
(15 min)	Title: "Functional Characterization of Two Novel BAHD Acyltransferases from *Populus trichocarpa*, SABT & BEBT, which are Potentially Involved in Salicinoid Phenolic Glycoside Synthesis"
4:45-5:00 PM	**OP-22: Kevin Potter**
(15 min)	Title: "Mechanistic Analysis of the Ent-Copalyl Diphosphate Synthases Required for Plant Gibberellin Hormone Biosynthesis Leads to Novel Product Chemistry"
5:00-7:30 PM	Dinner on your own
7:30-9:30 PM	Poster section and reception, McKimmon Center

Tuesday, Aug. 12 (Day 4), McKimmon Center

Morning

Time	Meeting activities
7:15-8:15 AM	Registration desk open
	Breakfast
Plenary Symposium: Botanic Medicines **(Chair: Dr. Fred Stevens)**	
8:15-9:00 AM	**P3: Dr. Nicholas Oberlies** (University of North Carolina, Greensboro, USA)
(45 min)	Title: "Chemistry of the Endophytic Fungi of the Medicinal Herb Milk Thistle (*Silybum marianum*)"
Symposium V: Botanical Medicines **(Chairs: Dr. Nicholas Oberlies and Dr. Jeremy Johnson)**	
9:00-9:35 AM	**KS-11: Dr. Douglas Kinghorn** (Ohio State University, USA)
(35 min)	Title: "Aglaia Cyclopenta [b]Benzofurans As Potential Anticancer Agents"
9:35-10:10 AM	**KS-12: Dr. Luc Pieters** (, University of Antwerp, Belgium)
(35 min)	Title: "Botanical Medicines Against Malaria"
10:00-10:20 AM	**Coffee break**
10:20-10:45 AM	**OP-23: Scott M.Laster**
(25 min)	Title: "Alkylamides from Echinacea and Their Effects on the Production of Inflammatory Mediators from Macrophages and Mast cells"
10:45-11:10 AM	**OP-24: Athar Ata**
(25 min)	Title: "New Enzyme Inhibiting Phytochemicals and their Pharmaceutical Applications"

11:10-11:25 AM	**OP-25: Pei Chen**
(15 min)	Title: "Differentiation of The Four Major Species (*C. burmannii*, *C. verum*, *C. cassia*, and *C. loureiroi*) of Cinnamons Using a Flow-Injection Mass Spectrometric (FIMS) Fingerprinting Method"
11:25-11:40 AM	**OP-26: Josh Kellogg**
(15 min)	Title: "Multi-target Functionality of Alaskan Seaweed in Combatting Hyperglycemia and Type 2 Diabetes"
10:40-11:55 AM	**OP-27: Lydia F Yamaguchi**
(15 min)	Title: "Changes in the Secondary Metabolites during Ontogeny of *Piper gaudichaudianum* Kunth"

Lunch 11:55 AM-1:00 PM

Afternoon-Evening, McKimmon Center

Symposium IV: Renewable Petro Biofuel from Plants **(Chairs: Dr. Danny J. Schnell and Dr. Deyu Xie)**	
1:00-1:35 PM	**KS-13: Dr. Danny Schnell** (University of Massachusetts, USA)
(35 min)	Title: "Approaches to Increase Carbon Capture and Redistribution to Fuel Molecules and Co-products in Camelina sativa""
1:35-2:10 PM	**KS-14: Dr. Edgar Cahoon** (University of Nebraska, USA)
(35 min)	Title: "Camelina: A Designer Oil Seed Crop for Metabolic Engineering of Advanced Biofuels and Bio-based Lubricants"
2:10-2:45 PM	**KS-15: Dr. Amy Grunden** (North Carolina State University, USA)
(35 min)	Title: "Increasing Photosynthetic CO_2 Capture in *Camelina sativa* with a Synthetic Carbon Fixation Cycle Composed of Selected Microbial Enzymes"
2:45-3:00 PM	**OP-28: Matthew J. Salie**
(15 min)	Title: "Discovering Novel Interacting Proteins with Heteromeric Acetyl-CoA Carboxylase"
3:00-3:15 PM	**OP-29: Hong Ma**
(15 min)	Title: "Synthetic Design of Pathways for Terpene Biofuel Production in Tobacco"
3:15-3:30 PM	**OP-30: Dr. Monica Borghi**
(15 min)	Title: "Metabolic Engineering of Camelina sativa to Produce Additives for Biodiesel"
Neish Young Investigator Award Winner (Chair: **Dr. Argelia Lorence)**	
3:30-3:55 PM	**Dr. Leslie Hicks** (University of North Carolina, USA)
(25 min)	Title: "Interrogation of Post-translational Regulation in *C. reinhardtii* via Mass Spectrometric Proteomics Approaches"
3:55-7:00 PM	Free time

7:00-10:00 PM	**Banquet at McKimmon Center** **Announcements of Neish Award Winner, Best Poster Winner, and Travel Award Winners**

PSNA business meeting: 4:00 PM-5:00 PM at McKimmon Center

Wednesday, Aug. 13 (Day 5), McKimmon Center

Morning

Time	Meeting activities
7:15-8:15 AM	Registration desk open
	Breakfast
Symposium VIII: Phytochemicals, Crops and Agriculture **(Chairs: Dr. Dan Sung, Dr. Baochun Li, and Dr. Peifeng Ren,)**	
8:15-8:50 AM ***(35 min)***	**KS-16: Dr. John Littleton** (University of Kentucky, USA) Title: "Selection of Mutant Plant Cells for Target-directed Biosynthesis of Bioactive Metabolites""
8:50-9:25 AM ***(35 min)***	**KS-17: Dr. Luke Mankin** (BASF Plant Science, L.P., Research Triangle Park, NC, USA) Title: "The Provisia™ Rice System: A New Vision in Red Rice Control (The Control of Grass Weeds in Rice)"
9:25-9:45 AM ***(20 min)***	**OP-31: Amanda Souza** Title: "A Metabolomics Based Approach for Understanding the Influence of Terroir in Wine Grape Juice Using LC-HR/AM""
9:45-10:00 AM ***(15 min)***	**OP-32: Adam Brown** "Studies on Bacterial Efflux Pump Inhibitors and Their Distribution in Land Plants"
10:00-10:15 AM ***(15 min)***	**OP-33: Jason McCallum** Title: "Anthocyanin and Pyranoanthocyanin Biosynthesis in Staghorn Sumac (*Rhus typhina* L.)""
10:15-10:35	**Coffee break**
10:35-11:10 AM ***(35 min)***	**KS-18: Dr. Marie Petracek** (Monsanto, St. Louis, MO, USA) Title: "Innovation in Agriculture: Mitigating the Effects of Climate Change"
11:10-11:25 PM ***(15 min)***	**OP-34: Michael Gutensohn** Title: "Metabolic Engineering of Monoterpene Biosynthesis in Tomato Fruits Via Introduction of the Non-canonical Substrate Neryl-diphosphate"

11:25-11:40 AM	**OP-35: Eva Knoch**
(15 min)	Title: "Hydroxynitrile Glucosides in Barley"
11:40 -11:55	**OP-36: Benjamin Delory**
(15 min)	Title: "Barley (*Hordeum distichon* L.) Roots Produce Volatile Aldehydes Derived from the Lipoxygenase/Hydroperoxide Lyase Pathway with a Strong Age-dependent Pattern

Lunch (11:55 AM-1:10 PM)

(Removal of Posters)

Afternoon, McKimmon Center

Symposium VIII: Phytochemicals, Crops and Agriculture **(Chairs: Dr. Nic Bate and Dr. Mark Berhow)**		
1:10-1:45 PM	**KS-20: Dr. Sean Cutler** (University of California, River side, USA)	
(35 min)	Title: "Agrichemical Control of Drought Tolerance Using Engineered ABA Receptors"	
1:45-2:20 AM	**KS-19: Elvira DeMeija** (the University of Illinois, USA)	
(35 min)	Title: "Phenolic Compounds from Fruits and Vegetables: Role in Chronic Diseases"	
2:20-2:35 PM	**OP-37: Li Tian**	
(15 min)	Title: "Vitamin A biofortification of wheat grains via a TILLING mutant-based approach"	
2:35-2:50 AM	**OP-38: Daniel Owens**	
(15 min)	Title: "Metabolism of Glyphosate and Aminophosphoric Acid in Glyphosate-Resistant and Conventional Canola (*Brassica napus L.*"	
2:50-3:00 PM	**Oral presentation winner announcement**	
(15 min)	**Closing remarks**	
	Adjournment	
3:00 PM	RAP editorial board meeting and incoming executive meeting	Room

Abstracts

Plenary Symposium: Plant Metabolic Biology

Richard A. Dixon, Ph.D.
Distinguished Research Professor
Department of Biology
University of North Texas
Denton, USA

Richard A. Dixon is Distinguished Research Professor in the Department of Biological Sciences at the University of North Texas, Denton. He was previously Distinguished Professor and Samuel Roberts Noble Research Chair, Senior Vice President and Founding Director of the Plant Biology Division at the Samuel Roberts Noble Foundation, Ardmore, Oklahoma, where he worked from 1988-2013. He received his Bachelor's and Doctoral degrees in Biochemistry and Botany from Oxford University (UK), and postdoctoral training in Plant Biochemistry at Cambridge University (UK). He was awarded the Doctor of Science degree for his research achievements by Oxford University in 2004. His research interests center on molecular biology and metabolic engineering of plant natural product pathways and cell walls in legumes and bioenergy crops. He has published over 420 papers on these and related topics in international journals, and has been named by the Institute for Scientific Information as one of the 10 most cited authors in the world in the plant and animal sciences. Professor Dixon is Co-Editor-in-Chief of the journal BioEnergy Research, a member of the Editorial Boards of four other international journals, and recipient of numerous awards including Oklahoma Scientist of the Year (2008) and, in 2012, the Groupe Polyphenols Scientific Prize. He is a Fellow of the American Association for the Advancement of Science, and was elected to membership of the US National Academy of Sciences in 2007, in which capacity he serves on the National Research Council Board on Agriculture and Natural Resources.

Plenary Symposium: Plant Metabolic Biology

[P-1] Biology Meets Phytochemistry- Lessons from Lignin Biosynthesis

Richard A. Dixon[1], Fang Chen[1], Lina Gallego-Giraldo[1], Xiaolan Rao[1], Nandika D'Souza[2], Qiao Zhao[3]

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Although the phenylpropanoid-derived plant cell wall polymer lignin can be synthesized in vitro by simple free radical coupling reactions, its synthesis and deposition in vivo are subject to a range of complex biological controls. Molecular biological approaches have therefore provided significant insights into the mechanisms of lignin biosynthesis and deposition in planta. The biosynthetic pathways leading to the formation of the cell wall polymer lignin have been extensively studied in recent years, and it now appears that plants exhibit substantial flexibility in their capacity to incorporate different building blocks into lignin. Natural lignins are generally composed of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, that are biosynthesized by polymerization of the three primary monolignols, p-coumaryl, coniferyl, and sinapyl alcohols, respectively; natural angiosperm lignins have only low levels (<~2%) of H-units. The content and composition of lignin can be drastically altered in transgenic plants in which different enzymes of the pathway have been targeted for down-regulation, sometimes, but by no means always, with severe impacts on plant development. Catechyl (C) and 5-hydroxyguaiacyl (5H) units that may derive from polymerization of the corresponding caffeyl and 5-hydroxyconiferyl alcohols are not found in 'normal' lignins. Until recently, these rare units were only found in the lignin of transgenic plants in which the first or second O-methylation reactions had been blocked. We serendipitously discovered a hitherto unsuspected lignin polymer (C-lignin) in the seed coats of vanilla orchid, and subsequently in the seeds of cacti, Cleome and Jatropha. C-lignin is a linear homopolymer formed by endwise β-O-4-coupling of caffeyl alcohol monomers onto the growing polymer, resulting in benzodioxane units. Although all cactus species examined never contained both C and G or S lignins in their seed coats, seeds of Cleome and Jatropha contain both C and G lignins which are, however, independent polymers that are deposited, at least in Cleome, at different times during seed coat development. RNA sequencing analysis has revealed potential mechanisms for C-lignin formation in the V. planifolia seed coat, but it is currently unclear whether plants can tolerate accumulation of C-lignin in vegetative tissues. As a further

example of the flexibility of lignin structures, stems of the Medicago truncatula CAD1 mutant contain lignin that is more than 95% composed of hydroxycinnamaldehyde units. These unusual lignins hold significant promise for development of added value co-products from biorefining. Finally, we have obtained genetic evidence in Arabidopsis indicating that laccase is critical for lignification in most tissues of the plant, and is not functionally redundant with peroxidase. These findings highlight the importance of multidisciplinary approaches to understanding and exploiting lignin biosynthesis for the bio-based economy.

Symposium I: Biosynthesis of Plant Natural Products

Vincenzo De Luca, Ph.D.
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St. Catharines, ON
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Dr. Vincenzo De Luca is a Professor and Tier 1 Canada Research Chair in Plant Biotechnology in the Department of Biological Sciences at Brock University. He received his PhD from Concordia University in Montreal Canada (1984) and was a Research Associate at the Plant Biotechnology Institute of the National Research Council of Canada (NRC, 1984-86). He has held government research positions at the NRC (1986-89), academic positions to the level of Professor at the Institut de Recherche en Biologie Végétale with the University of Montreal (1989-98) and was Principal Scientist 1 with Novartis (now Syngenta) in Research Triangle Park, NC (1998-2001). Over the past 30 years, his research has focused on explaining natural product biosynthesis and the cellular specialization involved in various medicinal and crop plants. His research is funded by several sources including the Natural Sciences and Engineering Research Council of Canada as well as the Canada Research Chairs Program. He has been awarded the 1991 C.D. Nelson Award of the Canadian Society of Plant Physiologists and the 2011 Brock University award for distinguished research and creative activity. He was the President of the Phytochemical Society of North America (1998-99) and is the current President of the Canadian Society for Plant Biologists (2013-15).

[KS-1] Discovery and Metabolic Engineering of Iridoid/secoiridoid and Monoterpenoid Indole Alkaloid Biosynthesis

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The Madagascar Periwinkle (Catharanthus roseus) has been developed into an increasingly widely used model system to study the cell and molecular biology of monoterpenoid indole alkaloid biosynthesis MIA (1) Recent successes in the molecular and biochemical characterization of the iridoid/secoiridoid [2-4] and monoterpenoid indole alkaloid pathways and their transport [5] have been achieved through rapid comparative bioinformatics guided identification of candidate genes combined with in planta virus induced gene silencing approaches and functional characterization of selected genes in the Catharanthus roseus model system. These discoveries have led to the complete elucidation of the pathways for strictosidine biosynthesis, the precursor of thousands of biologically active iridoids and MIAs. This achievement will speed up the discovery of related pathways in many more plant species and are leading to broad scale biochemical and molecular description of the reactions required for making thousands of iridoid/secoiridoids/MIAs. These studies have broad implications for advancing investigations about the evolutionary and ecological roles played by these metabolites in Nature as well as for their biotechnological production in plants/microorganisms.

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Symposium I: Biosynthesis of Plant Natural Products

Asaph Aharoni, Ph.D.
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Rehovot, Israel

Dr. Asaph Aharoni conducted his B.Sc. and M.Sc. studies at the Faculty of Agriculture of the Hebrew University of Jerusalem, Israel. In 1996 he began his Ph.D. studies in the CPRO-DLO Institute in Wageningen, The Netherlands. His doctorate work combined functional genomics, molecular biology and biochemistry for studying fruit development and ripening in strawberry. His main focus was the study of fruit flavor biosynthesis during the ripening process. His post-doctoral position was conducted in Plant Research International (Wageningen, The Netherlands), studying the genetic regulation of metabolic pathways in plants. His work involved the use of transposon tagging and metabolic profiling for discovery of genes controlling the biosynthesis of plant surface components (i.e. the cuticle) and metabolism of antioxidants (e.g. ascorbic acid). Furthermore, during his postdoc Asaph initiated and set-up metabolomics technologies as a tool for the comprehensive analyses of small molecules in plants. His work in this field had an important contribution to the implementation of these technologies for the study of plant biology and metabolism, particularly in the case of secondary metabolites. In August 2004 he started his own research group in the department of Plant Sciences at the Weizmann institute in Israel. Also today, his lab combines expertise in molecular biology, analytical chemistry and computational biology. The topic of his current research activity is the genetic regulation of metabolic pathways and its co-ordination with developmental and stress response programs in plant biology. The lab investigates several aspects of metabolic regulation including transport, transcriptional and post transcriptional control (**http://www.weizmann.ac.il/plants/aharoni**).

[KS-2] Deciphering the Steroidal Alkaloids Pathway in Tomato and Other Solanaceous Speceis through the Integration of Heterogeneous Data from Genetics, Informatics and Metabolomics

Asaph Aharoni

Department of Plant Sciences, Faculty of Biochemistry, Weizmann Institute of Science, P.O.B. 26, Rehovot, 76100, Israel

The regulation of metabolic pathways is constantly tuned in order to suit the needs of development and fitness. Our main research objective is to unravel networks of genes and proteins which coordinate the activity of metabolic pathways during plant development and stress response. An integrated investigation of several members of the Solanacea family (particularly tomato, potato and eggplant), rather than studying a single plant, provided us with unprecedented insights to metabolic biology in these species. Most if not all processes characterized, impact to a certain degree key quality, nutritional and post-harvest traits of these crop plants. Integrating cutting-edge transcriptomics and metabolomics tools together with genes co-expression assays were of great value in making several discoveries. In a recent example, combined co-expression analysis and metabolic profiling in tomato and potato led to the discovery of the multi-step, core pathway leading to the formation of the renowned Solanacea glycoalkaloids. This class of cholesterol-derived molecules represent important anti-nutritional compounds in these crop plants. In the presentation, I will highlight several technologies and genetic research tools and the invaluable knowledge on core metabolic pathways obtained through combining them in a single study. Most if not all could be applied in the coming years to the study of key traits in other, less studied fruit species.

Symposium I: Biosynthesis of Plant Natural Products

[OP-1] Protein-structure Function Relationships and Evolution of Triterpene-derived Homoterpene Volatile Formation

Reza Sohrabi[1], Somayesadat Badieyan[2], Jung-Hyun Huh[1], Liva Harinantenaina[3], David Kingston[3], Pablo Sobrado[2], and Dorothea Tholl[1]

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Despite their structural diversity, volatile terpenes are generally produced from a small number of core five- to fifteen-carbon intermediates. We have found unexpected plasticity in volatile terpene biosynthesis by showing that homo/norterpenes can arise from different biosynthetic routes in a tissue specific manner. While many angiosperms produce the homoterpene, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), by breakdown of the sesquiterpene alcohol, (E)-nerolidol, in aboveground tissues, we observed that Arabidopsis roots biosynthesize DMNT by degradation of the C30 triterpene diol, arabidiol, upon pathogen infection. Arabidiol breakdown is catalyzed by the cytochrome P450 monooxygenase CYP705A1, which belongs to an arabidiol triterpene biosynthetic gene cluster expressed in the root vascular tissue. To explore the mechanism of arabidiol cleavage by CYP705A1, we conducted homology modeling and substrate docking. The predicted coordination of arabidiol and the one-step C-C cleavage reaction support a mechanism equivalent to that catalyzed by the leafexpressed homoterpene synthase CYP82G1 with the substrate (E)-nerolidol. Sequence comparison with closely related CYP705A paralogs positioned in other triterpene biosynthetic gene clusters and homology modeling of these P450s suggest substantial divergence of substrate specificity associated with a lack of activity toward arabidiol. A scenario for the evolution of the CYP705A1 gene cluster based on genome synteny analysis is discussed.

[OP-2] Exploiting Natural Metabolic Variation to Discover a Novel D-amino Acid Racemase in Arabidopsis Thaliana

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D-amino acids (D-AAs), the enantiomorphs of proteogenic L-amino acids, are ubiquitous in all life forms and have only recently been discovered to function in more than the structural capacity in peptidoglycan and microbial bioactive compounds as originally thought. Surprisingly, D-AAs also act as neurotransmitters and endocrine signaling molecules in mammals, and as paracrine and autocrine effectors in bacteria. However, the function of DAAs in plants remains to be discovered. By taking advantage of the genetic and metabolic variation of 440 natural accessions of Arabidopsis thaliana, we have successfully used metabolic profiling and Genome Wide Association Studies to link differentially accumulated metabolites to genes. We identified a new metabolite, N-malonyl-D-allo-Isoleucine (NMD-Ile), and discovered a previously uncharacterized D-amino acid racemase involved in its biosynthesis. The structure of the NMD-Ile was elucidated by chemical analysis and the genetic locus controlling its accumulation was confirmed by reverse genetics and biochemical analysis. This work describes the first PLP-independent racemase found in higher eukaryotes and among the first racemase enzymes discovered in plants. This is an important discovery towards elucidating the function of D-AAs in plants.

Symposium I: Biosynthesis of Plant Natural Products

[OP-3] Delineation of the Lipophilic Flavone Biosynthetic Network in *Ocimum Basilicum* Trichomes

Anna Berim[1] and David R. Gang[1]

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Peltate trichomes of sweet basil (Ocimum basilicum L.) accumulate flavones with characteristic hydroxylations at positions 6 and 8 and up to four O-methylations. We previously analyzed flavonoid O-methylations and showed that they occurred in a defined order which implied that 6-hydroxylation must be preceded by 7-O-methylation. Indeed, the subsequently identified flavone 6-hydroxylase, CYP82D33, requires the 7-O-methyl group for activity. We then studied the origin of the free 7-hydroxyl moiety of nevadensin, a major lipophilic flavone in some basil lines, and found that it results from 7-O-demethylation by an oxoglutarate-dependent dioxygenase. The identification and isolation of the last missing enzyme in the network, the flavone 8-hydroxylase, was aided by analyses of its activity in crude trichome protein extracts. This reaction is catalyzed by an enzyme from a family of proteins not previously reported to be involved in specialized metabolism in plants. To test the performance of the isolated enzymes in vivo, up to six steps of the biosynthetic pathway were reconstructed in baker's yeast, which produced the expected lipophilic flavones when supplied with apigenin as precursor. Overall, our studies resulted in a complete biochemical description of an intricate metabolic network, and identified unprecedented components of flavonoid biosynthesis.

[OP-4] Quantitative Analysis of Glycerol in Arabidopsis Dicarboxylic Acid-rich Cutins Provides New Insights into Cutin Structure

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Cutin is a plant extracellular lipid polymer that serves as a protective barrier against biotic and abiotic stresses. Glycerol has been reported as a component of cutin, contributing up to 14% (w/w) of total released monomers. Partial hydrolysis reveals the presence of glycerol-aliphatic ester links, while glycerol-3-phosphate acyltransferases (GPATs) are essential for cutin biosynthesis. However, precise roles of glycerol in cutin assembly and structure remain uncertain. We have developed stable isotope-dilution GC/MS for quantitative analysis of glycerol with simultaneous determination of aliphatic monomers. To provide clues about the role of glycerol in dicarboxylic acid (DCA)-rich cutin, this methodology has been applied to compare wild-type (WT) Arabidopsis cutin with a series of mutants that are defective in cutin synthesis. The molar ratio of glycerol to total DCAs in WT cutins is 2:1. Glycerol content is strongly reduced in both stem and leaf cutin from all Arabidopsis mutants analyzed (gpat4/gpat8, att1-2 and lacs2-3). In addition, the molar reduction of glycerol is proportional to the molar reduction of total DCAs. These results imply a structural role for glycerol in DCA-rich cutin. Structural models consistent with these quantitative results are discussed.

Symposium II: Plant Metabolomics

John A. Ryals, Ph. D.
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Dr. Ryals received his Ph. D. in Molecular Biology from the University of Texas at Dallas in 1982 working with Prof. Hans Bremer on the global control of RNA synthesis in E. coli. As a post doctoral fellow in the lab of Prof. Charles Weissmann at the Institute for Molecular Biology, at the University of Zurich Dr. Ryals worked on human interferon gene expression., Dr. Ryals joined Ciba-Geigy (now Syngenta) in the Biotechnology Research Unit at Research Triangle Park in 1985 as a Senior Scientist and spent 13 years on defining the molecular biology of systemic acquired resistance in plants. Dr. Ryals worked in various researches and management positions including Head, Agricultural Biotechnology Research, Vice-President of Biotechnology, Vice-President, Research for Novartis Crop Protection, Inc. and Head of the Biotechnology and Genomics Center of Novartis, Inc. Dr. Ryals scientific interest at Ciba Geigy was the molecular biology of systemic acquired resistance. In 1997, Dr. Ryals founded Paradigm Genetics, Inc., an early systems biology company, and served as the Chief Executive Officer, Chief Science Officer and President and taking the company public in 2000. After leaving Paradigm Genetics in 2002, Dr. Ryals joined Metabolon, Inc. as co Founder, Chief Executive Officer and President. For the past 12 years, Dr. Ryals research interest has been in the development of metabolomics to aid in pharmaceutical drug discovery and development, healthcare and nutrition.

[KS-3] Insights into Plant Water Stress Pathway and Crop Diversity through Global Metabolomics

John A. Ryals

Metabolon, Inc. 617 Davis Dr, Durham, NC 27713, USA

We have developed an unbiased global metabolomics platform that can provide comprehensive coverage of biochemical pathways in biological systems. The platform is based on a combination of ultrahigh performance liquid chromatography/tandem mass spectrometry and gas chromatography/mass spectrometry systems. Rapid identification and quantitation of metabolites with high confidence are achieved using novel software and a comprehensive chemical reference library. After data generation, integrated tools for statistical analysis, pathway mapping, and data visualization can provide novel insights for understanding biological systems. The presentation will focus on using metabolomics to elucidate metabolic responses to water stress in Sporobolus stapfianus (a resurrection plant) and maize, and metabotypes of diverse maize and soybean lines.

Symposium II: Plant Metabolomics

Lloyd W. Sumner, Ph.D.
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Plant Biology Division
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Dr. Sumner acquired his B.Sc. degree in chemistry and mathematics in 1989 from Cameron University in Lawton, OK, USA and a Ph.D. in analytical chemistry in 1993 from Oklahoma State University in Stillwater, OK, USA. He then joined Texas A&M University, College Station TX, where he was the Director of the Mass Spectrometry Applications Laboratory and where he later served as the cofounder and Associate Director of the TAMU Laboratory for Biological Mass Spectrometry with Prof. David H Russell. He joined The Noble Foundation in 1999 and has risen to the rank of Professor within the Plant Biology Division. While at the Noble Foundation, Dr. Sumner has built a research program focused around the development and integration of large-scale biochemical profiling of plant metabolites, proteins, and transcripts (metabolomics, proteomics and transcriptomics) for the discovery and characterization of the molecular and biochemical components related to plant natural products biosynthesis. He also applies these integrated omics technologies for greater insight into the physiological and biochemical consequences of gene expression and system responses to genetic and environmental perturbations. In the process, he has published approximately 110 peer reviewed articles and book chapters; many with leading national and international collaborators. Dr. Sumner's research is supported by The Samuel Roberts Noble Foundation, and is or has been supported by NSF 2010, NSF Molecular and Cellular Biosciences, NSF Major Research Instrumentation program, NSF-JST joint Metabolomics for a Low Carbon Society, NSF Integrative Organismal Systems, and The Oklahoma Commission for the Advancement of Science and Technology. Dr. Sumner is currently a Fellow of The American Association for the Advancement of Science; Former Treasurer (2010-2012) and President (2008-2010) of the Metabolomics Society; 2013 Lifetime Honorary Fellow of the Metabolomics Society; Co-founding Member of the International Advisory Committee for Plant Metabolomics; Principal investigator of a new Plant, Algae, and Microbial Metabolomics Research Coordination Network (PAMM-NET), Adjunct Professor at Oklahoma State University Department of Biochemistry and Molecular Biology; and a 2007 Distinguished Alumni of Cameron University. Dr. Sumner serves as a Managing Editor for Plant Physiology, Front Pages Co-Editor and Editorial Board member for the journal Metabolomics, and review Editor for several plant related Frontiers journals.

[KS-4] Integrated Metabolomics, Gene Expression, and Gwas Identify New Saponin Biosynthetic Genes in Medicago Truncatula

Lloyd W. Sumner[1], Bonnie S. Watson[1], Zhentian Lei[1], DongSik Yang[1], Yuhong Tang[1], Derek Nedveck[2], Peter Tiffin[2], Nevin Young[2]

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Triterpene saponins are structurally diverse secondary metabolites found in many plant families, including the Leguminosae. They possess a broad spectrum of bioactivities ranging from allelopathy and anticancer activities to antifungal, antibacterial, anti-insect and anti-nutritive properties. In spite of their functional importance, the biosynthetic pathways for saponins remain largely uncharacterized. We are using an integrated metabolomics, correlated gene expression profiling and genome wide association studies (GWAS) for the discovery, prioritization, and characterization of novel saponin biosynthetic genes in the model legume Medicago truncatula which is known to accumulate a large variety of differentially glycosylated saponins. In this project, saponins from aerial and root tissues of close to 200 accessions in the Medicago Hapmap collection were profiled by UPLC- qTofMS. It was determined that the collection contained ecotypes with highly varied accumulation of saponins, both within the different tissues as well as between accessions. Eight lines with differential saponin accumulation were chosen for further characterization, including correlated gene expression analyses using RNAseq and genome wide association (GWAS) relative to the differential saponin accumulation. The correlated gene expression and GWAS results guided the selection and prioritization of gene candidates for subsequent cloning and pathway characterization. In vitro biochemical assays confirmed the activity of saponin biosynthetic enzymes. Additional molecular genetic confirmation was performed through the analysis of Tnt1 insertional mutantations within the targeted saponin genes and through the analysis of plants stably transformed with known and putative saponin genes. This presentation will describe the integrated technologies and approaches used and provide examples of novel gene discoveries.

Symposium II: Plant Metabolomics

[OP-5] Spatial Metabolomics: The Potential of Imaging Mass Spectrometry (ims) to Map the Distribution of Plant Metabolites

Berin Boughton[1], Jeffrey Spraggins[2], Allison Heskes[3], Irini Pateraki[3], Björn Hamberger[3], Tamil Chelvan Meenakshi Sundram[3], Ute Roessner[1]

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Imaging Mass Spectrometry (IMS) is an emerging technology in the biological sciences that maps the spatial (and temporal) distribution of biomolecules (metabolites, proteins, drugs and chemicals) in thin sections of tissue, whose development has largely focused on mammalian tissues. More recently, plant biologists have begun to adopt the technology to explore highly localized biosynthesis of plant secondary metabolites and explore metabolic control of plant growth and development during normal growth and in response to abiotic/biotic stresses. Biological systems are exquisitely compartmentalized into specialized cells, tissues and organs where biosynthesis of natural products occurs in specialized areas of plants. We are currently exploring methods to image plant metabolites in a variety of systems and demonstrate a Laser Desorption Ionization (LDI) IMS approach to elucidate the location of plant secondary metabolite biosynthesis within Vitex agnus-castus. We have also explored the distribution of metabolites in other tissues including thin sections of Eucalyptus sp. leaves and Triticum sp. roots using both an LDI and a Matrix Assisted Laser Desorption Ionisation (MALDI) approach.

[OP-6] Coupling Ion Chromatography with a New Q Exactive Orbitrap Mass Spectrometer For High Throughput Targeted Metabolomic Analysis

Junhua Wang[1], Terri Christison[1], Krista Backiel[2], Grace Ji[3], Shen Hu[3], Linda Lopez[1], Yingying Huang[1]

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Here we present a study using ion chromatography-Orbitrap MS for targeted metabolomic quantitative analysis of polar metabolites in oral cancer cells. Mass spectrometry based metabolomics approach has obtained increasing attention. Recently, a highly analytically sensitive platform coupling capillary ion chromatography (CapIC) with Q Exactive mass spectrometer has been successfully developed for nontargeted metabolic profiling of head and neck cancer cells [1]. The outstanding resolution of IC has led to the differentiation of many isomeric polar metabolites, and it has shown a broad coverage to glycolysis and TCA intermediates. In this work, we utilized a high performance ion chromatography (HPIC) system for higher throughput targeted analysis. Isotopically labeled standards are ideal external calibration references to spike into the metabolomic sample for targeted quantitative analysis in LC/MS experiment because of their similar ionization effect and chromatographic retention. Six stable isotope labeled standards available for TCA cycle were used for the targeted quantitative analysis. The corresponding endogenous metabolites in a large scale of samples were quantified easily against the standards by using the software tool Thermo Scientific™ Tracefinder.

A new high field Thermo Scientific™ Q Exactive HF™ quadrupole-Orbitrap mass spectrometer was operated under ESI negative mode for all detections. The HPIC/Q Exactive HF platform achieved five orders of magnitude linear range from 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000, 5000,to 10000 pg/µL (2 µL injection) with R2 = 0.99.UM1 and UM2 cells were initially established from the same tumor of a tongue cancer patient. However, UM1 cells are highly invasive whereas UM2 cells are less invasive. Similarly UMSCC5 cells are significantly more invasive than UMSCC6 cells. Experimentally, we observed that UMSCC5 cells consume a very large amount of glucose and secret a lot of lactate in cell culture. Based on our quantitative analysis, UM1 cells display significantly higher levels of TCA metabolites, including malate, fumarate, citrate, succinate and alpha-ketoglutarate. Similarly UMSCC5 cells express dramatically higher levels of these metabolites than UMSCC6 cells. These results suggests that highly invasive HNSCC cells possess a more active TCA cycle than less invasive HNSCC cells.

Symposium II: Plant Metabolomics

[OP-7] Towards Understanding of Glucosyltransferase Specificity in Citrus Paradisi

Shivakumar P. Devaiah[1] and Cecilia A. McIntosh[1,2]

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Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. Widely distributed in plants, food and traditional herbal medicines, more than 6000 flavonoids have been identified up to date. They are present mainly as glycosides whose phenolic hydrogen or hydrogens are substituted to sugar moiety. An increasing number of flavonoids have attracted much attention in relation to their biological activities, including anti-viral, anti-inflammatory, anti-bacterial, and vasodilatory activities. Present work is to understand the structure and function of a flavonol specific glucosyltransferase from Citrus paradisi. The study is one of the many steps towards custom designing of the protein. We employed homology modeling, site-directed mutagenesis and yeast expression system to generate mutants of glucosyltransferase and study their substrate specificity, regiospecificity and kinetic properties.

[OP-8] Haystack, a Web-based Tool for Metabolomics Research

Stephen Grace

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Liquid chromatography coupled to mass spectrometry (LCMS) has become a widely used technique in plant metabolomics for differential profiling, the broad screening of biomolecular constituents across multiple samples to diagnose phenotypic differences and elucidate relevant features. However, a significant limitation in LCMS-based metabolomics is the high-throughput data processing required for robust statistical analysis and data modeling for large numbers of samples with hundreds of unique chemical species. To address this problem, we developed Haystack, a web-based tool designed to visualize, parse, filter, and extract significant features from LCMS datasets rapidly and efficiently. Haystack runs in a browser environment with an intuitive graphical user interface that provides both display and data processing options. Total ion chromatograms (TICs) and base peak chromatograms (BPCs) are automatically displayed, along with time-resolved mass spectra and extracted ion chromatograms (EICs) over any mass range. Output files in the common .csv format can be saved for further statistical analysis or customized graphing. Haystack's core function is a flexible binning procedure that converts the mass dimension of the chromatogram into a set of interval variables that can uniquely identify a sample. Binned mass data can be analyzed by exploratory methods such as principal component analysis (PCA) to model class assignment and identify discriminatory features. The validity of this approach is demonstrated by comparison of a dataset from plants grown at two light conditions with manual and automated peak detection methods. Haystack successfully predicted class assignment based on PCA and cluster analysis, and identified discriminatory features based on analysis of EICs of significant bins.

Symposium II: Plant Metabolomics

[OP-9] Discovery of a Mammalian Endocannabinoid Ligand and Its Metabolites in Early Land Plants

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The endogenous arachidonate-based lipids that activate cannabinoid receptors have been well characterized in mammals. In plants only 12-18 carbon fatty acid ethanolamides have been identified so far and have been shown to modulate a number of physiological processes including seed and seedling development. However, since moss plants contain arachidonic acid, we hypothesized the occurrence of arachidonate-based metabolites in their tissues. Using selective lipidomics approach, we identified the presence of anandamide or arachidonylethanolamide (a 20C polyunsaturated fatty acid ethanolamide) and its precursors, in Physcomitrella patens that were previously not reported in plants. Comprehensive lipid profiles for protonema and gametophyte tissues of moss also revealed the occurrence of other saturated and unsaturated fatty acid ethanolamides and a distinct phospholipid and galactolipid composition. Further studies showed that anandamide, like abscisic acid, inhibits the growth of gametophytes more severely than saturated fatty acid ethanolamides. Our current studies are focused on understanding the physiological and developmental role of polyunsaturated fatty acid ethanolamides in nonseed plants. In conclusion, discovery of anandamide in moss provided us with an exciting possibility to identify fatty acid ethanolamide metabolic pathway in early land plants and elucidate receptor-mediated endocannabinoid signaling responses in plants that is akin to mammals.

[OP-10] Secondary Metabolite Richness in Piperaceae Species

Massuo J Kato[1], Lydia F Yamaguchi[1], NC Yoshida[1], AM Gaia[1], M Martins[1], Vítor Bueno A Costa[1], LY Valero-Gutierrez[1], CRO Oliveira-Jr[1], MV Correia[1], HH Fokoue[1], Gerardo Cebrian-Torreyon[1], TJ Massad[1], APM Egydio[2], ML Salatino[2], A Salatino[2]

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Species in the two giant genera, Piper and Peperomia, are characterized by diverse physiology, morphology, architecture, and biotic interactions with dispersers and herbivores. The genera are also represented across a wide spectrum of tropical ecosystems. Plant diversification is often thought to be driven by selective forces operating on easily observable morphological features, but secondary metabolites are also beginning to be recognized as being tightly linked with speciation. In Piper and Peperomia, these secondary compounds include amides, various meroterpenes, piperolides, pyrones, and polyketides, Although molecular phylogenetic studies using ITS and matK have been undertaken, there has been no previous attempt in Piper or Peperomia to analyse the distribution of these compounds in an evolutionary context. In this work, we conducted chemical profiling of crude extracts based on 1H NMR and ESI data combined with PCA, as well as detailed phytochemical characterization for species in these diverse genera. In spite of the importance of using combined tools to determine the occurrence of major classes of compounds, further phytochemical studies are still required to fully describe the chemical variation and occurrence of novel compounds such as protoflavonoids, polyketides, and meroterpenes. Studies of ontogenetic processes and plant-herbivore interactions have revealed additional factors, which may account for the high chemical variation among species.

Symposium II: Plant Metabolomics

[OP-11] A Novel Cloud Approach to Solving the Identification Bottleneck in the Untargeted Metabolomics

Robert Mistrik[1], Junhua Wang[2]

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Untargeted plant metabolomics, in combination with novel mass spectrometric techniques, allow the detection of thousands of low-concentration compounds that open important insights into physiologically relevant processes. However, majority of observed metabolites still remain unidentified. This bottleneck is greatly hindering the field of global metabolic profiling, since unidentified compounds cannot be placed into biochemical context. We will present a new type of spectral cloud providing the functionality required for identification of unknowns even if compounds are not present in the library. The first step in establishing the identity of an unknown is to determine possible substructures. The MSn spectra of various precursor ions and the MS stages of an unknown metabolite were searched against the newly developed mzCloud library (mzcloud.org) that contains substructurally characterized precursor ions of MSn spectra. Even if an unknown compound is not represented in the library, tandem spectra of molecules that belong to the same structural domain often provide unambiguous substructural pieces. Subsequently, identified substructure(s) were searched for structures in a publicly available structural database (PubChem) by restricting the search to consider only molecules that match the elemental composition determined from the pseudomolecural ion of the unknown.

Plenary Symposium: Plant Synthetic Biology

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Chris Paddon is a Principal Scientist at Amyris, Inc. in Emeryville, CA. He was project leader for the Semi-Synthetic Artemisinin project, and has subsequently led a number of projects at Amyris involving the use of synthetic biology for the production of natural products. He received his Bachelor's degree in Microbiology from The University of Surrey (UK), and doctorate in Biochemistry from Imperial College (London, UK). Following postdoctoral work at The National Institutes for Health (Bethesda, MD) he joined the pharmaceutical industry, working for Glaxo (now GSK; London, UK). He subsequently worked for Affymax (Palo Alto, CA) and Xenoport (Santa Clara, CA) before joining Amyris. He serves on the Scientific Advisory Board of Zagaya, a non-profit organization dedicated to the eradication of malaria, and furthering the impact of semi-synthetic artemisinin.

[P-2] Semi-synthetic Artemisinin: Using Synthetic Biology to Develop Industrial Production of an Essential Plant Natural Product

Chris J. Paddon

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There are estimated to be over 200 million clinical episodes of malaria annually, caused by the Plasmodium parasite, with approximately 660,000 deaths. Artemisinin is an antimalarial drug that is the key component of Artemisinin-based Combination Therapies (ACTs), the treatment recommended by the World health Organization for uncomplicated Plasmodium falciparum malaria. However, the supply and price of artemisinin (extracted from the plant Artemisia annua) has fluctuated greatly. The semi-synthetic artemisinin project was set up to develop an alternative source of artemisinin independent of the plant supply, resulting in stabilization of both the supply and price of the drug.

Initial strain engineering used E. coli to produce the artemisinin hydrocarbon precursor amorphadiene,[1] but efforts to functionally express the cytochrome P450 that oxidizes amorphadiene to artemisinic acid (the feedstock for chemical conversion to artemisinin) were unsuccessful. Subsequently, yeast was engineered to produce high concentrations of amorphadiene,[2] and following engineering of the enzymes responsible for its oxidation, high concentrations of artemisinic acid.[3] Fermentation and chemical development enabled industrial-scale production. Yeast strains developed by Amyris are now being used by the French pharmaceutical company Sanofi to produce semi-synthetic artemisinin for the developing world.[4] The creation of strains for the production of artemisinic acid represented the first use of synthetic biology for development of a pharmaceutical product. The development and potential uses for synthetic biology in the production of plant natural products will be discussed.

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Symposium VII: Phytochemicals, Pathogens and Insects

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Dr. Reuben Peters is a Professor in the Department of Biochemistry, Biophysics & Molecular Biology at Iowa State University in Ames, Iowa, USA (www.bbmb.iastate.edu/reuben-peters/). He received his PhD from the University of California at San Francisco with David Agard (1998), and was a Fellow of the Jane Coffin Childs Memorial Fund at Washington State University with Rodney Croteau (1998-2002). His research focuses on diterpenoid biosynthesis and physiological function. Their production proceeds via complex biochemical/enzymatic mechanisms, and the resulting natural products are important in host-microbe interactions (particularly for plants), with some having proven to be effective pharmaceutical drugs. Thus, this research has potential implications for both plant and human health. Accordingly, his research is funded by the US National Institutes of Health (NIH), US Department of Agriculture (USDA), as well as, previously, the US National Science Foundation (NSF). Reuben has received several awards, including Fellowships from the Jane Coffin Childs Memorial Fund for Medical Research and the Alexander von Humboldt Foundation, along with the Neish Young Investigator Award from PSNA.

[KS-5] Manifold Roles for Rice Diterpenoids

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Rice (Oryza sativa) is an important crop plant whose suspectibility to disease can critically impact food security. Among the means by which rice resists microbial pathogens is the production of diterpenoid phytoalexins, although the importance of such natural products biosynthesis is not fully understood. My group has taken a systematic approach towards elucidating both the underlying metabolic network and the physiological relevance of the resulting diterpenoids. Our work on diterpenoid metabolism has been based on the extensive sequence information available for rice (i.e., functional genomics), and includes the discovery of relevant biosynthetic gene clusters, as well as development of a synthetic biology approach in which we reconstitute rice metabolic pathways in E. coli, relying on the use of synthetic genes for functional incorporation of cytochrome P450 mono-oxygenases. Based on our comprehensive map for the early steps mediated by diterpene synthase in rice metabolism, we also are now taking a reverse genetic approach towards elucidating the biological function of the resulting diterpenoid natural products, including the application of genome editing technology as well as the identification of relevant lines from previous large scale insertional mutagenesis projects. Notably, the rice diterpenoids appear to serve as allelochemicals, as well as phytoalexins against important fungal and bacterial pathogens.

Symposium VII: Phytochemicals, Pathogens and Insects

[KS-6] Evolution and Impact of Cyanogenic Glucosides in Zygaena Moths

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Cyanogenic glucosides (CNglcs) are prevalent bioactive defense compounds in the plant kingdom. In the animal kingdom they are restricted to a few lineages, primarily to butterfly and moth species. The toxicity of the compounds stem from hydrogen cyanide released from the CNglcs by hydrolyzing enzymes upon tissue disruption. Six-spot burnet moth (Zygaena filipendulae, Zygaenoidea) larvae are able to sequester (selectively remove and incorporate into their own tissues) the CNglcs linamarin and lotaustralin from their food plant (Lotus corniculatus), as well as to carry out de novo biosynthesis of the exact same compounds from the parent amino acids valine and isoleucine. During the last 10 years we have developed the model system of the refined interactions between the six-spot burnet moth and its food plant Lotus to start to unravel the molecular basis for the evolution of the pathway and the impact of CNglcs on the different Z. filipendulae life stages. We have shown that Z. filipendulae larvae combine behavioural, morphological, physiological and biochemical strategies at different time points of feeding and digestion to avoid toxic hydrolysis of the CNglcs present in their Lotus food plant. The CNglcs are not only defense compounds, but are intimately integrated in all life stages such as mating, mate-calling and courting. The biosynthetic pathway is therefore carefully regulated through the different life stages in a sexdependent manor to secure an optimal content and ratio of CNglcs, regardless of the composition in the feed plant. We have identified the genes and biochemically characterized the encoded enzymes in the biosynthetic pathway in Z. filipendulae. A phylogenetic analysis revealed that although plants and Z. filipendulae synthesize CNglcs in essentially the same way, the pathways have evolved convergently in the two kingdoms.

[OP-12] Rhizosphere ginsenosides affect *Pyhtium irregulare* growth *in vitro* and *in vivo*

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Ginsenosides are triterpenoid saponins produced by American ginseng (Panax quinquefolius L.) that possess mild antifungal properties against pathogenic fungal species in vitro. However, growth of the oomycete Pythium irregulare, a major threat to commercial ginseng cultivation, is stimulated when exposed to ginsenosides. In the present study we tested the impact of ginsenosides in the rhizosphere on the ability of P. irregulare to cause disease on ginseng seedlings. Disease severity and Time to Infection (TTI) was evaluated non-destructively using measurements of leaf fluorescence as a proxy for stress. While both ginsenoside-treated and untreated ginseng plants showed signs of infection, the TTI for the untreated plants was earlier. The in vitro exposure of P. irregulare to purified ginsenoside extracts through disc diffusion assays demonstrated enhanced, but altered mycelial growth in a dose-dependent manner. Together these results suggest that when P. irregulare encounters ginsenosides in the rhizosphere, it undergoes an alteration in growth pattern, temporarily delaying infection, but also potentially building up inoculum. These results demonstrate that rhizosphere ginsenosides affect the growth pattern of P. irregulare in vivo, which likely impacts the severity of its pathogenicity.

[OP-13] Waxes Coating Fern Fronds: Fatty Acid Derivatives and Secondary Metabolites

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Fern fronds are coated by true cuticles resembling those of higher vascular plants. However, it is not clear how far wax compositions vary between fern species, and between ferns and gymno-/angiosperms. Here, the cuticular wax components of five fern species occurring in British Columbia were analyzed by GC–MS and GC–FID. Esters were found to be the most abundant compound class by weight in all species, with high chain length and isomer diversities. The fern waxes further comprised very–long–chain primary alcohols, fatty acids, aldehydes, alkanes, secondary alcohols, ketones, and some secondary metabolites such as fernene and β –sitosterol. The secondary alcohols and ketones identified in the fern waxes had typcial polyketide structures, different from those found in other vascular plants like Arabidopsis thaliana. This result, together with other characteristics of the fern wax mixtures, has significant implications on the evolutionary history of wax composition and biosynthesis.

Symposium VII: Phytochemicals, Pathogens and Insects

[OP-14] Exploring Plant Defense Theory in Tall Goldenrod

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Understanding the evolutionary reasons for patterns of chemical defense in plants is an ongoing theoretical and empirical challenge. The goal is to develop a model that can reliably predict how defenses are distributed within the plant over space and time. This is difficult given that evolutionary, ecological, and physiological processes and tradeoffs can operate over different spatial and temporal scales. We evaluate the major predictions of two leading defense theories, growth-differentiation balance (GDBH) and optimal defense (ODT). To achieve this, enemies, fitness components, terpenoids, and protease inhibitors were measured in Solidago altissima and used to construct conventional univariate and structural equation models (SEM). Leaf-tissue value indices extracted from an SEM revealed a strong correlation between tissue value and terpenoid defense that supports ODT. A tradeoff between serine protease inhibition and growth as well as an indirect tradeoff between growth and terpenoids manifested through galling insects supported the GDBH. Interestingly, there was a strong direct effect of terpenoids on rhizome mass, suggesting service to both storage and defense. The results support established theories but unknown genotypic traits explained much of the variation in defense confirming the need to integrate emerging theories such as pollination constraints, defense syndromes, tolerance, mutualisms, and facilitation.

[OP-15] Tobacco Sbip-428: A Sir2 Like Deacetylase, And Its Role In SA Mediated Pathway

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Salicylic acid (SA) plays an important role in various plant processes including disease resistance signaling. SA-Binding Protein 2 (SABP2) is an esterase like enzyme that catalyzes the conversion of methyl salicylate (MeSA) to SA and activating both local and systemic acquired resistance (SAR). SBIP-428 (SABP2 Interacting Protein-428), a SIR2 like deacetylase, has been found to interact with SABP2 in a yeast-two hybrid screening. Several recent studies have identified large scale post-translational modification of key cellular enzymes via acetylation, suggesting a key role for deacetylases in various signaling mechanisms. Recombinant purified SBIP-428 exhibits deacetylase activity using a commercial deacetylase assay kit. Further biochemical characterization is in progress to determine the effect of SABP2 and SA on the enzymatic activity. Acetylation of SABP2 was examined using recombinant SABP2 as well as native SABP2. Induction of SBIP-428 mRNA expression upon pathogen treatment was assessed. Arabidopsis thaliana homolog of tobacco SBIP-428 was identified and its corresponding T-DNA insertion mutant obtained for ABRC. A.t. mutant shows altered response to pathogen infection. Deciphering the role of SBIP-428 in the SABP2 dependent SA mediated defense pathway will help develop a better understanding of the regulation of SABP2 in the plant signaling pathway.

Symposium VII: Phytochemicals, Pathogens and Insects

[OP-16] Evolutionary Cues From Functional Switching Of Two Closely Related Class Ii Diterpene Synthases

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Class II diterpene synthases (diTPSs) catalyze the first committed step in diterpenoid biosynthesis by forming specific enantiomers of bicyclic copalyl/copalol-diphosphates from geranylgeranyl-diphosphate. The functional understanding of class II diTPSs has rapidly progressed with reported crystal-structures of the Arabidopsis ent-copalyl diphosphate synthase, involved in biosynthesis of gibberellin phytohormones in general metabolism. However, more functional studies are needed to clarify the basis for the great diterpenoid diversity of the specialized metabolism. We have identified two Tripterygium wilfordii class II diTPSs, TwTPS14 and TwTPS21 that diverge at 13 sites. Despite this similarity, the functionally expressed enzymes display distinct product profiles; TwTPS21 produces entcopal-8-ol diphosphate while the hydroxylated diphosphate product of TwTPS14 does not match any known diterpenoid intermediate. Here we show that the reciprocal exchange of two residues, 265H/Y and 325A/V, located in the active sites of models of TwTPS14 and TwTPS21, resulted in a complete product interchange, while substituting either of the residues gave mixed product profiles of hydroxylated diphosphates. Interestingly, two variants, TwTPS14_Y265H and TwTPS21_A325V, also produced the gibberellin precursor ent-copalyl-diphosphate.

This study exemplifies how specialized metabolism may have evolved from general metabolism in T. wilfordii and contributes to our understanding of the structure-function relationship of class II diTPSs.

[OP-17] Amides and Steroids from *Manekia obtusa* (Piperaceae) and Their Synergistic Activity Against Insect Herbivores

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The phytochemical study of the dichloromethane extract from Manekia obtuse (Piperaceae) resulted in the isolation of five substances: two amides, (S)-aegeline (**MO 1**) and cinnamamide (**MO 2**), and three steroids β-sitosterol, stigmasterol (**MO3A** and **MO3B**) and the estigmast-4-en-3-one (**MO 4**). We have developed a scalable enantioselective synthesis of aegeline (**MO 1**), isolated other co-occurring sterols and cinnamide, and evaluated their biological activity in the generalist (Spodoptera) herbivore bioassay and an immune assay on a specialist (Eois) herbivores. For the generalist, sitostenone-only diets reduced survival of individuals; however, this effect was ameliorated when mixed with sterols. Interestingly, the full mixture [sterols, cinnamide and aeglenine (1)] acted synergistically to enhance the toxicity to individuals - measured by lower survival accompanied by accelerated development times. An enantiomeric analog of aegeline (**N MO1**) was synthetized by our lab and evaluated in analogous mixture bioassays. Remarkably, this analog enhanced the survival of the generalist caterpillar relative to the control when evaluated alone. The positive effects that this analog had on the survival of the caterpillar were reversed when the analog was evaluated in a mixture with cinnamide, resulting in increased toxicity.. For the specialist, both aegeline (**N MO1**) and the analog (R)-1 demonstrated high bioactivity by completely inhibiting the specialist caterpillar's immune response both on their own and in mixtures. These preliminary results with the generalist support the hypothesis that synergistically acting mixtures can compensate for inactive molecules.

Symposium III: Plant Systems Biology

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Dr. Jörg Schwender is a tenured scientist in the Biosciences Department of Brookhaven National Laboratory, Upton, NY (http://www.bnl.gov/biosciences/staff/Schwender.php). He received his PhD from the Technical University Karlsruhe, Germany (1999), where he applied stable isotope tracer techniques to show that in higher plants the plastidial biosynthesis of terpenoids occurs by a formerly unknown non-mevalonate pathway. He then was a research associate at Michigan State University (2000-2005) working on the function of central carbon metabolism in lipid synthesis in developing oil seeds and developing ^{13}C-Metabolic Flux Analysis approaches in plants. At Brookhaven National Laboratory his research is funded mainly by the Department of Energy (Basic Energy Sciences). His research interests are in quantitative experimental analysis of metabolism and modeling of plant metabolism based on methods like Elementary Flux Modes Analysis, ^{13}C-Metabolic Flux Analysis and genome-scale constraint-based modeling, as well as systems biology approaches integrating transcriptome data with enzyme activity profiles, metabolic flux and targeted metabolic profiling.

[KS-7] Quantitative Analysis of Metabolism on a Network Scale Unlocks the Complexity of Central Carbon Metabolism

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Plant metabolism stands out for its remarkable complexity as well as redundancy of pathway functions. With now more than 50 published plant genomes available, exploration of in-silico representations of metabolism (genome scale metabolic models, GEM) comes into focus; with the prospect to give plant scientists the means to comprehensively explore metabolic capabilities of different model and crop plant species. However, by comparison to well-studied microbial model organisms it becomes clear that all plant genomes available to date are vastly underexplored and that the representation of all enzyme reactions by stoichiometric equations is challenging considering the extensive promiscuity in enzyme activity in particular in plant secondary metabolism. More challenges exist with regards to the diversity in cell type specific metabolism and the complex metabolic compartmentation. As opposed to pure in-silico approaches, we combine experimental and modeling approaches to gain predictive understanding of plant metabolism, with a focused on central (primary) metabolism and biosynthesis of cell mass. To understand carbon partitioning in developing oilseeds of rapeseed (Brassica napus) we developed a large scale, genome referenced metabolic model with emphasis on the biosynthesis of major cellular compounds. Based on Flux Balance Analysis we computationally explored pathway usage and analyze shifts in pathway usage by simulation of biomass component tradeoffs. Some model predictions can be compared to and validated by experimental data. We characterized 9 Brassica napus genotypes that represent genetic variability in seed storage product accumulation by a multi-omics approach. Developing embryos were grown in-vitro under well controlled conditions which allows performing ^{13}C-Metabolic Flux Analysis, metabolome- and proteome analysis as well as enzyme activity profiling in parallel. Across the genotypes, storage lipid content increased as a tradeoff with starch. Correlation analysis indicates that this biomass component tradeoff is orchestrated at the gene expression level as well as by posttranslational metabolic regulation.

Symposium III: Plant Systems Biology

Adrienne H.K. Roeder, Ph.D.
Nancy M. and Samuel C. Fleming Term Assistant Professor
Weill Institute of Cell and Molecular Biology and Plant Biology Department
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Dr. Adrienne Roeder is a Nancy M. and Samuel C. Fleming Term Assistant Professor in the Weill Institute of Cell and Molecular Biology and in the Department of Plant Biology at Cornell University, Ithaca, New York (http://plantbio.cals.cornell.edu/people/adrienne-roeder). She received her Ph.D. from the University of California, San Diego (2005), and was a Helen Hay Whitney and Moore Cell Center postdoctoral fellow at California Institute of Technology (2005-2011). Her research uses a systems biology approach to investigate the interface between cell growth, cell division, patterning and organogenesis. She uses the Arabidopsis sepal as a model system because the sepal organ size is highly reproducible, yet the size of the constituent cells is diverse and developmentally patterned. Her research focuses on the questions of how diversity is generated at the cellular level and how uniformity is achieved on the organ scale despite variability on the cellular scale. Her research is funded by the National Science Foundation and the Human Frontiers in Science Program.

[KS-8] A Systems Biology Approach to Understanding the Relationship between Plant Cell Size and Organ Size

Adrienne H.K. Roeder

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One of the biggest mysteries in both plant and animal development is how organ size is controlled. The development of plant floral organs is particularly robust; within a species, flower size is nearly invariant. Yet, the behavior of individual cells within the organs is often highly variable. For example, the outer sepal epidermis in Arabidopsis thaliana contains cells with a huge diversity of sizes ranging from giant cells about 1/5 the length of the sepal to small cells. Through live imaging and computational modeling, we have previously shown that variability in the time at which cells divide or enter endoreduplication (a specialized cell cycle in which the cell replicates its DNA, but fails to divide) accounts for the diversity of cell sizes, without perturbing the size of the organ (Roeder et al. 2010 PLoS Biology). This model rests on two assumptions: 1) The growth rate is uniform between cells, and 2) the choice to become a giant cell is random. Here, we test these two assumptions. First we examine the growth of individual cells and compare it with the growth of the tissue. We find that although the cellular growth rate is not uniform, the growth of individual cells can largely be predicted from the growth of the entire organ, suggesting the variability of individual cells contributes to the regularity of the overall organ. Second we find that the decision of a cell to become giant depends on the dosage of the transcription factor ATML1, suggesting the hypothesis that giant cells form based on stochastic fluctuations in ATML1 level. Thus, counterintuitively, our results suggest that cellular variability leads to reproducible organogenesis.

Symposium III: Plant Systems Biology

[OP-18] From Temporal Systems Biology to Regulatory Networks In The Microalga *Chlamydomonas Reinhardtii*

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Drastic alteration in macronutrients causes large changes in gene expression in the photosynthetic unicellular alga Chlamydomonas reinhardtii. A multi-omic investigation demonstrated that C. reinhardtii cells follow a biphasic response to nitrogen deprivation centered around the initiation of lipid accumulation between 4 and 6 h after N depletion. A drastic repatterning of metabolism also followed this biphasic modality, starting with a largely gluconeogenic metabolic state that transitioned to a glycolytic stage. Down-regulation of carbon assimilation and chlorophyll biosynthesis and an increase in nitrogen metabolism accompanied initiation of lipid biosynthesis. In addition, changes in transcript and protein levels of 414 predicted transcription factors and transcriptional regulators were monitored relative to other genes and classified by two separate measures: up-regulated versus down-regulated and early response versus late response relative to the two phases of polar lipid synthesis. Lipidomic and primary metabolite profiling generated compound accumulation levels that were integrated with the transcript dataset and transcription factor profiling to produce a transcriptional regulatory network. Evaluation of this proposed regulatory network led to the identification of several regulatory hubs that control many aspects of cellular metabolism, from N assimilation and metabolism, to central metabolism, photosynthesis and lipid metabolism.

[OP-19] Soybean 14-3-3 Proteins and Gmmyb176 Interactome: The Key Players Involved in the Regulation of Isoflavonoid Biosynthesis

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Isoflavonoids are a group of plant natural compounds synthesized almost exclusively by legumes and are abundant in soybean seeds. They play important roles in the induction of nod gene expression in Rhizobia that form nitrogen fixing nodules on soybean roots and plantmicrobial interactions. Isoflavonoids also contribute to the positive health effects associated with soybean consumption by humans and animals. By gene expression analysis, we identified CHS7 and CHS8 as genes that are critical for isoflavonoid synthesis. Expression of CHS8 gene is regulated by an R1 MYB transcription factor, GmMYB176, thus affecting isoflavonoid synthesis in soybean. The regulation of CHS8 expression and isoflavonoid biosynthesis by GmMYB176 requires combinatorial action of other additional factors. The recent work is focused on identifying the interacting proteins with GmMYB176 to achieve a detailed understanding of the regulation of isoflavonoid synthesis in soybean. Approaches including protein-protein interactions, functional genomics and molecular genetics are used to address these goals. Candidate GmMYB176 interactors such as bHLH, 14-3-3, kinases, WD40 and proteins with no functional domains are identified.

Symposium I: Biosynthesis of Plant Natural Products

Natalia Dudareva, Ph.D.
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West Lafayette, IN, USA

Natalia Dudareva is a Distinguished Professor in the Department of Biochemistry at Purdue University in West Lafayette, Indiana. She received her B.S. in Biology and Biochemistry and her M.S. in Biochemistry from Novosibirsk State University, Russia. She received a Ph.D. in Biochemistry and Molecular Biology from the Institute of Biochemistry in Kiev, Ukraine, as well as a Ph.D. in Plant Molecular Biology at the University of Louis Pasteur in Strasbourg, France (1995). Natalia did her postdoctoral research at the University of Windsor (Canada) and the University of Michigan, Ann Arbor (1995-1997). Her research focuses on the identification of biochemical and molecular mechanisms controlling the formation of an array of primary and secondary metabolites in plants, with emphasis on carbon flux distribution through two major metabolic networks (phenylpropanoid and terpenoid) using flowers as a model system. Natalia is elected fellow to the American Association for the Advancement of Science and has been acknowledged with a number of awards from Purdue University, including University Faculty Scholar, winner of the Agricultural Research Award and the Wickersham Chair of Excellence in Agricultural Research.

[KS-9] Aromatic Amino Acid Network: Biosynthesis, Regulation and Transport

Natalia Dudareva

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Phenolic compounds are ubiquitous throughout the plant kingdom constituting one of the largest and most diverse classes of plant secondary metabolites that have profound impacts on plant growth, development, reproduction and defense. All these phenolic compounds are derived from phenylalanine (Phe), an essential amino acid that humans cannot synthesize and must obtain directly or indirectly from plants. Phe biosynthesis in plants occurs via two alternative routes with either arogenate or phenylpyruvate as a key intermediate. Using petunia flowers as a model system, we have shown that plants predominantly synthesize Phe in plastids via the arogenate pathway while they can also utilize the microbial-like phenylpyruvate pathway. Moreover, flux through the phenylpyruvate route is increased when the entry point to the arogenate pathway is limiting. Interestingly, the phenylpyruvate pathway utilizes a cytosolic aminotransferase that strongly favors tyrosine as the amino donor. These results demonstrate that Phe biosynthesis is not limited to plastids and that there is an interconnection between aromatic amino acid catabolism and biosynthesis in planta. While Phe is primarily synthesized in plastids, it must be exported to the cytosol in order to participate in protein biosynthesis and serve as a precursor for phenolic compounds. Based on homology with E. coli PheP and co-expression with plant Phe metabolic genes, we have identified a gene encoding a Petunia hybrida plastidial cationic amino acid transporter (PhpCAT) that participates in plastidial Phe export. GFP fusion experiments confirm that the PhpCAT N-terminal presequence serves as a plastidial targeting peptide. Expression of PhpCAT correlates spatially, developmentally, and temporally with genes involved in biosynthesis of Phe and Phe-derived volatiles. RNAi down-regulation of PhpCAT resulted in reduced emission of cytosolically-synthesized Phe-derived volatiles while PhpCAT over-expression increased their levels. The impact of Phe export from plastids on regulating fluxes within the Phe metabolic network will be discussed.

Symposium I: Biosynthesis of Plant Natural Products

Jose M. Alonso, Ph.D.
Professor and University Faculty Scholar
Department of Plant and Microbial Biology
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Dr. Jose M. Alonso is a Professor and University Faculty Scholar in the department of Plant and Microbial Biology at North Carolina State, Raleigh, NC, USA (http://www4.ncsu.edu/~jmalonso/Alonso-Stepanova_Home.html). He received his PhD from the University of Valencia, Spain (1994), was postdoctoral fellow at the University of Pennsylvania, USA (1995-2000), and research associate at the Salk Institute for Biological Research, (2000-2001). His main interest is to understand the molecular circuits plants use to integrate environmental and developmental signals to produce specific responses. Towards this general goal his group has been focusing on the identification of the molecular "signal integrators" or "logic gates" involved in the interaction between two plant hormones, ethylene and auxin, in the regulation of root growth. His research has shown that ethylene activates the transcription of auxin biosynthetic genes in the root meristem (root tip) and then auxin is transported upwards to where it sensitizes the cells in the division zone enabling them to properly respond to ethylene. This research has been continuously funded by the National Science Foundation since 2003. Dr. Alonso is a member of the editorial board of The Plant Journal, an elected member of the North American Arabidopsis Steering Committee, Member of the Advisory Committee of The Arabidopsis Biological Resource Center and Director of the NCSU Plant Recombineering Center.

[KS-10] Auxin Biosynthesis and Its Regulation

Jose M. Alonso

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Auxin is an essential plant hormone that regulates nearly every aspect of plant life cycle. In the past three decades much has been learned about auxin transport, perception, signaling, and response. In contrast, the understanding of auxin biosynthesis and its regulation has lagged behind. Recent identification of several key auxin biosynthetic genes, TAA1/TARs and YUCs, revealed that plants control auxin production on a much finer spatial and temporal scale than previously thought. We are investigating how auxin biosynthesis is regulated focusing on the identification of cis- and trans-regulatory elements that control expression of the TAA1 gene. In parallel, variety of genetic and molecular approaches is being employed to test the physiological significance of the spatiotemporal changes in the levels of auxin biosynthetic enzymes. We are also exploring the roles of additional postulated routes of auxin production independent of the primary TAA1/TAR- and YUC-mediated IPyA branch. Finally, we are taking a systems approach to understand how the different routes of auxin biosynthesis are integrated and interact with the rest of the shikimate pathway at large. The latest developments in these different areas of auxin biology will be presented.

Symposium I: Biosynthesis of Plant Natural Products

[OP-20] Cad2 Deficiency Causes both Brown Midrib And Gold Hull And Internode Phenotypes In *Oryza Sativa*

Taichi Koshiba[1], Shinya Murakami[1], Takefumi Hattori[1], Mai Mukai[1], Akira Takahashi[2], Akio Miyao[2], Hirohiko Hirochika[2], Shiro Suzuki[1], Masahiro Sakamoto[3], Toshiaki Umezawa[1 4]

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Several brown midrib (bm) mutants have so far been isolated from the C4 grasses, maize, sorghum and pearl millet, but have not been detected in C3 grasses including rice (Oryza sativa). In the present study we characterized the cad2 (cinnamyl alcohol dehydrogenase 2) null mutant isolated from retrotransposon Tos17 insertion lines of Oryza sativa L. ssp. japonica cv. Nipponbare. This mutant exhibited brown-colored midribs in addition to hulls and internodes, clearly indicating both bm and gold hull and internode (gh) phenotypes. An OsCAD2 RNAi knock-down plant also exhibited brown coloration in the midrib. The enzymatic saccharification efficiency in the culm of the cad2 null mutant was increased by 16.1% than that of the control plants. The lignin content of the cad2 null mutant was 14.6% lower than that of the control plants. Thioacidolysis of the cad2 null mutant indicated the presence of cinnamaldehyde structures in the lignin. Taken together, our results show that deficiency of OsCAD2 causes the bm phenotype in addition to gh, and that the coloration is probably due to the accumulation of cinnamaldehyde-related structures in the lignin. Additionally, this cad2 null mutant is useful to silage purposes and biofuel production.

[OP-21] Functional Characterization Of Two Novel Bahd Acyltransferases From *Populus Trichocarpa*, Sabt & Bebt, Which Are Potentially Involved In Salicinoid Phenolic Glycoside Synthesis

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The salicinoid phenolic glycosides (PGs) are phytochemicals characteristic of the Salicaceae family, produced as a chemical defense against pathogenic attack and herbivory. The PGs are comprised of ester bound glucose, salicylic alcohol and phenolic moieties. Examples include: salicin, salicortin, tremuloidin, and tremulacin which accumulate to high concentrations in the leaves and bark of willows and poplars. Here we describe the transcriptional and phylogenetic analysis, cDNA cloning, heterologous expression, and in vitro functional characterization of two BAHD acyltransferases. Recombinant protein of one of these enzymes exhibited a wide range of activities with highest activity observed for co-substrates benzoyl-CoA with salicylic alcohol, yielding salicylbenzyl benzoate and was named benzoyl-CoA:salicylic alcohol O-benzoyl transferase (SABT). The second enzyme had a comparatively narrow range of activities. Its greatest affinity was observed for benzoyl-CoA with benzyl alcohol yielding benzyl benzoate and was named benzoyl-CoA:benzyl alcohol O-benzoyl transferase (BEBT). We interpret our data with reference to a previously hypothesized biosynthetic pathway for PG synthesis, and propose potential roles of these enzymes in the PG pathway.

Symposium I: Biosynthesis of Plant Natural Products

[OP-22] Mechanistic Analysis of the *Ent*-copalyl Diphosphate Synthases Required for Plant Gibberellin Hormone Biosynthesis Leads to Novel Product Chemistry

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Labdane related diterpenoids (LRDs) form a large class of natural products, with ~7,000 known. LRD biosynthesis begins with the class II protonation-initiated bicyclization of (E,E,E)-geranylgeranyl pyrophosphate (GGPP), most often with immediate deprotonation to yield the eponymous labdadienyl/copalyl diphosphate (CPP). The class II diterpene cyclase from Arabidopsis thaliana (AtCPS) catalyzes the production of ent-CPP in gibberellin phytohormone biosynthesis. The crystal structure for this enzyme was recently determined, and has been used here to begin investigating enzymatic structure-function relationships. Here we identify the catalytic base group, which is composed of an activated water molecule tightly bounded by an active site His and Asn dyad that are conserved in all class II diterpene cyclases involved in gibberellin biosynthesis. Substitution of Ala for either or both of these residues produces novel hydroxylated variants of ent-CPP. This result provides clear insight into the structure-function relationships of class II diterpene cyclases, and illustrates the plasticity of these enzymes that presumably underlies the observed extensive diversification of the labdane-related diterpenoids.

Plenary Symposium: Botanic Medicines

Nicholas H. Oberlies, Ph.D.
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Nick received his B.S. in Chemistry from Miami University (1992) and his Ph.D. in Medicinal Chemistry and Pharmacognosy from Purdue University (1997), where he studied under Professor Jerry L. McLaughlin. He then spent a year as a postdoctoral chemist at American Cyanamid, where he investigated leads with insecticidal, herbicidal, and fungicidal properties from natural sources. In 1998, he joined Research Triangle Institute, specifically to be mentored by Dr. Mansukh Wani and the now late, Dr. Monroe Wall, who are the co-discoverers of taxol and camptothecin. He rose through the ranks of RTI and eventually directed the Natural Products Laboratory. In 2009, he moved his group to the Department of Chemistry & Biochemistry at the University of North Carolina at Greensboro, who has a relatively new Ph.D. program in Medicinal Biochemistry that has an emphasis in natural products chemistry. There he leads a multidisciplinary effort to characterize and develop new chemical entities from natural sources. Over the past ten years, and often in close collaboration with Dr. Cedric Pearce of Mycosynthetix, Inc., his lab has worked to profile fungi for leads in diverse areas, including herbicidal, anticancer, and anthelmintic.

[P-3] Chemistry of the Endophytic Fungi of the Medicinal Herb Milk Thistle (*Silybum Marianum*)

Nicholas H. Oberlies

Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402

The Oberlies Lab has ongoing research projects that study fungal cultures, particularly those collected from leaf litter, for bioactive drug leads. We have also studied the chemistry of the medicinal herb, milk thistle [Silybum marianum (L.) Gaertn. (Asteraceae)], for over a decade. Recently, we have combined these pursuits in a project to study the endophytic fungi of milk thistle. Over forty fungal isolates were identified from milk thistle, comprising 25 OTUs based on ITS rDNA sequence data. Based on Maximum Likelihood analysis of partial LSU rDNA, these endophytes displayed phylogenetic affinities to four major classes of Ascomycota, which included the Dothideomycetes, Sordariomycetes, Eurotiomycetes, and Leotiomycetes. Chemistry studies on solid-substrate fermentation cultures led to the isolation and characterization of scores of compounds. Highlights of the chemical mycology of two of these will be presented, including stimulation of the production of flavonolignans and the DESI-MS imaging of polyhydroxyanthraquinones.

Symposium V: Botanical Medicines

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Professor and Jack L. Beal Chair
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Dr. A. Douglas Kinghorn holds the position of Professor and Jack L. Beal Chair in Natural Products Chemistry and Pharmacognosy at the College of Pharmacy, The Ohio State University. He received Ph.D. (1975) and D.Sc. (1990) degrees from The School of Pharmacy, University of London. From 1977-2004, he was a faculty member at the College of Pharmacy, University of Illinois at Chicago. Dr. Kinghorn is a Fellow of the Royal Pharmaceutical Society (London), the American Association of Pharmaceutical Scientists, the American Association for the Advancement of Science, and of the School of Pharmacy, University College London. He received the 2010 Norman R. Farnsworth Research Achievement Award of the American Society of Pharmacognosy (ASP) for lifetime contributions to natural products research. In 2011, Dr. Kinghorn was awarded an honorary D.Sc. degree from the University of Bradford in the U.K. He has authored or co-authored over 500 peer-reviewed research articles, review articles, and book chapters. His research interests are the isolation and structural characterization of bioactive natural products from higher plants, particularly potential anticancer agents, antileishmanial compounds, cancer chemopreventives, and taste-modifying substances. He is the Editor in Chief of the Journal of Natural Products and of the book series Progress in the Chemistry of Organic Natural Products (Springer-Verlag, Vienna and New York). Dr. Kinghorn has presented nearly 300 invited lectures in over 30 countries. He has directly supervised about 45 graduate students and over 60 postdoctoral students and visiting scholars.

[KS-11] Aglaia Cyclopenta[b]Benzofurans as Potential Anticancer Agents

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The genus Aglaia Lour. is the largest genus of the angiosperm plant family Meliaceae with more than 120 species distributed in areas of southeast Asia and the Pacific region, of which some are used medicinally.[1] Since the 1982 discovery by King et al. of rocaglamide,[2] the first member of the cyclopenta[b]benzofuran ("flavagline") class from A. elliptifolia, more than 100 naturally occurring derivatives of this compound class have been isolated and structurally characterized from over 30 Aglaia species.[3] Silvestrol and episilvestrol represent a modified form of the flavagline compound class in containing a dioxanyl ring, and were first reported in 2004 from Aglaia foveolata.[4] These compounds have attracted considerable interest in the area of natural products-based drug discovery in past two decades, particularly in terms of their potential utility as anticancer agents. Several cyclopenta[b]benzofurans have shown antiproliferative activity against various cancer cell lines at nanomolar concentrations as well as in vivo efficacy in tumor-bearing murine models.[3] It has been demonstrated that the protein translation factor eukaryotic initiation factor 4F (eIF4F) is a likely mammalian cellular target for the cyclopenta[b]benzofurans. In this presentation, current information will be provided on the distribution of the cyclopenta[b]benzofurans within the genus Aglaia, and on their potential as human anticancer agents.

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Symposium V: Botanical Medicines

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Luc Pieters is a professor in Pharmacognosy and Natural Product Chemistry at the Department of Pharmaceutical Sciences, University of Antwerp, Belgium. He is head of the research group "Natural Products & Food Research and Analysis" (NatuRA) (https://www.uantwerpen.be/natura). His main research interests are the phytochemical characterization of plant extracts used in traditional medicine, their pharmacological evaluation, and the isolation and identification of their active constituents. He holds an academic appointment in the Faculty of Pharmaceutical Sciences at the University of Ghent, Belgium. Luc Pieters is a former recipient of the Dr. Paul A.J. Janssen Award for Medicinal Chemistry, the Rhône-Poulenc Rorer Award of the Phytochemical Society of Europe (PSE), and the Pharmaton Boehringer Ingelheim Award for Phytotherapy. Luc Pieters is author or coauthor of over 230 publications in international scientific journals. Since 2007 he is the Editor-in-Chief of Planta Medica – Journal of Medicinal Plant and Natural Product Research, the official organ of the Society for Medicinal Plant and Natural Product Research (GA).

[KS-12] Botanical Medicines against Malaria

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Infectious diseases such as malaria remain a major burden in many tropical and developing countries, especially in Africa. In many Asian and African countries a large part of the population still depends on traditional medicine (mostly based on plants) for primary healthcare. This is due in part to limited access to synthetic drugs, but also for cultural reasons. Scientific investigations on traditional herbal medicinal products can result in the characterization of new lead compounds, to be developed as new drugs as such or after chemical modification, but can also be considered as a tool for the valorization of traditional medicine. The use of botanical medicines can be supported if their activity is scientifically confirmed, and if they are safe. This implies the need for extract characterization and quality control ("evidence based traditional medicine"). On the other hand, their use should be discouraged if the activity cannot be confirmed, or if there is a risk of (chronic) toxicity. Various research projects in Tanzania, Guinea-Conakry and DR Congo have resulted in the isolation of constituents with antiplasmodial activity, and the phytochemical profiling of medicinal plant extracts used against malaria. Tingenin B, a triterpene with a broad antimicrobial activity, was isolated from Elaeodendron schlechteranum (Celastraceae). Ormocarpum kirkii (Papilionaceae) contained a series of 3-3' linked biflavonoids such as (+)-chamaejasmin, which may be responsible for its antimalarial activity. The alkaloid strictosamide is the active principle from Nauclea pobeguinii (Rubiaceae). Nevertheless it should be considered that many natural products, e.g. glycosides, are pro-drugs that have to be deglycosylated and activated first, and that metabolisation processes should be taken into account in the search for active principles.

Symposium V: Botanical Medicines

[OP-23] Alkylamides from *Echinacea* and their Effects on the Production of Inflammatory Mediators from Macrophages and Mast Cells

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Echinacea is a medicinal herb used for the treatment of respiratory viral infections. Although its effects are controversial, current evidence suggests that Echinacea extracts provide relief by reducing production of inflammatory mediators, thereby reducing the symptoms and pathology associated with respiratory infections. Our laboratories are studying the constituents of Echinacea extracts that mediate these effects. Echinacea spp. produce a group of hydrophobic molecules known as alkylamides, which consist of a fatty acid of varying chain length and saturation, coupled to an isobutyl amine head group. Studies from our laboratories show that these molecules act broadly to suppress production of pro-inflammatory mediators from murine macrophages. Suppression occurs with a variety of different agonists including live influenza virus, LPS, and CpG DNA. Alkylamides also inhibit mediator production from RBL-2H3 cells, a mast cell-like cell line, suggesting that alkylamides may be useful for inhibiting inflammation in a variety of pathological situations. Interestingly, structure:function studies indicate that fatty acid chain length and level of saturation are critical determinants of alkylamide anti-inflammatory action. In addition, we find that modifications of the isobutylamide moiety produce changes in alkylamide activity. Current efforts are targeted at developing natural product analogs with improved properties for the treatment of inflammation.

[OP-24] New Enzyme Inhibiting Phytochemicals and their Pharmaceutical Applications

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Natural products derived from plants, marine organisms and microorganisms exhibit interesting anti-microbial, anti-viral, and anti-inflammatory activities. These bioactivities make natural products an important source for the discovery of new pharmaceutical agents, and more than 60% of the drugs available on the market are of natural product origin. One of the aspects of drug discovery process is the identification of small molecules with enzyme-inhibiting activities. Enzymes are essential to human life, mediating biochemical processes including metabolism, cellular signal transduction, cell cycling, and development. Malfunction in these biochemical systems often leads to disease that can be caused either by the dysfunction, overexpression, or hyper-activation of the enzymes involved. An understanding of diseases at the molecular level has provided several enzyme inhibitors in clinics. For instance, galanthamine, a potent acetylcholinesterase (AChE) inhibitor, is used to treat Alzheimer's disease. α-Glucosidase (EC 3.2.1.20) is a membrane bound enzyme and lies at intestinal cells. This enzyme catalyzes the final step of carbohydrates digestion by hydrolyzing the glycosidic bonds in carbohydrates to liberate free glucose. The resulting glucose is a source of an exaggerated rise in blood sugar causing postprandial hyperglycemia. This causes type 2 diabetes mellitus and affects approximately 2.1 billion people worldwide. The potent α-glucosidase inhibitors prevent the breakdown of carbohydrates in small intestine and prolong the absorption of glucose or carbohydrates in blood. These compounds may be used as chemotherapeutic agents in clinics for the treatment of diabetes and obesity. Due to the catalytic role of α-glucosidase in carbohydrate digestion, these inhibitors may also be used as therapeutic target for other carbohydrate mediated diseases including viral infections, cancer, HIV and hepatitis. Our recent chemical investigation of Aboriginal medicinal plants of Canada and fungi resulted in the identification of natural products exhibiting potent bioactivities including anti-microbial, anti-α-glucosidase and anti-AChE activities. In this presentation, isolation, structure elucidation of new bioactive natural products and their structure-activity relationships will be discussed.

Symposium V: Botanical Medicines

[OP-25] Differentiation of The Four Major Species (*C. burmannii, C. verum, C. cassia, And C. loureiroi*) of Cinnamons Using a Flow-Injection Mass Spectrometric (FIMS) Fingerprinting Method

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A simple and efficient flow-injection mass spectrometric (FIMS) method was developed to differentiate cinnamon (Cinnamomum) bark (CB) samples of the four major species (C.

burmannii, C. verum, C. aromaticum, and C. loureiroi) of cinnamon. Fifty cinnamon samples collected from China, Vietnam, Indonesia, and Sri Lanka were studied using the developed FIMS fingerprinting method. The FIMS fingerprints of the cinnamon samples were analyzed using principal component analysis (PCA). The FIMS technique only required one minute analysis time per sample. The representative samples from each of the four major species of cinnamon were further examined using an ultra, high-performance liquid chromatography high-resolution mass spectrometry system and the chemical differences between the four species were profiled. The results showed that the 1-minute FIMS fingerprinting method successfully differentiated the four cinnamon species studied.

[OP-26] Multi-target Functionality of Alaskan Seaweed in Combatting Hyperglycemia and Type 2 Diabetes

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Two factors underlying the development of type 2 diabetes are prolonged hyperglycemia due to increased carbohydrate consumption, and chronic inflammation in adipose tissue and macrophages. Alaska Native populations, who suffer disproportionately high rates of diabetes, have shifted away from traditional dietary foods, including seaweeds, which are rich sources of phytochemicals with potential to counteract diabetes. In this work, Alaskan seaweeds Fucus distichus (FD), Saccharina latissima (SL), Saccharina groenlandica (SG), Alaria marginata (AM), Pyropia fallax (PF), and Ulva lactuca (UL) were evaluated for their potential to decrease carbohydrate metabolism and ameliorate symptoms of hyperglycemic-linked inflammatory pathways. High levels of polyphenolics were discovered in medium-polarity fractions of AM, FD, and SG (326.8 - 557.2 µg phloroglucinol equivalents (PGE)/mg extract). AM and FD demonstrated selective inhibition of α-glycosidase and α-amylase, with significantly lower IC_{50} concentrations compared to acarbose. Fractions of AM, FD, SG, and SL reduced nitric oxide levels in LPS-induced RAW 264.7 macrophages, and mRNA expression assays demonstrated that AM, SL, and UL reduced levels for inflammatory cytokines IL-10, MCP-1, COX2, and TNF-α by 92%, 89%, 82%, and 85%, respectively. These results suggest that Alaskan algae may alleviate hyperglycemia and other type 2 diabetes biomarkers by depressing inflammation and regulating carbohydrate digestion.

Symposium V: Botanical Medicines

[OP-27] Changes in the Secondary Metabolites During Ontogeny of *Piper Gaudichaudianum* Kunth

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Understanding the changes in secondary metabolism during plant development and the consequences of these changes on associate herbivores is at the forefront of research in chemical ecology. Comparisons of seedling and mature chemical composition within the genus Piper have revealed that chemical changes in plants during early development appear to be species specific. In the case of P. tuberculatum, adult plants and seedlings grown in vivo have similar compositions of amides. However, P. solmsianum, P. regnellii, and P. gaudichaudianum, showed significant chemical changes during development, commonly producing allylphenols, apiole and dillapiole as seedlings and tetrahydrofuran lignans, dihydrobenzofuran neolignans and gaudichaudianic acid as mature plants. In this work, analysis using 1H NMR combined with principal component analysis (PCA) and HPLC-DAD of crude extracts was carried out in order to understand the metabolic changes during ontogeny of P. gaudichaudianum. Such remarkable differences in chemical composition could be associated with a flexible defensive strategy protecting the venerable seedlings to a variety of pathogens. FAPESP, CNPq, CAPES and PRP-USP

Symposium IV: Renewable Petro Biofuel from Plants

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Danny Schnell is professor in the Department of Biochemistry and Molecular Biology and a member of the Plant Biology Program at the University of Massachusetts, Amherst and co-director of The Institute of Massachusetts Biofuels Research (TIMBR). He received his B.S. degree in Life Sciences from the University of Nebraska, Lincoln in 1983, and completed his Ph.D. at the University of California, Davis in 1987. He was an HHMI postdoctoral fellow with Dr. Günter Blobel in the Laboratory of Cell Biology at the Rockefeller University between 1988 and 1993. In 1993, he took his first faculty position as an assistant professor in the Department of Biological Sciences at Rutgers University in New Jersey. In 2001, he moved to his present position, where he is a full professor and past head of the Department of Biochemistry and Molecular Biology. The major research interests of my laboratory focus on 1) the biogenesis (growth, division, adaptation and maintenance) of cellular compartments and macromolecular machines in eukaryotic cells, and 2) the metabolic engineering of chloroplast metabolism for the development of sustainable and renewable food and bioenergy crops. His work is funded by the National Science Foundation (NSF), the National Institutes of Health (NIH), and the Department of Energy (DOE). Danny served on the editorial board of Plant Physiology from 2002-2007 and is currently on the editorial boards of Molecular Biology of the Cell, The Journal of Cell Biology, and PLoS Biology. His work has been recognized by numerous awards, including election as a Fellow of the American Society of Plant Biologists (ASPB) and the American Association for the Advancement of Science (AAAS).

[KS-13] Approaches to Increase Carbon Capture and Redistribution to Fuel Molecules and Co-products in Camelina Sativa

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Camelina sativa has shown considerable promise as a dedicated oilseed biofuels crop because it requires low agronomic inputs and Camelina oil-based blends have been tested and approved as liquid transportation fuels. We are addressing key limitations to yield by engineering Camelina using an innovative systems approach to identify and integrate key regulatory/limiting factors that will 1) increase carbon fixation in photosynthetically active source tissues, 2) enhance fixed carbon transport from source to sink tissues, and 3) maximize allocation to sink tissues by increasing metabolic flux to seed oil production. We have addressed the first rate limiting step by addressing that hypothesis that photosynthetic carbon fixation is limited by CO2 diffusion to Rubisco, thereby decreasing the ratio of productive carboxylation (photosynthesis) to non- productive oxygenation (photorespiration). We tested this limitation by expressing components of the microbial carbon concentrating mechanisms in Camelina chloroplasts to concentrate CO_2 at Rubisco. Expression of CO2/$HCO3^-$ transporters increased CO2 assimilation by 20-30% in source tissues and resulted in 20-44% increases in seed yields, consistent with our hypothesis that CO2/$HCO3^-$ flux limits carbon fixation at Rubisco. We currently are integrating these traits with Camelina lines engineered for enhanced triacylglycerol and monoterpene synthesis to increase fuel molecule production. We will present data from combined transcriptomics and metabolomics analyses of these transgenic lines that reveal strategies for increasing allocation of fixed carbon to the production of commercially valuable products.

Symposium IV: Renewable Petro Biofuel from Plants

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Dr. Cahoon earned his M.S. from Cornell University in plant physiology and his Ph.D. from Michigan State University in plant biochemistry and molecular biology. He completed post-doctoral research in the Biology Department at Brookhaven National Laboratory. He was a senior research scientist at DuPont /Pioneer HiBred in Wilmington, DE from 1997 until 2002 and was an Associate Member and USDA-ARS scientist at the Donald Danforth Plant Science Center in Saint Louis from 2002 until 2008. He joined the faculty of the Center for Plant Science Innovation and Department of Biochemistry at the University of Nebraska-Lincoln (UNL) in December of 2008 and became Director of the UNL Center for Plant Science Innovation in August 2010. He was appointed George Holmes University Professor of Biochemistry at UNL in 2012. Dr. Cahoon and his team conduct research to understand the biosynthesis of a variety of lipidic compounds in plants, including vegetable oils and lipid soluble vitamins and antioxidants, and to modify or transfer the production of these compounds to crop plants through biotechnology. His lab also conducts studies on sphingolipid metabolism and function in plant membranes and signaling pathways. His research has impact in the areas of biofuels, nutritional biofortification of crops, and improvement of crop productivity and quality. He has published over 70 peer-reviewed papers and is an inventor or co-inventor on 30 issued US patents.

[KS-14] Camelina: A Designer Oilseed Crop for Metaboli Engineering of Advanced Biofuels and Bio-based Lubricants

Edgar B. Cahoon

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Camelina sativa (false flax) is an emerging Brassicaceae oilseed crop for semi-arid regions, including the North American Great Plains and United States Pacific Northwest. The growing interest in camelina is due largely to its potential for biodiesel and jet fuel production in geographic areas that are not well-suited cultivation of established oilseed crops such as soybean. Camelina is also not widely grown for food use and, as a result, does not compete with food crops for prime agricultural land. Our research is focused on developing camelina as an industrial oilseed crop for the production of designer vegetable oils for lubricants, biofuels, and high-value industrial oils. Camelina is an ideal crop plant for metabolic engineering because it can be genetically transformed by a simple, non-labor intensive floral vacuum infiltration of Agrobacterium. We have also developed an extensive metabolic engineering toolbox of seed-specific multi-gene expression vectors and camelina genomic resources. With these tools, we have initiated a biotechnological pipeline to generate camelina lines with improved fuel and lubricant properties and to field test biotechnologically-enhanced lines. Among the biofuel traits arising from this pipeline are oils with improved oxidative stability and fatty acid compositions tailored for jet fuel applications. The latter project has tapped the biotechnological potential of camelina for extensive candidate gene evaluation to identify novel enzymes specialized for the accumulation of oils with short- and medium-chain fatty acids.

Symposium IV: Renewable Petro Biofuel from Plants

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Dr. Amy Grunden is a Professor of Microbiology in the Department of Plant and Microbial Biology at North Carolina State University. Dr. Grunden's area of expertise is in the physiology and biotechnological application of extremophiles (organisms capable of thriving in diverse extreme environmental conditions). She received her Ph.D. in Microbiology and Cell Science from the University of Florida and has over 15 years of academic research experience. As part of her research program, she has developed the use of extremophile enzymes for crop improvement, biofuel production, and biodecontamination. Her research program has received support from DOD, DOE, NASA, NCBC, NSF, USDA, and DSM Nutritionals, Inc.

[KS-15] Increasing Photosynthetic Co2 Capture in *Camelina Sativa* with a Synthetic Carbon Fixation Cycle Composed of Slected Microbial Enzymes

Amy M. Grunden

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Biofuels are a promising alternative to fossil fuels due to their ability to lower greenhouse gas emissions, provide sustainable supplies of energy and create cleaner environments. Camelina sativa has features that make it an ideal energy crop such as its short growing season and ability to grow on marginal lands with little water and nutrient provision. Its seeds contain a high amount of oil (up to 40%), and the remaining protein rich meal can be used for livestock feed. This study is focused on increasing the carbon fixation capabilities in Camelina sativa in order to enhance its oil and biomass production. A novel synthetic carbon fixation pathway, called the SynCycle, has been generated in order to overcome the CO_2-uptake and assimilation limitations of the conventional Calvin-Benson cycle. The SynCycle, which is the shortest, energetically feasible reverse TCA cycle known, is composed of five microbially derived enzymes, which will lower the energy cost by 30% compared to the conventional Calvin-Benson cycle. The product of the SynCycle, glyoxylate, will serve as an intermediate in an engineered photorespiration bypass that feeds into the Calvin-Benson cycle. Candidate enzymes for the SynCycle were identified and kinetic properties of the SynCycle enzymes, singly and in combination, were determined using a variety of spectrophotometric, LC-MS, and NMR techniques. Functional production of individual SynCycle enzymes in planta was evaluated using a transient tobacco expression system. The cycle was also recently transformed into Camelina sativa, and a cultivar is being established for evaluation of biomass and seed oil production.

[OP-28] Discovering Novel Interacting Proteins with Heteromeric Acetyl-coa Carboxylase

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Lipids that are eventually stored in the seed as triacylglycerols are produced through de novo fatty acid synthesis. In plants, this pathway is plastid-localized and begins with the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA catalyzed by acetyl-CoA carboxylase (ACCase). In dicots and non-graminaceous monocots, plastid ACCase is a heteromeric complex comprised of four catalytic subunits: biotin carboxylase, biotin carboxyl carrier protein, and α - and β -carboxyltransferase. This complex is influenced by a myriad of post-translational mechanisms including redox status, phosphorylation, and feedback inhibition. We hypothesize that regulatory proteins are involved in one or more of these processes. To discover potential effector proteins, we isolated chloroplasts from Arabidopsis thaliana seedlings and performed co-immunoprecipitations using antibodies against three different ACCase subunits. Precipitated proteins were quantitatively identified using tandem mass spectrometry. All four ACCase subunits were identified in most replicates with high abundance. Additionally, the entire plastid pyruvate dehydrogenase complex (pPDC) as well as three other non-enzymatic proteins was identified at levels similar to the ACCase subunits. Orthogonal analysis of ACCase protein-protein interactions by Blue Native-SDS PAGE showed that pPDC and ACCase co-migrate. Additionally, yeast two-hybrid revealed a specific interaction between BCCP1 and one of the three non-enzymatic candidate interactors.

Symposium IV: Renewable Petro Biofuel from Plants

[OP-29] Synthetic Design of Pathways For Terpene Biofuel Production In Tobacco

Hong Ma[1], Yong Kyoung Kim[1], Yu Wang[2], Sheba Goklany[3], Eiji Takahashi[4], Yi-cheng Liu[1], Susie Y. Dai[5], Don Ort[4], Joseph Chappell[3], Xinguang Zhu[2], Joshua S. Yuan[1]

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Photosynthesis-driven hydrocarbon production represents one of the most energy-efficient routes for converting sunlight and CO2 to fuel molecules. In this study, we designed synthetic pathways in tobacco and achieved record-level production of squalene, a triterpene. Specifically, the squalene biosynthesis pathway, the MEP pathway and a synthetic photorespiration redirection pathway (C2 redirection pathway) were coupled to convert photorespiration product (glycolate) to pyruvate for terpene biosynthesis. This pathway design could reach two effects to synergize the accumulation of squalene: (1) directly channelling more carbons to the squalene biosynthesis pathway; (2) reducing the energy needs for recycling photorespiration products in mitochondria and peroxisome. As a result, engineering the synthetic pathway has led to an all-time high squalene production of 2700 ug/g FW, which is about 800 times greater than the wild type, and over four times greater than the level achieved by engineering terpene biosynthesis pathway alone. A global metabolite profiling assay showed that two major intermediates (pyruvate and malate) in this synthetic pathway increased 1.7-fold and 1.9-fold, respectively. A C-14 labeled glycolate feeding assay also suggested that the synthetic pathway was functional. This research thus established a novel approach to produce high level of terpenes toward biofuel, chemicals and pharmaceuticals in plants.

[OP-30] Metabolic Engineering of Camelina Sativa to Produce Additives for Biodiesel

Borghi Monica, Deyu Xie

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Camelina sativa L. Cranz (Camelina), also known as false flax, is an elective crop to produce biofuel for jets. Despite physical and chemical properties of Camelina oil meet most of the requirements to ensuring the safe usage of biodiesels, oxidative stability is poor, therefore the oil does not bear high temperatures or long time storage. To increase the oxidative stability of Camelina oil, we engineered the plant with the full length cDNA sequence of limonene synthase (LSpl) and a truncated form of it (LScyt), which lacks the leader sequence necessary for the chloroplastic localization of the protein. This strategy has been chosen to exploit cytosolic and chloroplastic pools of geranyl diphosphate, the precursor of many monoterpenes. Promoter of genes BANYULS and FRUITFULL have been preferred to drive the expression of LS in tissues close to the seed but physically separated from the embryo, to prevent the toxic effect of limonene towards embryo development. In addition, constitutive 35S promoter has also been used and the production of limonene in whole plant and seeds compared. Analysis of volatiles extracted from seeds and leaves has been performed via GC-MS and it revealed increased content of limonene in transgenic lines compared to wild-type.

Neish Young Investigator Award Winner

Leslie M. Hicks, Ph.D.
Assistant Professor
Department of Chemistry
University of North Carolina
Chapel Hill, NC 27599, USA

Leslie M. Hicks is an Assistant Professor of Chemistry (Analytical) at UNC-Chapel Hill. She received her B.S. in Chemistry at Marshall University (summa cum laude) and Ph.D. in Analytical Chemistry at the University of Illinois, Urbana-Champaign where she was the recipient of an NSF Graduate Research Fellowship. She was an Assistant Member and Principal Investigator at the Donald Danforth Plant Science Center and an adjunct professor in the Department of Biology at Washington University in St. Louis prior to assuming her current position. Research in the Hicks lab focuses on development and implementation of mass spectrometric approaches for protein characterization including post-translational modifications, as well as the identification of bioactive peptides/proteins from plants.

Interrogation of Post-translational Regulation In Chlamydomonas Reinhardtii Via Mass Spectrometric Proteomics Approaches

Leslie M. Hicks

Department of Chemistry, University of North Carolina,Chapel Hill, NC 27599, USA

The ability to exquisitely differentiate biological molecules dictating metabolism and its underlying biochemistry is a challenging and meaningful endeavor, as it underpins both fundamental biological research and applied bioengineering. With an interest in extending biological frontiers using advanced technologies, my laboratory aims to establish methods, methodologies, and concepts to set the foundation for clever, practical, and meaningful applications of mass spectrometry in addressing and answering important biological questions. The seminar will focus on our progress in the elucidation of post-translational modifications, specifically on developing and implementing methods for the identification and characterization of thiol-based regulatory switches as well as phosphorylation in Chlamydomonas.

Symposium VIII: Phytochemicals, Crops and Agriculture

John M. Littleton, Ph.D.
Professor
Department of Psychology
University of Kentucky
Lexington, KY, USA

John Littleton is currently a Professor in the UK Department of Psychology at the University of Kentucky as well as being Chief Scientific Officer of Naprogenix Inc., a start-up plant biotech company. Before coming to the US, he studied Human Physiology (BS) Neuropharmacology (PhD) and Medicine (MD) becoming Professor of Pharmacology in the University of London. In 1997 he left the University of London and started to develop a plant genomics drug discovery technology with Dr Deane Falcone in the Kentucky Tobacco Research and Development Center. By 2002, proof of concept had been obtained, and Naprogenix Inc was founded to commercialize the technology. This technology can be applied to any products a plant species can make and the company currently has several phase I or phase II STTR/SBIR awards supporting R & D on diverse natural products from native plants as non-toxic insecticides, nutraceuticals and pharmaceuticals. In 2011 Naprogenix spun off another company, Solidagex, to commercialize the first series of products obtained by using this technology.

[KS-16] Selection of Mutant Plant Cells for Target-Directed Biosynthesis of Bioactive Metabolites

John M. Littleton

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When bioactive metabolites in plants are too complex for chemical synthesis, this limits their potential uses. For example, low yields of potential pharmaceuticals in plants limit production, and the synthesis of compound libraries for screening against target proteins is difficult. An alternative is to use the genomic / biosynthetic capacity of the plant species. First, the therapeutic target protein is expressed in transgenic plant cells so that metabolites which interact appropriately with the target protein confer a survival advantage. In a gain-of-function mutant population those mutants which survive should be enriched in individuals over-producing the known "wild-type" active metabolite, or other unknown metabolites which are active at the target protein. An example is a native Lobelia species containing a previously uninvestigated alkaloidal inhibitor of the dopamine transporter (DAT), a target in Parkinson's Disease. Expression of the human (h)DAT in hairy root cultures of this species made these sensitive to MPP+, a cytotoxin which is accumulated intracellularly via the hDAT. Activation tagging mutagenesis was then used to generate mutant hairy roots from the transgenic hDAT line under continuous selection in MPP+. 120 MPP+-resistant mutant hairy roots were analyzed after 4 months on selection followed by two months off selection. As predicted, a large proportion show DAT inhibition well above wild-type cultures. Most of these are over-producing the major known active alkaloid, but, in several mutants, high levels of DAT inhibition do not correlate with this alkaloid, and previously undetected metabolites are present. This approach simply substitutes target-directed biosynthesis for the target-directed chemical synthesis of the pharmaceutical industry and may provide a novel technology for plant drug discovery.
Acknowledgements: Funding from KTRDC for proof of concept, and from KSTC, and NIAAA, NCI and NCCAM for proof of application. **Conflict of interest disclosure**. John Littleton is CEO of Naprogenix and owns equity in this company.

Symposium VIII: Phytochemicals, Crops and Agriculture

S. Luke Mankin, PhD, MBA
BASF Plant Sciences
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Dr. S. Luke Mankin is the Global Product Development Manager for Herbicide Tolerant Traits at BASF Corporation in Research Triangle Park, NC. He received his BA (1996) from the University of Chicago and his PhD (2000) and MBA (2007) from the NC State University. His work has evolved from optimization of plant transformation and transgene express to his current focus on herbicide tolerance and weed control. In his current role at BASF, Luke works on both GM and non GM methods for improving weed control and thereby global food security and farmers lives.

[KS-17] The Provisiatm Rice System: A New Vision in Red Rice Control (the Control of Grass Weeds in Rice)

S. Luke Mankin[2], D.R. Carlson[1], J. Harden[2], B. Luzzi[1], J. Stevenson-Paulik[1], J.B. Guice[2], C. Youmans[2], H. Hong[1], H. Castro[1], R. Sandhu[2], C. Hofelt[2], A. McKean[1], M. Scott[2], Dwight More[2]

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The Provisia™ Rice System is a new non-GM herbicide tolerant system under development by BASF which will augment the Clearfield® rice system, providing growers with another effective weed control technology and a new tool for resistance management. The system is a combination of Provisia herbicide with Provisia rice. Provisia herbicide is a postemerge graminicide which controls volunteer Clearfield rice, conventional rice types, red rice, weedy rice, and other common annual and perennial grasses, including barnyardgrass. It is not an ALS herbicide, and thus, provides another mode of action to combat ALS resistant grasses. In field trials, Provisia rice exhibited excellent tolerance to single and sequential Provisia herbicide applications. Optimum control of red rice and other grass species was obtained with sequential applications. Provisia herbicide can be tank-mixed with many common rice herbicides to provide broad spectrum control of broadleaf and grass weeds. Current research is focused on optimization of performance and weed control systems that mitigate the potential for the development of herbicide resistant weeds. BASF is working with multiple seed partners to bring the Provisia™ Rice System to the market in the latter part of this decade.

[OP-31] A Metabolomics Based Approach for Understanding the Influence of Terroir in Wine Grape Juice Using LC-HR/AM

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To better understand the influence of metabolic signatures by terroir and varietal character, a global profiling Metabolomic workflow was used for the analysis of wine grape juice sourced from two different vineyards. Grape juice samples were directly analyzed using the Dionex Ultimate 3000 coupled to a Q Exactive, and processed with SIEVE differential software. Distinct metabolic signatures were observed from the analysis of both positive and negative polarity components suggesting influence by terroir and varietal character.

Symposium VIII: Phytochemicals, Crops and Agriculture

[OP-32] Studies on Bacterial Efflux Pump Inhibitors and their Distribution in Land Plants

Adam Brown

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Multiple plants have been identified that inhibit bacterial toxic compound efflux pumps, thus synergizing the activity of antimicrobial compounds. This study sought to develop improved methods for the study of this phenomena, and to investigate the prevalence of efflux pump inhibitors in land plant lineages. Two improved assays were developed using both fluorimetry and mass spectrometry. These were employed to evaluate efflux pump inhibitory activity for a set of plant extracts and pure flavonoids. The fluorimetry-based assay was effective and rapid for some samples, but was confounded by quenching effects inherent in many of the samples tested. The mass spectrometry based assay circumvented these quenching issues, and was successful in quantifying the efflux pump inhibitory activity of a wide array of plant extracts and pure compounds. The data produced using the mass spectrometry-based assay when applied to plant extracts and to pure flavonoid standards was useful in demonstrating that the production of efflux pump inhibitors is more widely distributed in land plants than the previous literature suggests, and further that this prevalence is linked in part to the presence of flavonoids.

[OP-33] Anthocyanin and Pyranoanthocyanin Biosynthesis in Staghorn Sumac (rhus Typhina L.)

Chris W. Kirby, Mark H. Nabuurs, Jason L. McCallum

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Rhus typhina, also called the Staghorn Sumac, is a hardy, medium-sized, deciduous, dioecious shrub, native to the temperate north-eastern corner of North America. Historically, Staghorn sumac was used by Native peoples to treat infected wounds & throats, ameliorate abdominal pain, and to prepare a sour, albeit refreshing beverage. The medicinal properties of this plant have recently come under scrutiny, and our group has been involved in studying the anthocyanin content and profile of the brilliantly coloured fruits, of potential use in high-value nutraceutical, food-dye, or medicinal bioproducts applications. During development, the female inflorescences of Staghorn sumac change from greenish-yellow flower buds, through pink and red immature forms, to fully mature as dark burgundy fruits covered by a fine layer of pigmented hairs, with fruits often persisting overwinter. We recently discovered Staghorn sumac accumulates a highly unusual mixture of 7-O-methyl anthocyanins and 4-vinylcatechol pyranoanthocyanin adducts, which were fully characterized by UPLC-MS/MS and 2-D NMR methods. Such unusual anthocyanins presumably have unique biosynthetic origins. To shed further light on the origins of these unusual compounds, a temporal and tissue-specific study of anthocyanin biosynthesis from Staghorn sumac was conducted. Implications for pathway regulation and biosynthetic origins are discussed.

Symposium VIII: Phytochemicals, Crops and Agriculture

Marie Petracek, Ph.D.
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Marie Petracek got her BA from the College of St Teresa in Winona MN and PhD from the University of Minnesota in Genetics. Following post-doctoral studies on post-transcriptional control of light regulated gene expression in plants, she became as assistant professor at the Oklahoma State University in the department of Biochemistry and Molecular Biology in 1999. In 2005, Marie joined the Yield program in Biotechnology at Monsanto Company. Since then, she has served in a number of positions within the Yield program and then in 2012 became director of Yield Traits. The goal of the program is to identify and develop higher yielding corn and soy through the use of biotechnology. In 2013, Monsanto launched DroughtGard, the world's first biotech drought trait in corn.

[KS-18] Innovation in Agriculture: Mitigating the Effects of Climate Change

Marie Petracek

Monsanto Company, 700 Chesterfield, Parkway West, Chesterfield, MO 63017, USA

Multiple lines of evidence confirm that climate change represents a major challenge for agricultural systems to successfully meet accelerating global demand for safe, nutritious, and affordable food. These trends will likely induce geographic shifts in production regions, and will likely force farmers to choose different varieties or perhaps even different crops. A recent report from the IPCC highlights the threat that climate change represents to global food security. Monsanto and other companies are innovating to improve the ability of farmers to meet these challenges. It is essential for continued innovations in agriculture in order to meet growing crop demand in a way that uses as few resources as possible – including land, water, and energy. Recent investments in ag biologicals and information technology hold the promise of accelerated innovation, in order to help farmers continue to provide the nutritious food that the world needs – and doing so in a way that preserves the planet for future generations.

[OP-34] Fruits Via Introduction Of The Non-canonical Substrate Neryl Diphosphate

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Recently it was shown that monoterpenes in tomato trichomes are synthesized by phellandrene synthase 1 (PHS1) from the non-canonical substrate neryl diphosphate (NPP), the cis-isomer of geranyl diphosphate (GPP). As PHS1 accepts both NPP and GPP forming different monoterpenes, we overexpressed it in tomato fruits to test if NPP is also available in a tissue highly active in carotenoid production. However, PHS1 fruits produced only small amounts of GPP-derived PHS1 monoterpene products, indicating the absence of endogenous NPP. Therefore, NPP formation was achieved by diverting metabolic flux from carotenoids via expression of tomato neryl diphosphate synthase 1 (NDPS1). NDPS1 fruits produced NPPderived monoterpenes, while displaying reduced lycopene content. NDPS1 co-expression with PHS1 resulted in formation of monoterpenes, similar to those produced from NPP by PHS1 in vitro and in trichomes. Unexpectedly, PHS1 x NDPS1 fruits showed recovery of lycopene levels, suggesting that redirection of metabolic flux is only partially responsible for carotenoid reduction. In vitro assays demonstrated that NPP serves as inhibitor of geranylgeranyl diphosphate synthase, thus its consumption by PHS1 leads to recovery of lycopene levels. Monoterpenes produced in PHS1 x NDPS1 fruits contributed to direct plant defense against the herbivore Helicoverpa zea and the fungal pathogen Botrytis cinerea.

Symposium VIII: Phytochemicals, Crops and Agriculture

[OP-35] Hydroxynitrile Glucosides in Barley

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Barley contains five leucine derived hydroxynitrile glucosides (HNGs): epiheterodendrin (α), epidermin (β), sutherlandin (γ), osmaronin (γ) and dihydroosmaronin (γ). HNGs are known as plant defense compounds that include the cyanogenic glucosides, which can release hydrogen cyanide (epiheterodendrin is a cyanogenic glucoside). In barley, HNGs constitute more than 90% of the epidermal sugar. The total content of HNGs varies between barley cultivars while the ratio between the five compounds within one plant is constant. Epiheterodendrin is an undesired compound in malting barley used for whisky production in that the cyanide released during fermentation in combination with alcohol forms the carcinogenic compound ethyl carbamate. Breeders therefor select for lines low in HNGs. However, HNGs are defense compounds and we have shown that they play a role in relation to fungal attack. We are currently investigating the role of the individual compounds. In order to understand biosynthesis of HNGs in barley we identified and characterized five cytochrome P450 enzymes as well as two UDP-glucosyltransferases. So far we have identified biosynthetic enzymes for the α - and β -HNGs. The identification of the biosynthetic genes will aid in development of epiheterodendrin-free malting barley that still produces the β - and γ -HNGs for protection against pathogens.

[OP-36] Barley (Hordeum Distichon L.) Roots Produce Volatile Aldehydes Derived from the Lipoxygenase/ hydroperoxide Lyase Pathway with a Strong Age-dependent Pattern

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In chemical ecology, the roles played by root-emitted volatile organic compounds (VOCs) in biotic interactions and the quantitative analysis of such chemicals in root tissues remain poorly documented. In this context, this study aims at using a fully automated gas chromatography – mass spectrometry methodology allowing both identification and accurate quantification of VOCs produced by roots of a monocotyledonous plant species at five selected developmental stages from germination to the end of tillering. Results show that barley roots mainly produce four volatile aldehydes, namely hexanal, (E)-hex-2-enal, (E)-non-2-enal and (E,Z)-nona-2,6-dienal. These molecules are well-known linoleic and linolenic acid derivatives produced via the lipoxygenase/hydroperoxide lyase pathway of higher plants. Our findings contrast with analyses documented on aboveground barley tissues that mainly emit C6 aldehydes, alcohols and their corresponding esters. Multivariate statistical analyses performed on individual VOC concentrations indicate quantitative changes in the volatile profile produced by barley roots according to plant age. Barley roots produced higher total and individual VOC concentrations when young seminal roots emerged from the coleorhizae compared to older phenological stages. Moreover, results also show that the C6/C9 volatile aldehyde ratio was the lowest at the end of tillering while the maximum mean value of this ratio was reached in seven day-old barley roots.

Symposium VIII: Phytochemicals, Crops and Agriculture

Sean Cutler, Ph.D.
Associate Professor of Plant Cell Biology
University of California
Riverside, CA, USA

Sean Cutler received his PhD at Stanford University. He started his own research group as an Assistant Professor at the University of Toronto in 2002 and moved to the University of California, Riverside in 2007, where he is currently an Associate Professor of Plant Biology and Chemistry. He has received the American Society of Plant Biology's Charles Albert Shull Award (2011) and the Valent Biosciences Young Scientist Award (2010) and serves on the editorial boards of The Plant Journal and Molecular Plant. Dr. Cutler's current research focuses on using chemical genetics to understanding how plants respond to different forms of abiotic stress and dissecting the mechanism of action of an endogenous stress signaling molecule called abscisic acid. This information is being used to develop new genetic and chemical approaches for improving crop water use and drought stress tolerance.

[KS-20] Agrichemical Control of Drought Tolerance Using Engineered ABA Receptors

Sean Cutler

Plant Cell Biology and Chemistry, University of California, Riverside, CA 92521, USA

Rising temperatures and lessening water supplies are threatening agricultural productivity and have motivated efforts to improve plant water use and drought tolerance. During water deficit, plants produce elevated levels of abscisic acid (ABA), which improves water consumption and stress tolerance by controlling guard cell aperture and other protective responses. One attractive strategy for controlling water use is to develop compounds that activate ABA receptors, but agonists approved for use have yet to be developed. In principle, ABA receptors engineered to be responsive to an existing agrichemical would enable chemical control by a molecule in current use. Here, we describe an engineered variant of the ABA receptor PYR1 that possesses nanomolar sensitivity to the agrichemical mandipropamid and demonstrate its efficacy for controlling ABA responses and drought tolerance in transgenic plants. Furthermore, crystallographic studies provide a mechanistic basis for its activity and demonstrate the relative ease with which the PYR1 ligand-binding pocket can be altered to accommodate new ligands. Thus, we have successfully repurposed an existing agrichemical for a new use through receptor engineering. We anticipate that this strategy can be applied to other plant receptors and opens new doors for crop improvement.

Symposium VIII: Phytochemicals, Crops and Agriculture

Elvira Gonzalez de Mejia, Ph.D.
Professor and University Scholar
Department of Food Science and Human Nutrition
University of Illinois, Urbana-Champaign

Dr. Elvira Gonzalez de Mejia is a Professor and University Scholar at the University of Illinois campus Urbana-Champaign. She received a B.S. in Biochemical Engineering from the National Polytechnic Institute (Mexico), M.S. degree in Food Science and Technology, University of California, Davis and Ph.D. in Plant Biotechnology, National Polytechnic Institute (Mexico). Professor de Mejia joined the University of Illinois, Urbana-Champaign faculty in 2002 and she has published over 150 peer-reviewed publications, over 120 scientific presentations in the areas of Food Science, Food Toxicology, and Chemoprevention. The long-range goal of her research program is to enhance the health of individuals by the identification and evaluation of the benefits of bioactive compounds in plant foods. Her research is focused on plant food components with health benefits; analysis, chemical and biological characterization and mechanism of action of compounds in plant foods. Her scientific studies introduced new materials to improve human health. Her research is primarily funded by the USDA. Mentor of students and promoter of science among the general public for the National Academy of Science; Head and co-founder of several programs in Food Engineering and Food Science in different Universities in Latin America including the Doctoral program in Food Science in the central part of Mexico (PROPAC) where she implemented a vigorous educational and research program in Food Toxicology. Fellow of the Mexican Academy of Sciences; recognitions awarded by the Mexican Government and Foundations for her scientific contributions to Food Toxicology; fellow of the United Nations University. She has recently worked with the American Chemical Society to develop a second book on Hispanic Foods with special attention to the chemistry and biological activity of herbs and spices. As recognition of her international expertise and achievements in this latter area, Dr. de Mejia received the 2012 McCormick Award from the American Society of Nutrition. Dr. de Mejia's scholarly contributions have translated to leadership roles on journal editorial boards and in prominent professional societies.

[KS-19] Phenolic Compounds from Fruits and Vegetables: Role in Chronic Diseases

Elvira de Mejia[1,2], Jodee Johnson[2]

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Phenolic compounds in fruits and vegetables have anti-oxidant, anti-inflammatory and anti-carcinogenic properties. Inhibition of glycogen synthase kinase-3β (GSK-3β) inhibits nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB), which incorrect regulation is linked to cancer, inflammation, viral infection, and improper immune development. The objective was to examine the effect of phenolic compounds in fruits and vegetables on GSK-3β/NF-KB signaling pathway using *in vitro* and *in vivo* models. Flavonoids, specifically luteolin, apigenin and quercetin, were able to optimally bind within the catalytic site of GSK-3β and inhibit its activity. The inhibitory effects of flavonoids on GSK-3β enzymatic activity and on BxPC-3 pancreatic cancer cells proliferation significantly correlated ($r = 0.87$ for 24 h and $r = 0.86$ for 48 h) suggesting that flavonoids inhibited pancreatic cancer through inhibition of GSK-3β. Apigenin and luteolin enhanced, in vitro, the anti-cancer activity of chemotherapeutic drugs 5-fluorouracil, gemcitabine and oxaliplatin on BxPC-3 cells, and it was due to inhibition of GSK-3β/NF-KB pathway. Lut + Gem significantly lowered ($p = 0.048$) pancreatic tumor mass compared to control, when intraperitoneally-administered in an orthotopic mouse model. Lut, Gem, and Lut + Gem significantly reduced proliferating cell nuclear antigen expression (25%, 37% and 37%, respectively). Lut + Gem led to a significant reduction in the expressions of K-Ras (46%, $p=0.0006$), GSK-3β (34%, $p=0.014$), p(Tyr216)GSK-3β (16%, $p=0.033$), p(Ser311)NF-KB p65 (27%, $p=0.036$) and bcl-2/bax ratio (68%, $p=0.006$), while significantly increasing the expressions of cytochrome c (44%, $p=0.035$) and caspase 3 (417%, $p=0.003$). Lut plus Gem promoted apoptotic cell death in pancreatic tumor cells in vivo through inhibition of the K-Ras/GSK-3β/NF-KB pathway, leading to a reduction in bcl-2/bax ratio, release of cytochrome c and activation of caspase 3. Results demonstrated the potential of fruit and vegetable flavonoids to inhibit GSK-3β and protect against chronic diseases. This research was funded by 20/20 DNS.

Symposium VIII: Phytochemicals, Crops and Agriculture

[OP-37] Vitamin A Biofortification of Wheat Grains via a TILLING Mutant-based Approach

Xiaoqiong Qin, Shu Yu, Wenjun Zhang, Jorge Dubcovsky, Li Tian

Department of Plant Sciences, University of California, Davis, CA, USA

Vitamin A is an essential micronutrient that plays many important roles in human health. Since humans cannot carry out de novo synthesis of vitamin A, this important nutrient must be obtained from dietary sources. Inadequate dietary intake of vitamin A contributes largely to diseases associated with vitamin A deficiency and negatively impacts global public health. Improving provitamin A content of staple foods through biofortification will provide a sustainable solution to nutrient deficiency. Wheat is the most widely cultivated cereal crop and provides more than 16% of total dietary calories and proteins worldwide. Wheat grains contain high levels of carbohydrates, but are low in vitamins and nutrients. Our long term goal is to increase accumulation of β-carotene, the most efficient form of the provitamin A carotenoids, in wheat grains using a non-GMO Targeting Induced Local Lesions in Genomes (TILLING) mutant-based approach. Our strategy is to generate loss-of-function mutations (via TILLING) that block the catabolic and competing branch pathways for β-carotene accumulation. We will present our progress on cloning and characterization of carotenoid metabolic genes in wheat as well as mutant isolation from a tetraploid wheat TILLING mutant library.

[OP-38] Metabolism of Glyphosate and Aminophosphoric Acid in Glyphosate-resistant and Conventional Canola (Brassica Napus L.)

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Glyphosate (N-(phosphonomethyl) glycine) is the most used herbicide worldwide. Much of glyphosate's success can be attributed to the widespread adoption of transgenic, glyphosate-resistant crops. All currently available glyphosate-resistant crops contain a transgene encoding an invulnerable form of 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), the enzyme target of glyphosate. In glyphosate-resistant canola, a transgene (goxv247) for glyphosate oxidoreductase (GOX) is used in addition to cp4. GOX converts glyphosate to glyoxylate and aminophosphonomethyl glycine (AMPA) which are much less toxic to plants. The transgene for GOX alone is apparently insufficient to impart adequate glyphosate resistance. AMPA accumulates in the leaves of glyphosate-treated resistant and conventional canola, although the levels are higher in glyphosate-resistant canola. Conventional canola, soybean, and some other species apparently have an enzyme with GOX-like activity which results in the accumulation of AMPA upon treatment with glyphosate. In some cases, glyphosate-treated resistant soybean can produce enough AMPA with its non-transgenic GOX activity for the levels to become phytotoxic. The reason why the AMPA produced by the transgenic GOX in canola does not induce phytotoxicity remains to be explained. In this work, the phytotoxicity of AMPA in glyphosate-resistant canola and the metabolism and kinetics of glyphosate in resistant and conventional canola were examined.

Poster Abstracts

[P-529-1] Absolute Configuration of Syringylglycerol-8-*O*-4′-(Sinapyl Alcohol) Ethers (SGSE) and its Formation Mechanism from Sinapyl Alcohol with Soluble Enzyme Preparations of *Eucommia Ulmoides*

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In this study an enzymatic system, horseradish peroxidase (HRP)-hydrogen peroxide was used as catalysts for the enzymatic formation of enantiospecific syringulglycerol-8-O-4′ (sinapyl alcohol) ethers (SGSEs) from enzyme preparations of Eucommia ulmoides with sinapyl alcohol (SA) as a monolignol precursor. Reversed phase HPLC analysis of the erythro- and threo-SGSE showed that the ratio of erythro: threo was 47: 53. Both isomers were isolated by HPLC and their structural confirmation was done by 1H NMR spectra. Chiral column HPLC analysis of the erythro- and threo-SGSE showed that their enantiomeric compositions were as follows: (+)-erythro: (-)-erythro = 46.7:53.3 (6.6% e.e), and (+)-threo: (-)-threo = 45.2: 54.8 (9.6% e.e). To elucidate the stereochemistry of erythro and threo- SGSEs, we have determined absolute configurations of the four stereoisomers, (+)-erythro-, (-)-erythro-, (+)-threo-, and (-)-threo-SGSEs as (7S, 8R), (7R, 8S), (7S, 8S), and (7R, 8R), respectively, by Mosher's method through the 1H NMR spectroscopy of (R)- (+)-α -methoxy- α - trifluoromethylphenylacetate (MTPA) esters of α , γ and γ ′ positions and quantified exactly the ratio of these four stereoisomers. Therefore, composition of the four isomers was calculated as (7S, 8R)- (+)-erythro 22.14%, (7R, 8S)- (-)-erythro 25.26%, (7S, 8S)- (+)-threo 24.78%, and (7R, 8R)- (-)-threo 28.82% respectively.

[P-603-2] An Alternative Pathway Contributes to Phenylalanine Biosynthesis in Plants via a Cytosolic Tyrosine: Phenylpyruvate Aminotransferase

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Phenylalanine (Phe) is a protein building block and a precursor of numerous metabolites. While it was shown that plants predominantly synthesize Phe in plastids via arogenate pathway, the contribution of an alternative phenylpyruvate pathway, which is the main route for most microorganisms for Phe biosynthesis, has never been demonstrated. Here, to test the possibility of an alternative route for plant Phe formation, we generated RNAi transgenic petunia plants in which both the AROGENATE DEHYDRATASE 1 (ADT1) and PREPHENATE AMINOTRANSFERASE (PPA-AT) genes were simultaneously downregulated in petunia petals. While in PhADT1-RNAi line the levels of Phe and Phe-derived volatiles were reduced by 80%, those in the PhADT1xPhPPA-AT RNAi line were rescued to wild-type levels, indicating the involvement of an alternative Phe biosynthetic pathway. We have further isolated petunia phenylpyruvate aminotransferase (PhPPY-AT), which complements the E. coli Phe auxotrophic mutant. Transient downregulation of PhPPY-AT expression led to a 30-60% decrease in Phe-derived scent compounds in petunia flowers of wild-type and arogenate pathway knockdown lines. Biochemical characterization of purified recombinant PhPPY-AT showed that it prefers tyrosine as an amino donor. Together with cytosolic localization of PhPPY-AT, our studies demonstrate that the microbial-like phenylpyrvute pathway operates for plant Phe formation by cytosolic tyrosine:phenylpyruvate aminotransferase.

Poster Abstracts

[P-527-3] Analysis of Keto-Enol Tautomers of Indole-3-Pyruvic Acid

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Indole-3-pyruvic acid (IPyA) is one of the key biosynthetic intermediates on the pathway to auxin, an important plant hormone. Like all compounds containing hydrogen atoms in the a-position relative to a carbonyl, IPyA can exist as a mixture of keto and enol tautomers, which can complicate quantitation of this compound by standard analytic techniques. Separation of tautomers is not often consideredpossible due to rapid inter-conversion. However, we present here a detailed analysis of the tautomerism of IPyA and provide evidence that the two tautomers of IPyA may be readily separated by ultra-performance liquid chromatography (UPLC) and that the relative proportions of each form may be controlled using temperature and pH. The keto-enol tautomerism of IPyA should be taken into consideration during quantitative analyses of this compound and related compounds by liquid chromatography.

[P-555-4] Bioprospecting of Natural And Synthetic Cucurbitane Related Compounds

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Triterpenes constitute an extreme diverse class of natural products with a vast array of biological activities. They are derived from a limited set of characteristic skeletons and the structural diversity comes from a broad range of modification on the different carbon positions of the C30 skeletons. Many naturally occurring plant triterpenes are already being used as anticancer drugs, pesticide, and antimicrobial agents. TriForC (triforc.eu) is an EU-funded collaborative project on establishing an integrative and innovative pipeline for the exploitation of plant triterpenes. TriForC will establish a pipeline for the discovery, sustainable production and commercial utilisation of known and novel high-value triterpenes with new or superior biological activities. As one of the TriForC partners, we focus on a distinct group of triterpenes, cucurbitacins, with highly unsaturated and oxygenated properties. Cucurbitanes are naturally synthesized in the Cucurbitaceae family and derived from the cucurbitane skeleton. Cucurbitanes exhibits different bioactivity like anticancer, antidiabetic and insecticidal based on the different modification on the cucurbitane skeleton. To study the biological activity of naturally and unnaturally produced cucurbitane derived compounds, several species in the Cucurbitaceae family will be subjected to bioprospecting. For bioprodution naturally and novel cucurbitane derived compounds will be introduced to tobacco and microalgal platform by metabolic engineering.

[P-611-5] Characterization of Transgenic Camelina Plants Emitting Isoprene

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Isoprene (2-methyl-1,3-butadiene) is an organic, colorless, volatile compound produced and emitted by plants. The yearly production of isoprene emissions by vegetation is around 600 million tons, with half that coming from tropical broadleaf trees and the remainder coming from shrubs. Although recent studies indicate that isoprene can enhance thermo-tolerance and quench oxidative stress, its function in herbaceous plants is still largely unknown. In this study Camelina sativa (L.) Cranz (Camelina), a species of the Brassicaceae family mainly cultivated as an oilseed crop in Europe and in North America, has been transformed with the isoprene synthase gene (PcISPS) from Populus x canescens (Grey poplar) which resulted in increased isoprene emission. Measurements of the expression of key regulatory genes of the plastidial 2-C-methyl-D-erythriol-4-phosphate (MEP) pathway, chlorophyll content and efficiency of the photosystem are also presented. Finally, the potential and pitfalls of the manipulation of the isoprene emission in herbaceous plants is discussed.

Poster Abstracts

[P- 622-6] Characterization of Two Amyrin Synthases Responsible for Triterpenoid Biosynthesis from *Ilex asprella*

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Ilex asprella, a medicinal herb widely used in south China, contains a large amount of triterpenoid saponins, most of which are of ursane-type. Based on the results of transcriptomic analysis, two amyrin synthases (AS) genes, IaAS1 and IaAS2, were cloned from the I. asprella root. Functional characterizations were carried out by heterologous expression in yeast Saccharomyces cerevisiae. Analysis of the resulting products by GC and GC-MS showed that both ASs are multifunctional amyrin synthases, producing α-amyrin and β-amyrin at different ratio. IaAS1, which mainly produces α-amyrin, is the second triterpene synthases so far identified in which the level of α-amyrin produced is ≥ 80%. Meanwhile, IaAS2 mainly synthesizes β-amyrin, with a yield of 95%. The relative expression levels of both AS genes were consistent with total saponin content patterns in eight different tissues of I. asprella. Finally, phylogenetic analysis and multiple sequences alignment of the two ASs and several well-characterized oxidosqualene cyclases from other plants were conducted to further elucidate their evolution relationship.

[P-602-7] Cloning and Characterization of a Norbelladine 4′-O-Methyltransferase Involved in the Biosynthesis of Galanthamine in Narcissus Sp. Aff. Pseudonarcissus

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Galanthamine is a plant alkaloid used to treat the symptoms of Alzheimer's disease. This compound is produced by members of the family Amaryllidaceae including Narcissus species. Using BLAST to find O-methyltransferases in a Narcissus sp. aff. pseudonarcissus transcriptome, a collection of candidate genes was obtained for the methylation of norbelladine to 4′-O-methylnorbelladine in the galanthamine biosynthetic pathway. Using HAYSTACK, expression profiles of the methyltransferase genes were compared to the pattern of galanthamine accumulation in Narcissus sp. aff. pseudonarcissus leaf, flower, and bulb tissues. One of the candidate methyltransferase genes fit the HAYSTACK model. This methyltransferase cDNA was expressed in E. coli, the protein purified by affinity chromatography and found to convert norbelladine to 4′-O-methylnorbelladine.

[P-547-8] Effects of Amino Acid Sequence Insertion on the Substrate Preference of a Citrus Paradisi Glucosyltransferase

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Glucosyltransferases (GTs) are enzymes which perform glucosylation reactions, which involve attaching a UDP-activated glucose molecule to acceptor molecules specific to the enzyme. The enzyme which our lab focuses its research on is a flavonol-specific 3-O-GT found in Citrus paradisi, or grapefruit (Cp3GT). This enzyme is part of the class of enzymes known as flavonoid GTs, which are responsible for, among other things, the formation of compounds which can affect the taste of citrus. Our lab focuses its research on performing site-directed mutagenesis on Cp3GT in an attempt to discover the residues important for substrate and regiospecificity. In this study, we are testing the basis of substrate septicity of Cp3GT. We hypothesize that incorporation of five amino acids specific to Citrus sinensis GT (CsGT) into Cp3GT at 308th position may facilitate mCp3GT to use anthocyanidins as one of the substrates. We report our findings thus far concerning the addition of specific residues to the Cp3GT's amino acid sequence based on an alignment with the sequence of a putative flavonoid GT found in Citrus sinensis.

Poster Abstracts

[P-574-9] Elucidating the Molecular Mechanism of Lignin-Modification-Induced Dwarfism by Characterizing Suppressors of an Arabidopsis Lignin-Deficient Mutant

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Production of biofuels from lignocellulosic biomass is a promising approach to reduce our reliance on non-sustainable and environment-harmful fossil fuels. One of the major obstacles for this process is the difficulty in accessing the sugars embedded in cell wall due to the presence of lignin, a phenylpropanoid polymer. Past studies have been successful in attempting to manipulate lignin for substantial enhancement of cell wall digestibility. However, this came with a cost of significant plant growth reduction and loss of biomass. To understand the mechanism underlying this lignin-modification-induced dwarfism (LMID), we have carried out a suppressor screen for an Arabidopsis lignin mutant ref8, which is severely dwarf and sterile. From 280 M2 pools, 22 suppressor lines that show an alleviated growth phenotype were isolated. Using bulked segregant analysis and next -generation sequencing, we have identified a candidate growth inhibition relieved (GIR) gene, GIR1. We confirmed that this is the causal gene by complementation as well as generation and analysis of the double mutant of an independent knockout allele and ref8. The GIR1 gene has been previously identified several times from different genetic screens including ABA sensitivity, miRNA activity, and trichome density. It was shown to encode an importin beta protein that mediates protein transport from the cytosol into the nucleus. We are currently working to understand the molecular mechanism by which GIR1 mediates the ref8 dwarf phenotype.

[P-609-10] Evolutionary Cues from Functional Switching of Two Closely Related Class II Diterpene Synthases

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Class II diterpene synthases (diTPSs) catalyze the first committed step in diterpenoid biosynthesis by forming specific enantiomers of bicyclic copalyl/copalol-diphosphates from geranylgeranyl-diphosphate. The functional understanding of class II diTPSs has rapidly progressed with reported crystal-structures of the Arabidopsis ent-copalyl diphosphate synthase, involved in biosynthesis of gibberellin phytohormones in general metabolism. However, more functional studies are needed to clarify the basis for the great diterpenoid diversity of the specialized metabolism.

We have identified two Tripterygium wilfordii class II diTPSs, TwTPS14 and TwTPS21 that diverge at 13 sites. Despite this similarity, the functionally expressed enzymes display distinct product profiles; TwTPS21 produces ent-copal-8-ol diphosphate while the hydroxylated diphosphate product of TwTPS14 does not match any known diterpenoid intermediate.

Here we show that the reciprocal exchange of two residues, 265H/Y and 325A/V, located in the active sites of models of TwTPS14 and TwTPS21, resulted in a complete product interchange, while substituting either of the residues gave mixed product profiles of hydroxylated diphosphates. Interestingly, two variants, TwTPS14_Y265H and TwTPS21_A325V, also produced the gibberellin precursor ent-copalyl-diphosphate.

This study exemplifies how specialized metabolism may have evolved from general metabolism in T. wilfordii and contributes to our understanding of the structure-function relationship of class II diTPSs.

Poster Abstracts

[P-606-11] Genetic and Biochemical Characterization of Acyl-Lipid Desaturase 1

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Lipid desaturases can be classified into three major classes based on substrate specificity: acyl-ACP, acyl-CoA (ACD), and acyl-lipid desaturases (ALD). The previously named fatty acid desaturase enzyme family (FAD2, FAD3, FAD4, FAD6, FAD7, FAD8) is acyl-lipid-specific and therefore falls into the ALD category. Because glycerolipids have variable head groups, acyl chains, and acyl positions on the glycerol backbone the specific interactions between ALDs and their substrates may vary as well. The Arabidopsis genome encodes eight additional putative ALDs which share sequence similarity with cyanobacteria ALDs and mammalian ACDs: At1g06080 (ADS1), At1g06090, At1g06100, At1g06120, At1g06350, At1g06360, At2g31360 (ADS2), and At3g15870. In this study we characterize the ADS1 gene using several T-DNA knockout lines. Leaf fatty acid composition analysis showed that the 18:1 content in ads1 was lower than wild type (Columbia 0). Additionally, lipidomics comparison demonstrated that MGDG (34) content in ads1 was lower than wild type. Finally, ADS1 was confirmed to be plastid-localized by observing the subcellular localization of EYFP-tagged protein. The ongoing study will be focused on assessing the functional role of ADS1.

[P-522-12] Investigating Potentially Key Residues Which Imparts the Substrate and Regiospecificity of a Flavonol-Specific 3-O-Glucosyltransferase from Grapefruit

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Most naturally-occurring flavonoids are found in glucosylated form. Glucosyltransferases (GTs) are enzymes that catalyze the transfer of glucose from a high energy sugar donor to an acceptor molecule. Citrus paradisi flavonol-specific glucosyltransferase (Cp-F3-O-GT) is recognized for its rigid substrate and regiospecificity. In this work, homology modeling, site-directed mutagenesis, and biochemical analyses of the recombinant mutant Cp-F3-O-GT proteins were used to investigate potential amino acid residues that might be responsible for the enzymes strict regiospecificity while also investigating its substrate specificity. The single point mutations of three amino acid residues within the grapefruit F3-O-GT identified through sequence alignment and homology modeling were performed. Analyses of the enzyme activity of the recombinant mutant F3-O-GT proteins revealed that the single point mutations of serine 20 to leucine (**S20L**) and proline 297 to phenylalanine (**P297F**) rendered the recombinant enzymes inactive with flavonol substrates at 6% and 12% respectively relative to wild-type. However, the mutation of glycine 392 to glutamate (**G392E**) remained active and glucosylated the flavonol acceptors quercein (Km app= 11 µM; Vmax = 5.7 pKat/µg) relative to the wild-type (Km app= 93 µM; Vmax = 41.7 pKat/µg), and kaempferol (Km app= 7 µM; Vmax = 3.8 pKat/µg) relative to the wild-type (Km app = 39 µM; Vmax = 4.2 pKat/µg). The mutant enzyme also did not show broadened acceptor substrate specificity as it also favored flavonols as the preferred acceptor substrate. The optimum pH of the mutant enzyme was 8.0 similar to the wild-type F3-O-GT. Activity of the mutant enzyme was stimulated by NaCl and KCl, but inhibited by Cu2+, Zn2+, Fe2+ as well as UDP with an apparent Ki of 10µM. Product identification to determine glucosylation position is being investigated for a possible change in regiospecificity.

Poster Abstracts

[P-610-13] Isolation and Evaluation of Antioxidants from Leaves of Two Italian Salt-Stressed Olive (olea Europaea L.) Cultivars

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The olive tree (Olea europaea L.) is an important crop in the Mediterranean Basin where drought and salinity are two of the main factors affecting crop productivity. It is well know that olive oil and olive leaf tea lends important economic and dietetic benefits to the people of that region, and since olive trees are considered a tolerant species to salinity, sporadic irrigation with sea water is currently under evaluation as a viable method to overcome prolonged periods of drought. Given the importance that metabolites with nutraceutical properties have for human health, the major aim of this research is to investigate the added nutraceutical value of leaves from plants exposed to irrigation with salty water. HPLC-PAD-ESI/MS data and measurements of the expression of key regulatory genes of the phenylpropanoid metabolic pathway reveal that olive leaves under salt-stress increased their content of metabolites with nutraceutical properties (Kaempferol, Luteolin, Quercetin). This last achievement represents a milestone in metabolomic studies because it is the first one conducted on salt-stressed olive tree leaves, which are an important by-product with economic relevance in terms of olive tea leaf.

[P-614-14] Manipulation of IPP and DMAPP Flux by Regulation of Isopentenyl Pyrophosphate Isomerase (IPPI) Location in Cytosol and Chloroplast Changed the Terpenoid Metabolism in *Artemisia Annua*

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Artemisia annua is a common type of wormwood, which has a camphor-like scent due to abundant monoterpene and sesquiterpene. Artemisinin (sesquiterpene lactone endoperoxide) is an effective antimalarial component from Artemisia annua. In our study, IPPI, a key gene in the biosynthesis of terpenoid was cloned, biochemical analysis showed that the N-terminal truncation IPPI (lack of transit peptide) catalyzed IPP to DMAPP, IPPI can also catalyze the reverse reaction from DMAPP to IPP, equilibrium favored the forward reaction, the products ratios of IPP: DMAPP are 1: (7.0-7.3). We generated transgenic Artemisia annua overexpressing IPPI lack of transit peptide: (-tp)IPPI and IPPI with transit peptide. Transient expression in Nicotiana benthamiana showed that (-tp)IPPI localized in cytosol and IPPI with transit peptide mainly localized in chloroplast. Overexpression of (-tp)IPPI in transgenic plants accumulated more monoterpens and sesquiterpene (up to 1.45-and 1.47-fold, respectively), and significantly increased the amount of artemisinin, Arteanniun b and deoxyqinghaosu (up to 3.5-, 2.6-and 3.5-fold, respectively) compared with control. Enhanced expression of chloroplast-localized IPPI decreased monoterpens, sesquiterpenes.

Key Words

Artemisia annua; Artemisinin; isopentenyl pyrophosphate isomerase; isoprenoid biosynthetic pathway; overexression

Poster Abstracts

[P-615-15] Metabolic Profiling of Commercial Tobacco, KY171 and Narrow Leaf Madole Overexpressing AtPAP1

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Anthocyanin is a flavonoid that provides many beneficial effects to plant and human health such as protection of plants from environmental stress and attraction of pollinators. It also has strong antioxidative activity that provides prevention against chronic diseases in human being. Anthocyanin biosynthesis has been extensively studied especially in model plants Arabidopsis. The PAP1 gene (production of anthocyanin pigment) encodes a MYB transcription factor that plays a key role in regulation of anthocyanin biosynthesis. It forms a regulatory complex with a basic helix-loop-helix (bHLH) domain protein and a WD40 repeat protein and regulates the structural gene expression in anthocyanin biosynthesis. Many anthocyanin structures have also been determined. Here we reported the metabolite profiling in commercial tobacco varieties overexpressing AtPAP1. Our results showed that the PAP1 transgenic plants turned deep purple color. The total anthocyanin content reached about 700 µg / g, FW. The major anthocyanin was determined as cyaniding 3-O-rutinoside by GC-MS with standard. The carbon flow toward the anthocyanin biosynthesis will be discussed.

[P-586-16] New Biosynthetical Insights in *Peperomia Pellucida*

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Peperomia pellucida (L.) HBK (Piperaceae) is an herbaceous plant widespread in the in the tropics with several biological properties including antimicrobial, analgesic, and anti-inflammatory properties. Previous phytochemical investigations have described the rare dinorlignan pellucidin A. In the present work, we conducted further phytochemical investigations of P. pellucid which resulted so far in the isolation of 2,4,5-trimethoxycinnamic acid, 2,4,5-trimethoxystyrene, 2,4,5-trimethoxybenzaldehyde, dillapiol, 2,3,5-trimethoxyflavone in addition to pellucidin A. The biosynthesis of pellucidin A is of particular interest because of its unique cyclobutane moiety. Thus, we evaluated L-[2-13C]-phenylalanine, [8-13C]-2,4,5-trimethoxystyrene, and [8-13C]-2,4,5-trimethoxycinnamic acid as biosynthetic precursors of pellucidin A. Fully developed plants were fed with several precursors for different time periods and the crude extracts of leaves were analyzed by GC/MS. The L-[2-13C]-phenylalanine, the first precursor in the pathway, and [8-13C]-2,4,5-trimethoxystyrene, a putative direct precursor were both incorporated into pellucidin A. These results show that pellucidin A is formed by the dimerization of two unities of 2,4,5-trimethoxystyrene, instead of 2,4,5-trihydroxystyrene as supposed in previous works.

FAPESP, CNPq, PRP-USP and CAPES

Poster Abstracts

[P-538-17] Swift Regulation of Genes Involved in the Biosynthesis of Cyanogenic Glucosides in Cassava (*Manihot Esculenta*)

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The important crop plant, cassava (Manihot esculenta) contains potential toxic cyanogenic glucosides in all tissues. Upon tissue disruption e.g. in the course of food preparation or as a result of insect attack, the cyanogenic glucosides are degraded resulting in release of toxic hydrogen cyanide. A diurnal variation of cyanogenic glucoside content with peak levels around dusk and dawn has been observed in cassava. The diurnal variation may be a result of light mediated regulation of the pathway, represent turnover of cyanogenic glucosides in the presence of light, a circadian clock or a combination of these. To study this, the cyanogenic glucoside content and expression of genes involved in the pathway have been monitored throughout a 24 hour period. The cyanogenic glucoside content was shown to peak in the morning and the afternoon and decrease during the light period and during the night. Transcript and protein levels of genes involved in the biosynthesis and degradation of cyanogenic glucosides showed a clear diurnal variation. In addition, fluctuations in light intensity during the daytime was accompanied by significant changes in transcript, protein and cyanogenic glucoside levels. Thus cyanogenic glucoside formation and turnover is much more dynamic that previously thought most likely reflecting previously unrealized functions of cyanogenic glucosides.

[P-552-18] α-Onocerin is an Unusual Triterpene in *Ononis spinosa*.

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Triterpenes constitutte a structurally diverse group of metabolites derived from the cyclization of squalene derived intermediates. Triterpenes are synthesized by a plethora of plant families; however, some species can synthesize unique triterpene skeletons not yet detected in other families of the plant kingdom.

Ononis spinosa is a species in the *Fabaceae* family. Extracts from the plant is known to have diuretic, anti-inflammatory and analgesic activities. *Ononis spinosa* is able to produce a unique symmetric triterpene denominated α-onocerin, although it is not known if the properties mentioned above are attributable to this compound. Rowan and Dean (1971) showed that protein extracts from leaves and roots of *Ononis spinosa* produce α-onocerin using squalene-2(3),22(23)-diepoxide as substrate and not 2(3)-oxidosqualene. However no specific protein(s) or genes responsible for the cyclization of squalene-2(3),22(23)-diepoxide is known.

TriForC (triforc.eu) is an EU-funded collaborative project on establishing an integrative and innovative pipeline for the exploitation of plant triterpenes. In attempts to increase the available resources for triterpene industrial use, we aim to elucidate the biosynthesis of α-onocerin. Additionally, specific bioassays testing only α-onocerin are few in the literature, thus α-onocerin is being tested for several agricultural and medicinal bioassays.

Poster Abstracts

[P-613-19] A Two-Step Precursor Ion Scanning-Based LC-MS/MS Method for Structural Elucidation of Flavonoids

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Flavonoids are very important plant secondary metabolites. Their formation is of ecological significance to plants. For example, flavonoids were found to inhibit the larval growth of Heliothis zea that cause damage of tobacco plants. To human being, flavonoids are potent nutrients, given that numerous studies have shown their antioxidantive, antiinflammation, and anti-cardiovascular disease activities. To be able to understand the bioavailability and functions of plant flavonoids, it is necessary to reveal their exact chemical structures. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a powerful approach for structural elucidation of metabolites. In this report, a two-step precursor ion scanning-based LC-MS/MS method was developed for the structural elucidation of plant flavonoids. The two-step precursor ion scanning method is goal-oriented and can increase the sensitivity of flavonoid search. The developed method was used for the structure elucidation of flavonoids in tobacco flowers and leaves and 17 flavonoids were identified. This method was proved to be very effective and can be used for the identification of flavonoids in other plants.

Poster Abstracts

[P-591-20] Analysis of Triacylglycerol in Seed Oils Using Q Exactive Orbitrap Mass Spectrometer and Lipid Search for Automated Data Processing

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Summary

Here we present a new software tool, Lipid Search, for lipid identification and differential analysis in seed oils using an ultra-high resolution Orbitrap mass spectrometer.

Introduction

Healthier oils are becoming increasingly important for overall human health and nutrition. The nutritional composition of seed oils is closely linked to their polyunsaturated fatty acids (PUFA) content. Ultra-high resolution (R >100,000) LC-MS and MS/MS methodology can provide important information for rapid determination of triacylglyceride (TAG) profiles, which can aid in the development of next-generation healthy oil products. Data analysis of several hundred TAG species in a typical oil sample can be a bottleneck in reducing this high resolution data to useful information. Thus, robust data processing software combined with high quality MS data is needed for rapid and accurate analysis of oils.

Methods

Profiling of TAGs in seed oils was performed by LC-MS using a high-performance Orbitrap hybrid mass spectrometer. LC-MS and MS/MS data were initially obtained at 140,000 and 70,000 mass resolving power, using a Q Exactive1. However, in some cases this MS resolution was not sufficient to resolve the minor mass differences between double bonds and di-13C species. The LC/MS HPLC gradient was 100% Acetonitrile to 80% 3:1 IPA/MTBE (5mM ammonium acetate) in 60 min using a C30 Acclaim column (2.1x150mm, 3µm) operated at 300µL/min. MS and MS/MS high resolution data was analyzed with Lipid Search software to identify the TAG species.

Preliminary Data

The analytical workflow described here uses new Lipid Search software for lipid identification through a database search of the accurate masses of precursors and the fragment ions predicted for each potential adduct form of the lipids in the database. Relative quantitation and statistical analysis can be performed, along with a graphical summary of the chromatographic and spectral data for each lipid result. The new workflow also aligns and merges the LC-MS data obtained on different samples to obtain a relative quantitative comparison of the sample set, allowing statistical comparison between the groups compared to control. The identified

lipids were quantified in each oil sample to provide a relative comparison of the TG species. The resolution required to separate the [M+NH4]+ adduct of TAG 50:1 from the di-13C isotope of TAG 50:2 is 187,000 FWHM; LC-MS experiments demonstrate these are sufficiently resolved to prevent any significant contribution from the interfering isotopes. Thus, ultra-high resolution may be used to provide relative quantitation of TAG species without the need for isotopic correction which significantly simplifies the analysis of the data. In addition, more sophisticated experiments may be developed with hybrid Orbitraps to ensure the identification occurs for the species of interest. For example, a mass tag for the difference between two different adduct ions (Na and NH4) may be employed to trigger data dependent MS/MS scans without acquiring the spectra of redundant isotopic peaks. Using the strategies described here, the replicate analysis of canola, grape seed, peanut, soybean and sunflower oils contained over 2000 TAG ions combined and yielded identification of over 400 different species providing a very detailed map of the differences in the oil profiles and the relative amounts of PUFA containing species. This fully automated analysis quickly provides higher quality data than with current analytical methods.

(1) D Schwedler, D Peake, J Gilbert, J Flook, B McNew, D Gachotte, S Greenwalt and J Balcer, New Lipid Analysis Tools Using Quadrupole – Orbitrap Instrumentation, presented at the 60th Annual Conference on Mass Spectrometry and Allied Topics, May 20 - 24, 2012, Vancouver.

Poster Abstracts

[P-592-21] Characterization of Arachidonylethanolamide Metabolic Pathway in Moss

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Arachidonylethanolamide (AEA) is a bioactive lipid ligand for mammalian cannabinoid receptors (CB). Thus far, AEA was reported to occur only in animals and was shown to regulate a wide range of physiological responses. Our recent finding of the occurrence of AEA in moss has led us hypothesize that AEA might mediate stress responses in plants, similar to that in animals. In mammals, AEA is generated from hydrolysis of N-acylphosphatidylethanolamine (NAPE) by a NAPE-specific phospholipase D (NAPE-PLD), and degraded by a fatty acid amide hydrolase (FAAH) and this metabolic pathway is highly conserved among eukaryotes. Here, using in silico approach, putative genes encoding for AEA pathway enzymes, were identified in moss. Full-length coding sequences for putative NAPE-PLD and FAAH were isolated from Physcomitrella patens and were cloned and expressed into a heterologous expression vector. Biochemical characterization of AEA pathway enzymes is underway and is expected to lead to generation of AEA metabolite mutants in moss. Such mutants will allow for elucidation of the role of AEA in development of moss and mediating stress responses. Overall, this study will provide novel insights into functional and evolutionary role of lipid-mediated signaling in plants.

[P-564-22] Further Characterization of the Nitrogenous Metabolome of Black Cohosh (Actaea Racemosa L.)

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The roots/rhizomes of black cohosh (Actaea racemosa L. (syn. Cimicifuga racemosa [L.] (Nutt.)) have been used traditionally by Native Americans to treat colds, rheumatism as well as for alleviating menopausal symptoms such as hot flashes. Guided by a hypothesis that alkaloids and other nitrogen-containing compounds are active constituents, we have been exploring the nitrogenous metabolome of black cohosh using mass spectrometric dereplication. Previously, we reported on the identification of 73 nitrogen-containing compounds, many of which were new natural products. As an extension of this work, herein we describe identification of another series of nitrogenous compounds. Among the newly identified compounds, several tetrahydro and dihydroisoquinolines were identified based on comparison of their product ion spectra with those the previously identified salsolinol and norsalsolinol. New guanidino compounds such as benzoyl arginine or N-hexanoyl arginine were identified based on comparison of tandem mass spectra with previously identified guanidino compounds. In total, more than twelve constituents, many of which are new natural products, were identified and their presence established by comparison with synthetic authentic standards. This study further extends the knowledge of the nitrogenous metabolome of black cohosh and provides possible new markers for standardization of black cohosh supplements.

Poster Abstracts

[P-525-23] Mutagenesis of a Flavonol- 3-O-Glucosyltransferase and the Effect on Enzyme Function

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Flavonoids are an important group of secondary metabolites found in plants and have a wide variety of properties. Some play a role in flower pigmentation, while others have antimicrobial properties. Glucosylation is an important modification of flavonoids and is mediated by glucosyltransferases. In this process, the enzyme transfers glucose from UDP-glucose to a specific position on the flavonoid. Previous study from the lab characterized a glucosyltransferase from C. paradisi that is flavonol specific. In this study an attempt has been made to study the structure and function of this flavonol specific glucosyltransferase using site directed mutagenesis. The glutamine residue at position 87 of the Cp-3-O-GT enzyme was changed to isoleucine, the analogous residue in the 3-O-glucosyltransferase of Clitoria ternatea. Similarly, the histidine at position 154 was changed to tyrosine. We hypothesize that these mutations will change substrate specificity. The glutamate at position 88 was changed to an aspartic acid. We hypothesize that this will change the regiospecificity of the enzyme, as aspartic acid is the analogous residue found in some 7-O-glucosyltransferases. Finally, we introduced a double mutation with glutamine 87 becoming isoleucine and glutamate 88 becoming aspartic acid, with the hypothesis that both regiospecificity and substrate specificity will be changed.

[P-560-24] Unlocking the Code of Saponin Structure-Activity Relationships for Use in Pest Management

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Saponins are steroid and triterpenoid glycosides that constitute a structurally diverse class of plant defense compounds. Due to their detergent-like properties, saponins can disrupt cell membranes of herbivores and pest, cause cell death, and ultimately kill them. Despite the promising potential of saponins for use as bio-pesticide, little is known about which saponin structures are toxic to which specific herbivores or pests. This study aims to elucidate the relationship between saponin chemical structures and their biological activities and evolution. The plant system used in this study is Barbarea vulgaris, which is the only genus in the Cabbage family known to produce saponin. Previously we have identified several biosynthetic genes of saponins as well as genome regions (QTL) that determine resistance of saponin in B. vulgaris (Augustin et al. 2012; Kuzina et al. 2011; Kuzina et al. 2009). We will use genomics, transcriptomics and metabolomics tools to elucidate the biosynthetic pathway of saponins, and metabolically engineer desired or novel saponins in model plants with combinations of biosynthetic genes. Purified saponins from engineered plants will be used in bioassays with a range of pest species, to unlock the code of which saponin structures have a given specific biological activity.

Poster Abstracts

[P-531-25] What Determines Product Specificity of Oxidosqualene Cyclases in Triterpenoid Biosynthesis?

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In the wild crucifer Barbarea vulgaris the production of triterpenoid saponins is correlated with resistance towards crucifer crop pests and they therefore have a potential as a biopesticides1,2. The proposed biosynthetic pathway of triterpenoid saponins branches off from the sterol biosynthesis with 2,3-oxidosqualene as the shared precursor. The triterpenoid backbone structures are further modified mainly by cytochromes P450 and glycosyltransferases, thereby creating a vast structural diversity. 2,3-oxidosqualene is cyclized by oxidosqualene cyclases (OSC) to a limited number of triterpene backbone structures such as α-amyrin, β-amyrin, and lupeol. Despite high sequence identity the OSCs produce different backbone structures and also differ in the ratios of the different compounds produced. Our current research is focused on improving the activity of the saponins as biopesticides by e.g. targetting other organisms. One of the strategies is to elucidate the relationship between the structure and the activity of the OSC, as this will enable us to control the backbone structures formed by the OSC and also their ratios.

[1]**Kuzina *et al.* (**2009): Identification of defense compounds in Barbarea vulgaris against the herbivore Phyllotreta nemorum by an ecometabolomic approach. Plant Physiology.Vol. 151, pp. 1977-1990.
[2]**Agerbirk et al.** (2003): A saponin correlated with variable resistance of Barbarea vulgaris to the diamondback moth Plutella xylostella. Journal of Chemical Ecology. Vol. 29, pp. 1417-1433

[P-589-26] What Does Chemistry Tell Us About Peperomia Evolution?

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Peperomia (Piperaceae) is one of the most diverse genera among the basal Angiosperms with

1600-1700 species; in spite of its diversity it has received relatively little attention in phytochemical studies, and only 30 species have been thoroughly investigated. The chemistry of these species is unique and is characterized by 2-acylcyclohexane-1,3-diones, meroterpenes (polyketide chromenes), secolignans (peperomins) and few amides. The main objective of our study was to correlate the molecular phylogeny of Peperomia obtained using matK and ITS DNA sequences with the chemical profile of crude foliar extracts. PCA and HCA discriminations based on NMR data of the crude extracts were most effective in differentiating the species studied, which separated into two broad groups based on chemistry. The first group was defined by lignoids and included P. blanda (tetrahydrofurans) and P. glabella (peperomins).

The second group contained P. tetraphylla (amides) and P. trineura (2-acylcyclohexane-1,3-dione). Further molecular analyses with chalcone synthases (CHS) sequences produced a phylogeny similar to that obtained using matK and ITS. Detailed phytochemical study of this set of species is forthcoming in order to better characterize the clades and to overlay chemical profiling on molecular data.

FAPESP, CNPq, PRP-USP and CAPES

Poster Abstracts

[P-520-27] Differential Transcriptome Analysis of Developing Trichomes in Ocimum Basilicum.

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Peltate trichomes are the site of biosynthesis of flavor- and fragrance-defining compounds in sweet basil (Ocimum basilicum). Of the four studied basil chemotypes, line SW exhibits the most complex metabolic profile, accumulating both phenylpropanoids and terpenoids at comparably high levels. To obtain insights into regulatory and metabolic processes accompanying trichome development, we collected RNA from developing trichomes of line SW using laser microdissection, and sequenced the resulting cDNA using Illumina technology. More than 1.2 billion reads were assembled into 167,506 contigs, 38% of which were assigned a match by BLAST search against UniProt. Of the >1,000 contigs significantly overexpressed in immature trichomes defined as development stage 1, those with the highest expression were involved in DNA and protein biosynthesis and processing. In contrast, differentially expressed contigs from trichomes assigned to stages 3 and 4 were predominantly related to the biosynthesis of essential oil components. Tissue assigned to development stage 2 is likely to represent capitate trichomes and is enriched in transcripts involved in carbohydrate metabolism. Importantly, numerous transcription factors and putative transporters were identified among differentially expressed genes in different development stages. These new sequencing data are an excellent basis for a plethora of investigations into trichome physiology.

[P-619-28] Illumina Sequencing Based Transcriptomic Analysis for Ramie and Development of Est-ssr Markers for Economically Important Traits

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Ramie (Boehmeria nívea L.Gaud) is a traditionally economic fiber crop in China and has been cultivated for more than 2000 years. We used a high-throughput Illumina sequencing approach to sequence this plant. Sequence assembly obtained approximately 48,547contigs. The average length of sequences is 639 base pair nucleotides. Sequences were annotated by using public reference genomes curated by NCBI, NR, COGs, Swiss-Protand KEGG. Sequence assembly obtained 29,689 unigenes, led to21,607 proteins from an analysis at the Swiss-Prot database and 10,903 proteins from an analysis at the COG database. GO classification analysis was carried out to understand functions of genome-wide involvement in biological processes, cellular components and molecular functions. The resulting data showed419 unigenens involved in cell wall, membrane and envelope biogenesis, and 274unigenes associated with cellulose, hemicellulose, lignin, and pectin metabolism. A few of candidate genes with potential roles involved in cellulose, hemicellulose, lignin, and pectin biosynthesis were selected for RT-PCR analysis to show their tissue specificity. In addition, 11,320 potential EST-SSR were identified from 8,254 unigenes. Forty-six pairs of high quality PCR primers were designed and used for further assessment of EST-SSR. Of these, 41 primer pairs could successfully amplify cDNA fragments. These data are significant for us to understand molecular biology of ramie.

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Poster Abstracts

[P-621-29] Accumulation of Medium Chain Fatty Acids in Transgenic *Camelina sativa* Seed for Biofuel Production

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Camelina sativa is an important oilseed crop for biofuel production since it could grow with less water and fertilizer on the marginal land. A major potential application of Camelina seed oil is to be used for jet fuel production. Jet fuel specifications and conversion of oil to alkanes is most efficient and economic with medium-chain, saturated fatty acids. Acyl-acyl carrier protein (ACP) thioesterase (FatB) catalyzes the hydrolysis of acyl-ACP to free fatty acids and ACP. The free fatty acids are exported out of the plastid and subsequently esterified to coenzyme A, then react with Glycerol-3-phosphate in ER, finally stored as triacylglycerol (TAG) mainly in oil bodies. Seed oil of California bay (Umbellularia californica) contains up to 30% C10:0 and 60% C12:0 (lauric acid) fatty acids. We expressed the lauroyl-acyl carrier protein thioesterase (FatB) from California bay into Camelina and obtained up to 38% C12:0 and C14:0 fatty acids in seed oil of transgenic Camelina. In addition, acyl chains esterified to acyl carrier protein (ACP) are elongated by the sequential addition of two-carbon at a time. The elongation of C16:0-ACP to C18:0-ACP is mediated by β-ketoacyl-ACP synthase II (KAS II). We generated two RNAi constructs to reduce formation of C18:0-ACP in order to increase medium chain fatty acids in transgenic Camelina seed oil.

[P-620-30] Introduce Photorespiratory Bypass in Camelina Sativa, an Important Biofuel Crop

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In C3 plants, about 25% of the carbon fixed by photosynthesis is lost by photorespiration. Carbon losses through photorespiration have been successfully reduced in Arabidopsis by introducing the bacterial catabolic pathway that competes for glycolate, a photorespiratory intermediate (Kebeish et al., 2007). This method decreases carbon flux through photorespiration (photorespiratory bypass), and increases CO_2 concentration in the chloroplasts. We have overexpressed photorespiratory bypass in the chloroplasts of an oil seed crop, Camelina sativa. This pathway successfully increased the CO_2 concentration in the chloroplasts and resulted in 10-20% increase in photosynthesis in transgenic Camelina. The transgenic plants grew at a faster rate, had more and larger leaves than wild type controls. The transgenic plants also showed earlier flowering and had a greater number of pods and 50-70% higher seed yield. The seed oil yield per plant also increased by 15-35%. We are currently analyzing the transcriptome and metabolite profiles of the leaves from plants containing photorespiratory bypass, and comparing that with the WT leaf transcriptome. Studying the transcriptome and metabolism changes from plants having photorespiratory bypass would help us understand the effects of higher chloroplastic CO_2 and lower photorespiration on physiological processes that lead to higher seed oil yield in Camelina.

[P-612-31] Manipulation of Terpene Biosynthetic Pathways by Ectopically Expressing an Animal Geranyl Diphosphate Synthase Gene in *Camelina sativa*

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Camelina sativa is an important industrial oil crop, and its oilseeds can produce great starting materials for biofuel. Additives such as terpenes are critical in improving crude oil products of Camelina to well-adopted biofuel. However, naturally synthesized terpenes in plants are in low abundance. It has been shown that the production of terpenes is not only regulated by the level of terpene biosynthetic enzymes, but also highly contributed by the level of supplied substrates in the cell. Geranyl diphosphate (GPP), generally considered as the pivotal precursor of monoterpene biosynthesis, is synthesized by the head-to-tail condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) at the action of geranyl diphophate synthase (GPPS). In order to increase the production of GPP, we ectopically express an animal GPPS in Camelina. The overexpression of GPPS enhances vegetative growth and promotes early flowering in transgenic plants. Metabolic analysis in leaf, flower and stem demonstrates that the production of triterpene is higher in transgenic plants than that in wild-type plants, indicating the metabolic flux in terpene biosynthesis is increased as the result of expressing GPPS. Our data suggest that we have generated an ideal plant material which may produce more terpenes used for biofuel.

Poster Abstracts

[P-580-32] Capsaicinoid Content in a Daily Diet in the US Southwest

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The Southwestern (SW) diet features chiles in many meals, often multiple times per day. Chiles (Capsicum sp.) contain at least two classes of bioactive compounds, capsaicinoids and carotenoids, which are present in varying amounts and specific chemical types in different chilebased foods. Multiple in vitro studies suggest a chemopreventive role for capsaicinoids in carcinogenesis. However, the relevance of these studies are difficult to interpret without baseline ranges for chile consumption among frequent and seldom consumers. A focused food recall tool was used to measure dietary chile intake among 163 participants living in Las Cruces New Mexico. The majority of these individuals (73%) had consumed chile at least once, and 10% had eaten chile at three different meals in the previous 24 h. The chile containing foods consumed by these individuals were also reported. The content of the most abundant capsaicinoids, capsaicin and dihydrocapsaicin, in these food types were determined using automated hexane extraction and GC-FID separation and detection. From these results we can estimate the range of capsaicinoids available for oral absorption in a typical diet and compare those concentrations with the concentrations needed for health beneficial effects.

[P-544-33] Chemical Investigation of Secondary Metabolites from Freshwater Ascomycetes

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Fungi are prolific producers of secondary metabolites that have various medical applications. This therefore provides a very good reason to focus on the exploration of fungi for new drug candidates. Freshwater Ascomycetes (FWA) are a distinct ecological group that is of special interest due to the relative scarcity of information about them. Fungi in this ecosystem are assumed to create a wide array of biologically active chemicals conferring an adaptive value to the producing organism. To date, 70 FWA fungal species has been collected and described from different geographical regions across North Carolina. These fungi were grown in culture in the laboratory and extracted for their secondary metabolites. Compounds isolated consist of a diverse collection of chemical structures, including peptaibols, xanthones, epidithiodioxopiperazine, and polyketides, to name a few. To evaluate the practical value of the isolated compounds and extracts, biological testing, including the brine shrimp test and a series of antimicrobial assays were utilized.

[P-542-34] Dinsentangling Immunomodulating Activity of *Echinacea Purpurea* Extract

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Echinacea purpurea has traditional been used as a treatment for infection; however its efficacy is often the subject of controversy due to contradicting study results. While some studies have shown this common plant extract suppresses the immune response (anti-inflammatory), others have shown it enhances it (immunostimulatory). It is our hypothesis that the in vitro immunostimulatory response observed from E. purpurea extracts is a result of the presence of endophytic bacteria living within the plant, while the anti-inflammatory activity results from secondary metabolites produced by the plant itself. To test our hypothesis we used bioactivity directed fractionation the separate the immunostimulatory constituents from the anti-inflammatory constituents. Lipopolysaccharide (LPS) levels were used to determine the influence that endophytic bacteria have on the immunomodulating activity of the plant extract. We also grew a series of sterilized plants and tested them for immunomodulating activity. Our results revealed that were successfully able to separate the immunostimulatory activity from the anti-inflammatory activity. Fractions possessing immunostimulatory activity showed high levels of LPS, while the fractions possessing anti-inflammatory activity contained small molecule secondary metabolites produced by the plant. Our studies on sterilized plants showed a strong correlation between the bacterial load of the plants and the immunostimulatory effect.

Poster Abstracts

[P-543-35] Isolation of Secondary Metabolites from the Fungal Endophytes of *Silybum Marianum*

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Recent studies in our research group have focused on examining the role of fungal endophytes from ethnobotanical herbs such as milk thistle (Silybum marianum) by investigating the production of secondary metabolites from this unique ecological group. In continuation of this project, a subset (ten) of crude extracts from solid-substrate fermentation cultures obtained from the leaves of milk thistle were subjected to chemical separation. Several compounds belonging to a variety of structural classes were isolated and identified by using NMR and mass spectrometry techniques. Interesting biological activities have been reported in literature for many of these compounds.

[P-516-36] Polyoxypregnane Glycosides from the Roots of Marsdenia Tenacissima

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Marsdenia tenacissima is a perennial climber widely distributed in Yunnan province of China. The roots of this plant are not only used as a traditional Dai nationality herb medicine 'Dai-Bai-Jie' but also used as the main medicinal materials for preparing a series of preparations in China. For the first time, a systematic phytochemical study was performed on the roots of M. tenacissima. Consequently, 42 polyoxypregnane glycosides were isolated from the 95% alcoholic extract from the roots of M. tenacissima by the combination of various separation techniques. Based on the analyses of mass spectrometry and NMR spectroscopy, the structures of all compounds exhibiting the structural patterns of C21-steroid diester derivatives with the oligosaccharide sugar moiety consisting three or four unites were elucidated. Among them, 39 polyoxypregnane glycosides were new compounds.

[P-571-37] Ursane Triterpenoids and Their Anti-inflammatory Activity

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The pentacyclic triterpenes α-amyrin (**1**) and ursolic acid (**5**) were isolated from the Mexican medicinal plants Bursera copallifera and Asterohyptis setellulata, respectively. Various derivatives of α-amyrin (**1**) were synthetized, and the natural compounds and derivatives were evaluated on the TPA-induced ear edema on mice model, at the dose of 1 mg/ear, for test their anti-inflammatory activity. Results showed that all the compounds inhibited the ear edema; being α-amyrinone (**2**) and α-amyrin lactone (**4**) the most active compounds with inhibition percentages of 81.18 % and 79.28% respectively. Furthermore, the inhibitory activity of the NO production on lipopolysaccharide (LPS)-stimulated mouse peritoneal macrophages, was determined at the non-cytotoxic concentration (30 µg/mL). In this bioassay, compound **2**, **4,** and **5** exhibited inhibition of NO production up to 76.5, 99.0, and 87.5% respectively.

Poster Abstracts

[P-576-38] Abscisic Acid Regulation of Wound-Induced Suberization

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Suberin is a complex macromolecule deposited in response to wounding to prevent desiccation and pathogen infection, and comprises two distinct but covalently-linked domains. One domain is polyphenolic in nature, assembled from hydroxycinnamic acids, hydroxycinnamoyl amides and monolignols; the other domain is polyaliphatic in nature, assembled primarily from fatty acids, ω-hydroxy fatty acids, α,ω-dioic acids, 1-alkanols and glycerol. In Solanum tuberosum, the phenolic and aromatic domains of suberin are deposited in a coordinately regulated manner, with the phenolic domain being laid down in advance of the aliphatic one. Several lines of evidence from other research groups point to the involvement of the plant hormone abscisic acid (ABA) in the regulation of wound-induced suberization in potato tubers. In the present work, we treated potato tuber discs with the ABA biosynthesis inhibitor fluoridone and monitored the deposition of suberin aliphatics over time. A parallel group of discs were treated with fluoridone plus exogenous ABA. LC-MS analysis of ABA confirmed the inhibition of de novo ABA biosynthesis in fluoridone-treated discs. GC-MS analysis of suberin aliphatics revealed a nearly complete inhibition of suberin deposition in fluoridone-treated discs, while the application of exogenous ABA accelerated suberin deposition relative to water-treated controls. Expression analysis of suberin biosynthesis related genes by semi-quantitative RT-PCR revealed altered patterns of gene expression in response to the fluoridone and fluoridone + ABA treatments, consistent with an up-regulation of suberin biosynthesis genes by ABA.

[P-535-39] Biosynthesis of Brassicicolin A, A Phytotoxin from Plant Pathogen *Alternaria Brassicicola*

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Brassicicolin A is a host-selective phytotoxin produced by the cruciferous plant pathogen Alternaria brassicicola (Schewein.) Wiltshire. The chemical structure of brassicicolin A contains a mannitol with all hydroxyl groups esterified with two acetyl, two 2-hydroxyisopentanoyl and two 2-isocyanoisopentanoyl residues. A simple retrobiosynthetic analysis suggests the various precursors of brassicicolin A, however, no such work has been published to date. Toward this end, the biosynthetic precursors of brassicicolin A were investigated using the following isotopically labeled compounds: 13C6-glucose, 15N-valine, and 2H8-valine. Isotopically labeled brassicicolin A was obtained from fungal cultures incubated separately with each of the labeled compounds. After incubation of cultures, extraction and separation of metabolites, samples of brassicicolin A obtained from cultures incubated with the labeled compounds and from control cultures were analyzed using various spectroscopic techniques. 13C NMR and INADEQUATE spectra of brassicicolin A obtained from cultures incubated with 13C6-glucose showed several carbon resonances resulting from 13C-13C coupling of adjacent carbons, which were due to the six 13C-labeled carbons present in mannitol and in attached residues. Furthermore, spectroscopic data including 1H NMR, 15N-NMR and HPLC-ESI-MS of brassicicolin A indicated that both 2-hydroxyisopentanoyl and 2-isocyanoisopentanoyl residues are derived from valine.

[P-532-40] Identification of Bioactive Compounds from the Endophytic Fungi of *Asimina triloba*

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Despite their abundance and diversity, endophytic fungi have been sparsely explored, and therefore, there is a growing interest in the endophytes of medicinal plants. Although, the North American paw paw tree [Asimina triloba (L.) Dunal (Annonaceae)] has been studied extensively due to its production of cytotoxic Annonaceous acetogenins, the endophytic fungi that live within it have yet to be examined. This project is exploring the various fungi that grow in paw paw in search for new bioactive compounds. To date, over 275 fungal isolates have been made from the seeds, stems, and leaves of paw paw. HPLC, NMR, and HRMS were used for isolation and identification of fungal secondary metabolites. This poster will highlight some of the key compounds characterized to date from paw paw endophytes.

Poster Abstracts

[P-533-41] Metabolism of the Cruciferous Phytoalexins Brassilexin and Rutalexin by the Plant Pathogen *Alternaria Brassicicola*

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Brassilexin and rutalexin are phytoalexins produced by Brassica species such as canola (Brassica napus, B. rapa, B. juncea), broccoli (B. oleracea var. botrytis) and rutabaga. Phytoalexins are plant metabolites produced de novo in response to different kinds of stress including pathogen attack, and not present in healthy plants. In addition to their antifungal activity, some cruciferous phytoalexins are able to inhibit detoxifying enzymes produced by cruciferous pathogenic fungi. Both brassilexin and rutalexin inhibit the fungal growth of important cruciferous pathogens. In this work, the metabolism and potential detoxification of brassilexin and rutalexin by A. brassicicola was investigated and the antifungal activity of metabolites against A. brassicicola was determined. Results of this work showed that both phytoalexins were metabolized by A. brassicicola in ca. 24 hours. Interestingly, rutalexin was found to induce phomapyrone G, a metabolite produced by A. brassicicola, and to be hydrolyzed to an intermediate that was condensed with phomapyrone G. The final products of metabolism of brassilexin and rutalexin were substantially less toxic than the phytoalexins, hence it is concluded that A. brassicicola is able to detoxify both phytoalexins efficiently.

[P-534-42] Metabolite Profiles of *Alternaria Brassicicola* in Culture and *In Planta*

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Alternaria brassicicola (Schwein.) Wiltshire, together with A. brassicae, causes Alternaria black spot (also called dark leaf spot) in almost all organs of Brassica species. A comprehensive investigation of the metabolite profiles of A. brassicicola was carried out under various culture conditions including modifying carbon and nitrogen sources. Based on analyses of culture extracts by HPLC-DAD-ESI-MS, nitrates appeared to affect the production of depudecin and α-acetylorcinol, which increased with decrease in the concentration of KNO3 or NaNO3, and in the absence of nitrates. These results suggest that nitrogen sources affect the biosynthesis of depudecin, a histone deacetylase inhibitor, and α-acetylorcinol. In iron deficient conditions, A. brassicicola produced larger amounts of siderophores than in control cultures (ferric citrate 2 μM) or in high concentration of ferric citrate (200 μM). In addition, leaf extracts of infected plants (Brassica juncea, B. napus, and Sinapis alba) were analyzed by HPLC-ESI-MS. Although MeOH extracts of infected leaves did not show fungal metabolites, spore germination fluids obtained from infected plant leaves contained several fungal metabolites (siderophores and phomapyrone A). These results indicate that siderophores may facilitate fungal colonization by depriving host plants of iron.

Poster Abstracts

[P-573-43] Wound-Triggered Anthocyanin Accumulation is Impaired in Arabidopsis CYP94 Mutants

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Plants synthesize jasmonic acid and its derivatives (JA) in response to various biotic and abiotic stresses. JA acts as a signal activating or inhibiting biochemical pathways, transport processes, transcriptional regulatory networks and developmental processes related to defense. Jasmonoyl-isoleucine (JA-Ile) is the endogenous bioactive form of JA that is perceived by SCFCOI1 - JAZ (JASMONATE ZIM-DOMAIN) receptor complex. Tissue damage activates JA-Ile biosynthesis that in turn triggers JAZ protein degradation. JAZs are transcriptional repressors that bind to the MYB/bHLH/WD-repeat complex to repress anthocyanin biosynthesis and trichome initiation and thus the proteolytic degradation of JAZ releases MYB/bHLH/WD-repeat from repression to activate secondary metabolite synthesis. Arabidopsis cyp94b1cyp94b3 mutant hyperaccumulates JA-Ile as a consequence of blocked JA-Ile turnover. Contrary to the expectation that JA-Ile hyperaccumulation will lead to increased sensitivity to mechanical wounding resulting in increased secondary metabolite accumulations, cyp94b1cyp94b3 was more resistant to wound-induced secondary metabolite accumulation. The results imply missing components of the current wound signaling model and present novel system to study JA-mediated phytochemical synthesis.

[P-595-44] Melanogenesis Inhibition Activity in Zebrafish (*Danio Rerio*) Embryos of Extracts and Oils from *Pistacia Chinensis* Bunge

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Pistacia is one of the genera of the family Anacardiaceae in the order Sapindales. Many Pistacia species have been used as well-known folk medicines. Pistacia chinensis Bunge is one of the native plants of Taiwan with the potential of medicine plants. The objectives of this project are to evaluate the antityrosinase activity and melanogenesis inhibition activity in zebrafish (Danio rerio) embryos of ethanolic extracts and essential oils from P. chinensis twig and leaf. Results from the evaluation, extracts and oils of P. chinensis leaf and twig exhibited mushroom tyrosinase inhibition activity. Melanogenesis inhibition activity in zebrafish of oils from P. chinensis leaf and twig were better than those of ethanolic extracts. At a concentration of 100 microgram/mL, P. chinensis leaf and twig oils inhibited 20.9% and 31.4% of melanin production of zebrafish embryos, respectively.

[P-593-45] Mosquito Larvicidal Phytochemicals of Supercritical Fluid Extract from *Cryptomeria Japonica* Bark

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Mosquitoes are the vectors of causative agents for diseases such as dengue fever, malaria and yellow fever etc.; they pose the greatest threat to public health of human. It is imperative to control mosquito vector population in order to decrease the spread of these diseases. Exploration of more effective natural mosquito larvicides from plants for mosquito control is worthy of further research. Mosquito larvicidal activities of phytochemicals of supercritical fluid extract from Cryptomeria japonica bark were evaluated in this study. Supercritical fluid extract (SFE) showed high mosquito larvicidal activity, both LC50 values of SFE against Aedes aegypti and Ae. albopictus larvae were lower than those of positive control, rotenone. Five diterpenoids were isolated and identified from SFE. Mosquito larvicidal activities of these diterpenoids were also examined. Results indicated that supercritical fluid extract from Cryptomeria japonica bark and its phytochemicals possess high mosquito larvicidal activity, and are highly potent to be used as natural pesticides.

Poster Abstracts

[P-461-46] Phenolic Plant Metabolites as Bioactive Food and Feed Additives

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Functional additives in food and animal feed formulations are gaining acceptance as consumers and producers recognize the health benefits associated with certain natural plant products. Phenolic compounds in particular have emerged as a class of compounds with antioxidant, antibacterial, and antifungal properties. Small phenolic compounds such as ferulic and coumaric acids are ubiquitous in plants and may be obtained from lignocellulosic biomass while larger phenolic structures such as catechins are available from peanut skins, a by-product of peanut processing. The initiative to develop alternative fuels such as ethanol from biomass presents an opportunity to generate an inexpensive supply of ferulic and coumaric acids as co-products for food and feed applications. Similarly, the availability of peanut skins as a low-cost material from the peanut processing industry provides a source of catechins. The growth of the market for these phenolic compounds is expected to provide additional revenue for peanut processors and bioethanol producers as demand increases for functional additives in the food and feed industries.

[P-517-47] Refining NIR Calibrations for Total Carbohydrate Composition and Isoflavones and Saponins in Ground Whole Soy Meal

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Although many near infrared (NIR) spectrometric calibrations exist for a variety of components in soy, current calibration methods are often limited by either a small sample size on which the calibrations are based or a wide variation in sample preparation and measurement methods, which yields unreliable results. Over the past two years our lab has used defined analytical methodology to measure 1) isoflavones and saponins in soy samples, and 2) the soluble carbohydrates (sucrose, raffinose, stachyose, verbicose, glucose and fructose) and "insoluble" carbohydrates (such as starch, cellulose, pectin and other structural carbohydrates) by hydrolysis to the monomer form and derivation to measure total insoluble monomers and total uronic acids. This analysis was done in triplicate on over 500 crop samples from the 2011 harvest and over 600 samples from the 2012 harvest. NIR calibrations have been developed from scans performed on the ground whole soy meal on three different NIR instruments using three different calibration development packages.

[P-585-48] Response to Salt of a Rice Diversity Panel

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Rice is one of the most important crops worldwide and represents one of the main foods for 70 percent of the world's population. Rice is grown in countries with long coastal regions including India, China, and the USA. In these countries there are areas where rice production is being negatively affected by soil salinity. Rice is naturally salt-sensitive, although some varieties are more tolerant than others. This work is part of a collaborative effort between the Lorence and Walia Laboratories, in which we are applying high throughput plant phenotyping approaches to identify novel sources of salt tolerance within a diversity panel. A selected group of 100 rice lines including salt-tolerant and salt-sensitive types was sent to the Lorence team to analyze their response to salt stress at the early vegetative stage using a powerful nondestructive phenomics system called Scanalyzer HTS. The Lorence group has developed phenotyping protocols for the identification and characterization of salt tolerant lines, and I will present my main findings applying those protocols to the rice lines that I analyzed during my summer internship.

Poster Abstracts

[P-594-49] Role of Micrornas During Storage Root Development in Sweet Potato

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Sweet potato (Ipomoea batatas) is one of the major food and vegetable crops around the world. Sweet potato produces two types of roots: storage roots and fibrous roots. A storage root also called tuberous root is a modified lateral root, enlarged to function as a storage organ, which is the plant part for human food and/or vegetable. However, the molecular mechanism controlling lateral roots to become storage root is still unclear. In this study, we systematically investigate the potential role of 16 conserved microRNAs (miRNAs) during the storage root development; miRNAs are an extensive class of small regulatory RNAs controlling almost all biological and metabolic process in plants. Our results show that some miRNAs expressed at an organ-dependent manner. For example, the expression level of miR 156 and miR 162 was significantly lower in storage roots than that in leave and fibrous roots. This suggests that miRNAs may play a role during storage root initiation and development. Sweet potato-specific miRNAs may play unique role during this process.

[P-515-50] Secondary Metabolite Production by Fungal Endophytes of Canadian Fruit Crops

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The potential role of fungal endophytes in the protection of grasses and conifers against insect and fungal diseases has been demonstrated. In this study, we examine the secondary metabolites isolated from fungal endophytes of grape, raspberry, blueberry and cranberry plants collected in Ontario and Nova Scotia. The plants and berries of these high-value crops are highly susceptible to a variety of fungal and bacterial diseases as well as insect pests. 250 fungal endophyte isolates were grown and a number of extracts exhibited antifungal and antibacterial activity. Strains isolated from grape and raspberry leaves have led to the characterization of a number of new compounds. Fifteen Xylaria sp. fungal endophytes isolated from lowbush blueberry plants were shown to produce griseofulvin. This is of interest because the same Xylaria sp. was isolated as an endophyte from needles of pine trees in the surrounding forest demonstrating a pine-blueberry forest ecotype. Additionally, known antibacterial and antifungal compounds were isolated from blueberry, grape and cranberry endophytes. The bioactivity of the compounds isolated to date suggests that they play a role in defence against diseases and pests and could potentially be used in agriculture for crop protection.

[P-599-51] The Effects of Drought and Salt Stresses on Gaba Metabolic Pathway in Bean (P*haseolus Vulgaris* L.) Seedlings

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Drought and salinity are abiotic stresses reduce the plant efficiency all over the world. Soil salinity is increasing with drought. Therefore, understanding the mechanisms of plants against abiotic stress conditions is an effective method to increase stress tolerance and productivity. γ-aminobutyric acid (GABA) is proposed to be a signaling molecule involved in nitrogen metabolism and protection against oxidative damage in response to various abiotic stress conditions. The aim of our study was to examine the role of GABA response in bean (Phaseolus vulgaris L. cv.Akman 98) to salt and drought stresses. Glutamate dehydrogenase (GDH) activity, GABA accumulation and lipid peroxidation (MDA) were determined. Our data showed an increase in MDA under multiple stress conditions in bean seedlings. A possible explanation is that GABA shunt could be a key metabolic pathway to adapt to drought, salt and multiple stress conditions.

Poster Abstracts

[P-486-52] The Patagonian Wild Raspberry (Rubus Geoides): A Source of Bioactive Antioxidants Characterized by HPLC-DAD-MS/MSN

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The Chilean wild raspberry (Rubus geoides, Rosaceae) is a native species occurring from southern Chile to Patagonia and Tierra del Fuego. It has a pleasant taste and attractive color. The fruits were consumed since prehistoric times by Amerindians who called it "Miñe-miñe". An HPLC-DAD-MS/MSn method was developed for polyphenol profiling in R. geoides samples from different locations of southern Chile. Fruits were collected in Araucania, Aysen and Magallanes. The antioxidant activities of methanol and phenolic-enriched-extracts (XAD) were assessed for their ability to scavenge DPPH and ABTS radicals and their reducing power (FRAP). Total phenolic, total flavonoid and total anthocyanin content was determined. A commercial raspberry (R. ideaus) sample was included for comparison. High antioxidant activity was found for the fruits in agreement with previous reports on other native Chilean berries and northern hemisphere currants. Several anthocyanidins and flavonol glycosides were tentatively identified by HPLC-MS-MSn. Further work is underway to obtain a more complete picture of the phenolic antioxidants in fruit, including isolation of the main constituents for NMR characterization.

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[P-700-53] Enhancement of Vitamin E Concentrations in Soybean and Camelina Seeds for Improved Oil Oxidative Stability

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Vitamin E, which comprises tocopherols and tocotrienols, not only provides nutritional value to seeds but is also important for oxidative stability of vegetable oils. In monocots, tocotrienol synthesis occurs by prenylation of homogentisate (HGA) with the unsaturated C20 isoprenoid geranylgeranyl diphosphate (GGDP), a reaction catalyzed by HGA geranylgeranyl transferase (HGGT). In contrast, tocopherols are synthesized by prenylation of HGA with the saturated C20 isoprenoid phytyl diphosphate (PDP) via HGA phytyltransferase (HPT) activity. Seed-specific expression of barley HGGT in soybeans increased vitamin E content up to 10-fold by accumulation of primarily δ- and γ-tocotrienols. Co-expression of γ-tocopherol/tocotrienol methyltransferase (VTE4) gene with HGGT in soybean seeds shifted vitamin E composition to more nutritionally significant α- and β-forms. Successful crosses of high vitamin E soybean lines with high oleic and high γ-linolenic acid (GLA)/stearidonic acid (STA) lines were generated for oil oxidative stability testing. In camelina, seed specific expression of barley HGGT resulted in ~5-fold increase in vitamin E content. Enhancements in vitamin E concentrations in camelina seeds were also obtained by increasing levels of the HGA substrate through seed specific co-expression of Arabidopsis HPPD and bifuctional E.coli TyrA to enhance HGA synthesis and by RNAi suppression of the HGA oxygenase (HGO) gene to reduce HGA catabolism. These constructs were also introduced into a camelina line engineered for seed-specific HGGT expression to measure the effect of combining increases in HGA pool sizes with enhanced HGA prenylation on vitamin E production.

Poster Abstracts

[P-701-54] Volatile Metabolic Profiles In Cuban Oregano (*Plectranthus Amboinicus*) Analyzed By SPME-GC-MS

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Cuban oregano (Plectranthus amboinicus, in the Lamiaceae family) is native to Africa. It is a potent aromatic medicinal herb because of a wide array of volatiles produced in tissues in its native ecosystems. Presently, it is widely grown in tropical regions. In this study, we hypothesize that the greenhouse growth likely alters its aromatic compound profiles identified from its native growth environment. Leaves with different ages and positions were collected for volatile analysis. Solid phase microextraction (SPME) is used to extract volatiles, which are analyzed using gas chromatography-mass spectrometry (GC-MS) analysis. We have observed numerous volatiles. Examples of main components include caryophyllene, copaene and naphthalene, which are strong anti-microbial compounds. Meanwhile, we observed the differentiation of volatile profiles in different tissues. This research enhances the understanding of medicinal volatile profiles of Cuban oregano when grown in different conditions from its native environment.

[P-702-55] Analysis Of Polysacchride In Medicinal *Ganoderma lucidum*

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Ganoderma lucidum is a medicinal mushroom that has been used for more than 2000 years in Traditional Chinese Medicines. Its food body is one of the largest ones in the fungus kingdom. Over the past hundreds of years, medicinal applications have showed differential functions among varieties. To compare medicinal efficacy, we have collected multiple germplasm varieties. In this report, we use different phytochemical approaches coupled with food body morphology characterization to understand medicinal polysaccharide levels in one germplasm, namely Xiang Chi Zhi #1 (XCZ#1). Hot water alone, hot water in ultrasound, supercritical CO2, and hot enzymatic buffer were used to extract fresh powders of food body samples. Each extraction was technically repeated twice. Detailed steps will be discussed in our poster. The resulting extractions were analyzed to characterize the level of polysaccharides. The level of polysaccharides from the hot water in ultrasound was approximately 10.7%, higher than those from other methods. In addition, we observed that extracts from the fresh samples showed strong antioxidative activities. These data will enhance further understanding polysaccharide compositions in XCZ#1 and be helpful to investigate other varieties.

[P-597-56] Bioengineering and Regulation of Monoterpenoid Production in the Glandular Trichomes of Mints

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To study the causes for the differences in oil composition among different mint cultivars, we performed next-generation transcriptome analyses with glandular trichomes. Promoter sequences were obtained for the most highly expressed genes in these specialized cells from peppermint. These promoters were fused to the gene encoding menthone:menthol reductase, and constructs were transformed into Erospicata which is devoid of (-)-menthol but resistant to Verticillium wilt (unlike peppermint). In certain transgenic Erospicata lines (-)-menthone was converted to (-)-menthol. However, the isolated promoters were not equally effective in driving the expression of transgenes in a glandular trichome-specific fashion. Besides, transcriptome data showed that IPR expression level (responsible for (+)-isopulegone production as an intermediate in (-)-menthol biosynthesis pathway) is critically low just in spearmint. Detailed analysis of a putative glandular trichome specific promoter (upstream of IPR) in several mint species revealed that an IPR gene copy is located in reverse orientation upstream of the 5'-end of the promoter. Since transcribed inverted repeats trigger DNA methylation of identical sequences in plants, we conducted both leaf and glandular trichome-based DNA bisulfite sequencing of IPR. Interestingly, our results show that cytosine methylation of IPR in glandular trichomes is the reason of inactivation of this gene in spearmint.

Poster Abstracts

[P-703-57] Transcriptional and Phenotypic Salt Stress Responses in Oryza sativa Are Dependent on Time and Temperature

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Plants have an internal clock that mediates almost all daily activities. This circadian clock can be entrained using external light and temperature cues. The stimulation of the plant with light/dark cycles and warm days and cold nights establishes an internal control mechanism that allows the plant to anticipate and adapt to changes in its environment. The circadian clock is highly conserved between plant species and leads to a higher probability of survival and increased number of progeny. Study of the circadian clock in *Arabidopsis thaliana* indicates that it plays an important role in mediating stress responses. We are investigating the role of the circadian clock in response to salt stress in *Oryza sativa*. In particular we are interested in understanding the contribution of recurring light/dark (photocycles) and day/night temperature differences (thermocycles) in modulating the response to salt stress. Our approach incorporates phenotypic and transcriptional data to generate a new understanding of the salt stress response in *Oryza sativa*. A better understanding of the response to stress in these entrainment conditions may elucidate ways to combat decreases in rice yield due to global climate change.

[P-704-58] RNAi-induced Gene Silencing to Provide Genetic Evidence of Early Steps of Artemisinin Biosynthesis in *Artemisia annua*

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Artemisia annua L. (Qing Hao in Chinese Ping Ying) is an indigenous medicinal plant from China. To date, this plant provides the only natural resource to produce artemisinin, the effective antimalarial medicine. Artemisinin is an endoperoxide sesquiterpene lactone. Over the past two decades, research efforts have provided biochemical evidence to show early steps from amorpha-4, 11-diene to artemisinic acid and dihydroartemisinic acid, two of which are considered being the precursors of artemisinin. Two fundamental genes, *ADS* and *CYP*, were demonstrated to control the steps to artemisinic acid and hydroartemisinic acid by biochemical and transgenic analyses. However, given that the heterogeneous property of plants leads to difficulties and uncertainties to select mutants and cause natural variations, the genetic evidence for the presence of the pathway remains to be explored. We have bred a homozygous population, which provides an ideal model to investigate genetics of artemisinin biosynthesis. In this study, we introduced ADS-RNAi and CYP71-RNAi constructs to *A. annua* to silence the expression of *ADS* and *CYP* genes. The RT-PCR showed a significant decrease in *ADS* expression in transgenic plants compared with the wild type plants. HPLC analysis showed that artemisinin contents were significantly decreased in two ADS-RNAi transgenic lines, showing that *ADS* genetically controls its step to artemisinin *in Planta*. In this presentation, we will discuss the significance of genetic evidence for the final understanding of artemisinin biosynthesis in plants. This work was financially supported by North Carolina Biotechnology Center.

Index

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

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