55th Annual Meeting of the Phytochemical Society of North America
August 6-10, 2016
University of California, Davis

Eric Conn Symposium
Integrated Omics: Technology and Application
Phytochemical Metabolism
Functional Foods and Botanical Medicine
Synthetic Biology and Metabolic Engineering
Plant, Microbe, Insect Interactions
Phytochemical Signaling
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Greetings

Dear Colleagues,

We are delighted to welcome you to the 55th annual meeting of the PSNA! The mission of the PSNA is to inspire and foster research and development in the chemistry, molecular biology, biochemistry and systems biology of plant metabolites, their impact upon plant, animal and human physiology and pathology, renewable energy, and their economic utilization and value (www.psna-online.org/). PSNA meetings provide participants with exposure to the cutting-edge research of leading international scientists, but remain small enough to offer an interactive and intimate environment conducive to knowledge exchange and strategic discussion. This year’s meeting is particularly special as we will have a symposium dedicated to the pioneer work of an outstanding plant natural product biochemist and a former UC Davis professor, Dr. Eric Conn.

A highlight of the conference will be the presentation of the Arthur C. Neish Young Investigator Award to young scientists who just started to develop their independent career. The Society’s effort to stimulate early careers in phytochemistry is also exemplified by the “Phytochemistry/PSNA Young Investigator Research Grant Award”, which is sponsored by Elsevier and presented biannually to a dynamic young scientist within ten years of receiving their doctoral degree and currently leading an independent research program in the broader areas of phytochemistry at a university, or at a government- or not-for-profit research institute. A young member’s luncheon has also been organized, in the form of panel discussion with a diverse group of scientists, which will expose students and postdocs to various career options.

Special thanks go to all of our sponsors for providing generous support for the conference! We look forward to many inspiring presentations and thought-provoking discussions at the conference. We also hope that you will find time to explore the beautiful UC Davis campus and many exciting neighboring areas of Davis.

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Conference Program

All events take place at the UC Davis Conference Center. Your registration includes the conference program, the welcome reception on Saturday, continental breakfast Sunday-Wednesday, coffee breaks Sunday-Wednesday, lunch Sunday-Tuesday, poster sessions on Sunday and Monday, and the banquet on Tuesday.

Saturday, August 6, 2016

3:00-7:00 pm  Registration desk open
6:00-8:00 pm  Welcome Reception

Sunday, August 7, 2016

7:30-8:00 am  Continental breakfast
8:00-8:15 am  Opening remarks
8:15-10:05 am  Symposium I: Eric Conn Symposium
  Chairs: David Gang (Washington State University) and Argelia Lorence (Arkansas State University)
  8:15-9:00 am Birger Møller (University of Copenhagen)
  Cyanogenic glucosides: from the field to the single molecule
  9:00-9:45 am Eve Wurtele (Iowa State University)
  Orphan genes: An untapped reservoir of novel traits
  9:45-10:05 am Lucia Montini (University of Copenhagen)
  Dhurrin, a small compound that can do great things
10:05-10:25 am  Coffee break
  Chairs: Mark Berhow (USDA/ARS/NCAUR) and Lloyd Sumner (University of Missouri)
  10:25-10:55 am Xu (Sirius) Li (North Carolina State University)
  Identification of a residue responsible for UDP-sugar donor selectivity of a dihydroxybenzoic acid glycosyltransferase from Arabidopsis natural accessions
  10:55-11:25 am Hiroshi Maeda (University of Wisconsin, Madison)
  Relaxation of tyrosine pathway regulation during the evolution of betalain pigmentation in Caryophyllales
  11:25-11:55 am Philip Bates (University of Southern Mississippi)
  Deciphering the control of fatty acid fluxes through the lipid metabolic network for enhanced plant oil bioengineering
11:55 am –1:00 pm  Lunch
1:00-2:50 pm  Symposium II: Integrated Omics: Technology and Application
  Chairs: Mark Berhow (USDA/ARS/NCAUR) and Lloyd Sumner (University of Missouri)
  1:00-1:45 pm Oliver Fiehn (University of California, Davis)
  Using metabolomics to address biochemical diversity and function in plants
  1:45-2:30 pm Dan Kliebenstein (University of California, Davis)
  Adaptive metabolites solve the fluctuating environment via genetic variation and large regulatory networks
2:30-2:50 pm Sangeeta Dhaubhadel (Agriculture and Agri-Food Canada) Omics insights into regulation of isoflavonoid biosynthesis in soybean

2:50-3:10 pm Coffee break

3:10-5:10 pm
3:10-3:30 pm Sanem Hosbas Coskun (National Institute of Standards and Technology) In-vivo and in-vitro antidiabetic effect of Achillea setacea Waldst. & Kit. and detection of major phenolic compounds by LC-MS/MS and NMR techniques
3:30-3:50 pm Lloyd Sumner (University of Missouri) Integrated metabolomics identifies a novel M. truncatula DDMP-transferase (MtDPT) and its role in saponin biosynthesis
3:50-4:10 pm Mark Berhow (USDA, ARS, NCAUR) Rapid analysis of the chemical composition in plant products: Correlating rapid spectral analysis with comprehensive phytochemical composition
4:10-4:30 pm Herana Seneviratne (Washington State University) Identifying and localizing plant secondary metabolites in specific cell types in situ: Integrated MALDI imaging, ion mobility separation and collision-induced dissociation approaches
4:30-4:50 pm Michael Paulsmeyer (University of Illinois, Urbana) Genetic mapping of a new anthocyanin phenotype in maize
4:50-5:10 pm Michael Rush (University of Illinois, Chicago) Structure elucidation of procyanidin oligomers by MALDI TOF-TOF – characteristic ions for identifying A and B type procyanidins from MS² data

5:30-7:30 pm Poster session I
Monday, August 8, 2016

7:30-8:00 am Continental breakfast

8:00-8:15 am Conference announcement

8:15-10:05 am Symposium III: Phytochemical Metabolism
Chairs: Xiao-Ya Chen (SIBS, Chinese Academy of Sciences) and Deyu Xie (North Carolina State University)
8:15-9:00 am Ian Graham (University of York)
Molecular breeding of medicinal crops and discoveries along the way
9:00-9:45 am Guodong Wang (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences)
Genome mining for sesterterpenoid discovery in Brassicaceae plants
9:45-10:05 am Reinhard Jetter (University of British Columbia)
The structures, biosynthesis and genetics of wheat wax polyketides

10:05-10:25 am Coffee break

10:25 am-12:05 pm 10:25-10:45 am Mingzhuo Li (Anhui Agricultural University)
Functional characterization of a tea (Camellia sinensis) R2R3-MYB suppressor that downregulates the phenylpropanoid and shikimate pathways
10:45-11:05 am Mark Lange (Washington State University)
De-mystifying chemical diversity in the Thunder God Vine - new avenues for the production of triptolide, a diterpene epoxide for treating kidney disease and cancer
11:05-11:25 am Craig Schenck (University of Wisconsin, Madison)
Phylogeny-guided structure-function analysis of tyrosine biosynthetic enzymes in legumes
11:25-11:45 am Ying Wang (South China Botanical Garden)
Carotenoid and anthocyanin biosynthesis and accumulation in fleshy fruits of Lycium L.
11:45 am-12:05 pm Bernd Schneider (Max Planck Institute for Chemical Ecology)
Nudicaulins - a unique group of flower pigments of Papaver nudicaule:
Biosynthesis and potential ecological function

12:05-1:30 pm Lunch

12:15-1:15 pm Executive Committee meeting

12:15-1:15 pm Career panel/workshop

1:30-3:00 pm Symposium IV: Functional Foods and Botanical Medicine
Fred Stevens (Oregon State University) and Elvira de Mejia (University of Illinois, Urbana)
1:30-2:15 pm Andy Waterhouse (University of California, Davis)
Gut metabolites of dietary phenolics
2:15-3:00 pm Thomas Prisinzano (University of Kansas)
Salvia divinorum: A unique CNS active plant

3:00-3:20 pm Coffee break
3:20-4:20 pm  3:20-3:40 pm Ines Paraiso (Oregon State University)
Biotransformation of xanthohumol and related flavonoids by intestinal bacteria
3:40-4:00 pm Richard van Breemen (University of Illinois, Chicago)
Studies of pharmacokinetic interactions between drugs and botanical dietary supplements used by menopausal women
4:00-4:20 pm Claudia Maier (Oregon State University)
Modulation of the brain metabolome by an aqueous extract of Centella asiatica

4:20-4:30 pm  Group photo

4:30-5:30 pm  Society business meeting

5:30-7:30 pm  Poster session II
Tuesday, August 9, 2016

7:30-8:00 am  Continental breakfast

8:00-8:15 am  Conference announcement

8:15-10:05 am  Symposium V: Synthetic Biology and Metabolic Engineering
Chairs: Dae-Kyun Ro (University of Calgary) and Philipp Zerbe (University of California, Davis)
8:15-9:00 am  Björn Hamberger (Michigan State University)
Discovery of terpene biosynthetic pathways in plants: Synthetic biology tools for production
9:00-9:45 am  John Dueber (University of California, Berkeley)
Use of an enzyme-coupled biosensor to engineer a BIA fermentation pathway from glucose in Saccharomyces cerevisiae
9:45-10:05 am  Jose Celedon (University of British Columbia)
Transcriptome and metabolite analysis across xylem development in Sandalwood trees reveal the final step in sandalwood oil biosynthesis

10:05-10:25 am  Coffee break

10:25 am-12:05 pm  10:25-10:45 am  Deyu Xie (North Carolina State University)
Enhancement of amorphadiene pathway for antimalarial medicines in Artemisia annua
10:45-11:05 am  Dae-Kyun Ro (University of Calgary)
Molecular evidence for protein complex in natural rubber biosynthesis in lettuce (Lactuca sativa)
11:05-11:25 am  Simona Florea (University of Kentucky)
Genome editing through chromosome end knock-off: Reshaping the alkaloid profiles in Epichloë coenophiala
11:25-11:45 am  Toshiaki Umezawa (Kyoto University)
Modification of lignin aromatic composition in Oryza sativa for biomass refinery
11:45 am -12:05 pm  Kyle Pelot (University of California, Davis)
Elucidation of diterpene synthases in Salvia divinorum toward novel plant-derived therapeutics

12:05-1:00 pm  Lunch

1:00-2:50 pm  Symposium VI: Plant, Microbe, Insect Interactions
Chairs: Dan Kliebenstein (University of California, Davis) and Sangeeta Dhaubhadel (Agriculture Agri-Food Canada)
1:00-1:45 pm  Andre Kessler (Cornell University)
Volatile-mediated information transfer in Tall Goldenrod, Solidago altissima: Ecological consequences and evolutionary aspects
1:45-2:30 pm  Beth Sattely (Stanford University)
A gene-centric approach for the discovery and engineering of plant chemistry
2:30-2:50 pm  Quang Huy To (University of Saskatchewan)
Nasturllexins and cyclonasturlexin, novel phytoalexins from Nasturtium officinale, originate from parallel biosynthetic pathways

2:50-3:10 pm  Coffee break
3:10-3:50 pm 3:10-3:30 pm **Yezhang Ding** (University of California, San Diego)
Terpene synthase ZmTps21 is responsible for a previously undetected β-selinene derived antimicrobial phytoalexin in maize (*Zea mays*)
3:30-3:50 pm **Carlos Sanchez Arcos** (Max Planck Institute for Chemical Ecology)
Non-targeted and targeted metabolomic approaches reveal differences in legume chemistry before and after infestation with pea aphid host races

3:50-7:00 pm Free time

7:00-9:00 pm **Award Banquet**
Wednesday, August 10, 2016

7:30-8:00 am Continental breakfast

8:00-8:15 am Conference announcement

8:15-10:05 am Symposium VII: Phytochemical Signaling

Chairs: Kent Chapman (University of North Texas) and Dhirendra Kumar (East Tennessee State University)

8:15-9:00 am Gregg Howe (Michigan State University)
Phytochemical signaling in plant defense
9:00-9:45 am Savithramma Dinesh-Kumar (University of California, Davis)
Emerging perspectives on chemical modulators of autophagy for disease control
9:45-10:05 am Kent Chapman (University of North Texas)
Structural insights into the biochemical properties of Arabidopsis fatty acid amide hydrolase

10:05-10:25 am Coffee break

10:25-11:55 am Mike Sullivan (US Dairy Forage Research Center, ARS-USDA)
A reverse genetics approach to elucidating substrate specificity of a hydroxycinnamoyl-CoA hydroxycinnamoyl transferase using transgenic alfalfa
10:45-11:15 am Clare Casteel (University of California, Davis)
The role of vector-borne viruses in altering host plant defenses
11:15-11:35 am Dhirendra Kumar (East Tennessee State University)
SIP68 is a putative glucosyltransferase enzyme with a likely role in plant stress response
11:35-11:55 am Yousuke Takaoka (Tohoku University)
Development of JAZ-subtype selective agonist based on Coronatine

Noon Conclusion of PSNA conference, lunch on your own
Eric Conn’s contributions to the field of phytochemistry span an illustrious career of more than 45 years. The majority of this time was spent in the University of California, first at the Berkeley campus and later at Davis. His research efforts have impacted most significantly within the field of natural product biochemistry focusing particularly on phenols, coumarins and cyanogenic glycosides. His pioneering studies of the shikimate pathway, the phenylpropanoid pathway, and the localization and compartmentalizing of numerous secondary natural products and their respective metabolic enzymes have gained him well-deserved world wide recognition. Indeed, thousands of publications have been produced because of his discovery of the enzyme phenylalanine ammonia lyase (PAL). The range of his work has been very broad. Early work in his career described fundamental processes involved in the oxidation/reduction of NADP⁺ and associated reactions which are now standard textbook dogma. In recent years, he has contributed extensively to chemotaxonomic investigations into cyanide containing genera, especially the *Acacia*. His publication record includes nearly 200 manuscripts, including 28 peer reviewed papers in the journal *Phytochemistry*. Additionally, he has served as President of American Society of Plant Physiology, as well as President, Editor-In-Chief, and Life Member of the Phytochemical Society of North America.

Birger Lindberg Møller is a professor of Plant Biochemistry at the University of Copenhagen, Director of the Center for Synthetic Biology and Head of the VILLUM research center “Plant Plasticity”. A major research interest is the development of crop plants for the future with focus on the role of bio-active natural products in plant resistance to abiotic and biotic stresses. Key classes of natural products studied are hydroxynitrile glucosides and diterpenoids with focus on their synthesis, turn-over, storage, and role in plant insect and plant microbe interactions. BLM is an elected member of the Royal Danish Academy of Science and Letters and member of the International Human Rights Network of Academic and Scholarly Societies, Washington. In 2007, BLM was awarded the Villum Kann Rasmussen Research Prize, the largest Danish research award (350,000 Euro). In 2013, BLM was awarded an ERC Advanced Grant and in 2015 an ERC PoC grant.

[S1-1] Cyanogenic glucosides: from the field to the single molecule

Birger Lindberg Møller
Plant Biochemistry Laboratory, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark

For more than 420 million years, plants, insects and microbes have co-evolved based on a chemical arms race including deployment of refined chemical defense systems by each player. Cyanogenic glucosides (α-hydroxynitrile glucosides) are one class of defense compounds
produced by numerous plants (e.g. sorghum, barley, wheat, cassava, clover, flax, almonds, eucalypts). Following tissue disruption e.g. caused by a chewing insect, the cyanogenic glucosides are hydrolyzed and release toxic hydrogen cyanide to protect the plant from generalist herbivores. Many fungi are not deterred by hydrogen cyanide which they rapidly convert into carbon dioxide and ammonia. In sorghum, dhurrin is formed from tyrosine in a pathway catalyzed by two multifunctional ER-bound cytochrome P450s (CYP79A1 and CYP71E1), an ER-bound P450 oxidoreductase (POR) and a soluble UDP-glucosyltransferase (UGT85B1) with E- and Z-oximes as key intermediates. In vivo studies based on transient expression in Nicotiana benthamiana demonstrated that all enzymes were active when expressed as eGFP fusion proteins. Protein-protein interactions and channeling was studied in planta using fluorescence lifetime imaging microscopy, fluorescence correlation spectroscopy and metabolite analysis demonstrating that the enzymes catalyzing dhurrin biosynthesis are organized within dynamic metabolons. Using the styrene maleic acid (SMA) copolymer, discrete lipid particles (SMALPs) were excised from the ER membrane enabling purification of the dhurrin metabolon by affinity chromatography and characterization by mass spectrometry based proteomics. Functional importance of identified protein-protein interactions and lipid environment was studied by reconstitution of the dhurrin pathway in proteoliposomes. UGT85B1 binding to liposomes was dependent of the presence of CYP79A1 and CYP71E1. A model for the organization of the dhurrin metabolon in multi-enzyme clusters is presented. In vitro studies show that CYP71E1 is very sensitive to oxygen. In vivo inactivation of CYP71E1 e.g. by the oxidative burst following a fungal infection would result in accumulation of the E-oxime which is a fungal toxin. In contrast, pathogenic fungi are able to detoxify Z-oximes. The genes encoding the biosynthetic enzymes for cyanogenic glucosides are clustered on the plant genomes. Specialized insects like the Six-Spot Burnet Moth (Zygaena filipendulae) manage to sequester cyanogenic glucosides from their food plant and to use the plant defence compound in their own defence against predators. The insect de novo synthesizes the cyanogenic glucosides if the amounts obtained by sequestering are low. Sufficient levels are important because cyanogenic glucosides play numerous additional intimate roles in the mating process of the insects e.g. as nuptial gifts. In plants, cyanogenic glucosides may function as storage reservoirs of reduced nitrogen (ammonia) and sugar as demonstrated by the operation of two pathways for endogenous turn-over of cyanogenic glucosides avoiding the release of hydrogen cyanide. Forage sorghum contains the cyanogenic glucoside dhurrin and following adverse growth conditions, the amounts of HCN released may be toxic to grazing livestock. In collaboration with Australian researchers, biochemical screens and TILLING approaches have been used to identify a single amino acid change in the CYP79A1 enzyme that resulted in an inactive enzyme and acyanogenic forage sorghum plants.

Recent literature:

Eve Syrkin Wurtele received her Ph.D. from UCLA, and did a postdoctoral fellowship with Eric E. Conn at UC-Davis. After a stint at a biotechnology company, she joined Iowa State University. Wurtele's research centers on the interplay between metabolic and regulatory signals. She is particularly interested in how new genes (orphans) arise and participate in these processes. The research is revealing the complex networks that mediate accumulation of proteins, starches, oils, and specialized natural products. Wurtele also directs the award-winning computer game, MetaBlast (metablast.org). She received the 2012 Pioneer Dupont Award for Iowa Woman of Research Innovation and Leadership.

[S1-2] Orphan genes: An untapped reservoir of novel traits

Eve Syrkin Wurtele
Iowa State University, Ames, IA, USA

The premise that new genes can arise from non-genic DNA sequences is borne out from massive DNA and RNA sequencing data. This concept sharply contrasts with the long-accepted view that novel gene functions primarily arise from a slow process of accumulated mutations and rearrangements of already-established genes. We hypothesize that a major role of orphan genes is to regulate the defense and metabolic responses that enable evolutionary adaptation to new environments. Here, I highlight one orphan gene, QQS, detailing its function and how it is being harnessed for improving crops. In doing so, I describe experimental and computational tools that facilitate predictive understanding of the origin and function of orphan genes in driving evolutionary adaptation. I end by explaining my sincere debt to my postdoctoral mentor, Professor Eric Conn.

References
Dhurrin is a specialized metabolite belonging to the class of cyanogenic glucosides (CNGLCs) and is constitutively produced in *Sorghum bicolor* L. Moench. In Sorghum, dhurrin occupies a fundamental place in the plant defence system due its ability to release toxic hydrogen cyanide (HCN) upon tissue disruption. Besides this defensive role, dhurrin may also function as an important source molecule of whereby carbon and nitrogen may be remobilized and recycled during certain environmental growth-condition upon plant demand. However, despite its role *in planta*, our knowledge about how, when and why dhurrin is endogenously metabolized is very limited. In this study we compared the effects of different environmental stresses on the dhurrin metabolism to elucidate its role in plant growth, development and response to abiotic stresses.

In different experiments, several sorghum lines, varying for their ability to produce dhurrin, were exposed to high light intensity (1000 mmol m⁻² s⁻¹), high temperature (60 °C) and different water availability (optimal, limited and re-watered conditions). Comparative metabolomic profiling, namely LC-MS, GC-MS and MALDI-MS, was carried out at specific time points and plant growth stages to monitor changes in the dhurrin and turnover products content and their distribution at the single cell level. By combining several metabolomic approaches at different plant levels, we were able to investigate if and how dhurrin and its turnover derivatives are involved on abiotic stress response and emphasize the important multi-functionality of specialized metabolites in crops.

**Neish Award Speaker**

Xu (Sirius) Li is an Assistant Professor at the Plants for Human Health Institute, North Carolina State University. Before joining NCSU in 2011, he worked as a postdoc in Clint Chapple’s laboratory at Purdue University. He received his PhD degree in biochemistry in 2005 from Iowa State University where he was awarded a Plant Science Institute Fellowship. He obtained a BS degree in plant molecular and developmental biology, and a MS degree in plant physiology, both from Peking University in China. Dr. Li’s research focuses on plant specialized (secondary) metabolism. He has been developing an integrated metabolomics, genetics, and genomics platform for discovery of plant metabolic pathways using the model plant Arabidopsis thaliana. His long-term goal is to apply this platform to understand the biosynthesis of health-promoting phytochemicals and to manipulate metabolic pathways in crops, vegetables, and fruits to enhance their values for human health.

**[S1-4] Identification of a residue responsible for UDP-sugar donor selectivity of a dihydroxybenzoic acid glycosyltransferase from Arabidopsis natural accessions**

Han-Yi Chen¹², Xu Li¹²
¹Plants for Human Health Institute, North Carolina State University, Kannapolis, NC, USA
²Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA

UDP glycosyltransferase plays a major role in the diversity, reactivity, and regulation of plant specialized metabolites by catalyzing the transfer of the sugar moiety from activated UDP-sugars to various acceptors. Arabidopsis UGT89A2 was previously identified from a genome-wide association study as a key factor that controls the differential accumulation of dihydroxybenzoic acid (DHBA) glycosides in distinct Arabidopsis natural accessions, including
Col-0 and C24. The in vitro enzyme assays indicate that these distinct metabolic phenotypes reflect the divergence of UGT89A2 enzyme properties in the Col-0 and C24 accessions. UGT89A2 from Col-0 is highly selective toward UDP-xylose as the sugar donor, and the isoform from C24 can utilize both UDP-glucose and UDP-xylose but with a higher affinity to the glucose donor. The sequences of the two isozymes only differ at six amino acid residues. Examination of these amino acid residues in more natural accessions revealed a strong correlation between the amino acid polymorphism at the position 153 and the DHBA glycoside accumulation pattern. Site-directed mutagenesis that swapped residue 153 between UGT89A2 from Col-0 and C24 reversed the UDP-sugar preferences, indicating that the residue 153 plays an important role in determining sugar donor specificity of UGT89A2. This study provides insight into the key amino acid changes that confer sugar donor selectivity on UGTs, and demonstrates the usefulness of natural variation in understanding structure-function relationship of enzymes involved in specialized metabolism.

Neish Award Speaker

Hiroshi Maeda is assistant professor in the Department of Botany at University of Wisconsin-Madison. He received BS and MS degree in Biotechnology at Osaka University. He then moved to the US and obtained PhD at Michigan State University in 2006, working with Dr. Dean DellaPenna on tocopherol (vitamin E) functions in photosynthetic organisms. After working as postdoc with Dr. Natalia Dudareva at Purdue University on phenylalanine and benzenoid volatile biosynthesis in petunia flowers, he started his current position at UW-Madison from the fall 2011. Dr. Maeda’s laboratory has been investigating evolutionary diversification of the tyrosine biosynthetic pathway in various plant species. Dr. Maeda was the recipient of the Anton Lang Memorial Graduate Student Award from MSU DOE-Plant Research Laboratory in 2006 and the Eric Conn Young Investigator Award from the American Society of Plant Biologists in 2011.

[S1-5] Relaxation of tyrosine pathway regulation during the evolution of betalain pigmentation in Caryophyllales

Samuel Lopez-Nieves¹, Ya Yang², Samuel Brockington³, Hiroshi Maeda¹
¹Department of Botany, University of Wisconsin–Madison, Madison, WI, USA
²Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA
³Department of Plant Sciences, University of Cambridge, Cambridge, UK

Betalain pigments are unique to the plant order Caryophyllales and synthesized from the aromatic amino acid L-tyrosine (Tyr). However, it is unknown how Tyr-derived betalain pigments evolved to replace the otherwise ubiquitous phenylalanine-derived anthocyanins. Here, we investigated the Tyr biosynthetic pathway in table beets (Beta vulgaris L.), which produce high levels of betalains. Like most plants, B. vulgaris synthesizes Tyr via plastidic arogenate dehydrogenases (TyrA/ADH), which were encoded by two ADH genes (BvADHα and BvADHβ). However, unlike BvADHβ and other plant ADHs that are strongly inhibited by Tyr, BvADHα exhibited relaxed sensitivity to Tyr. Phylogenetic analysis combined with recombinant enzyme characterization further revealed that Tyr-insensitive BvADHα orthologs arose in conjunction with betalain pigmentation in the Caryophyllales. Our results indicate that relaxation of Tyr pathway regulation is intimately associated with the evolution of betalain pigmentation, highlighting the significance of upstream primary metabolic regulation for the diversification of specialized plant metabolism.
Neish Award Speaker

**Philip Bates** received his B.S. in Biochemistry here at UC Davis in 2002 and did undergraduate research with John Labavitch. He went on to join the lab of John Ohlrogge at Michigan State University where he started his career in plant lipids and received his Ph.D. in Biochemistry in 2008. From there he continued to work in the field of lipids with John Browse at Washington State University as a postdoc. In 2013 Philip accepted an assistant professorship in the department of chemistry and biochemistry at The University of Southern Mississippi. In 2014 the International Plant Lipid Symposium presented him with the Paul K. Stumpf Award, which is named after the UC Davis professor that is patriarch of the plant lipid biochemistry field. In 2015 the University of Southern Mississippi granted him the Nina Bell Suggs Endowed Professorship for exceptional junior faculty.

[S1-6] *Deciphering the control of fatty acid fluxes through the lipid metabolic network for enhanced plant oil bioengineering*

Philip Bates  
The University of Southern Mississippi, Hattiesburg, MS, USA

The rising demand for plant oils as food, fuels, and chemicals indicates that we need to produce more plant oils, and tailor the plant oil compositions for specific uses. However, plant oil bioengineering is hindered by the fact that plant oil biosynthesis involves a complex metabolic network with multiple subcellular compartments, parallel pathways, cycles, and pathways that have a dual function to produce essential membrane lipids and storage oils. It is tempting to assume that manipulation of a gene found at a metabolic map branch point will produce a desired change within lipid metabolism. However, with few exceptions the effect of gene manipulation on membrane or storage lipid amounts or compositions cannot be accurately predicted. One reason for this limitation within plant oil bioengineering is the inadequate understanding of the quantitative fluxes of fatty acids through various branches of the plant lipid metabolic network, and the gene products which control flux through these branch points. We utilize an in vivo metabolic labeling approach to quantitate the flux of fatty acids through the lipid metabolic network in plant seeds and leaves. By combining lipid flux analysis with genetic mutants and the expression of transgenes we are beginning to understand: (1) which gene products control fatty acid fluxes through the lipid metabolic network; (2) which reactions act as bottlenecks to plant oil engineering; and (3) how plants can work against the metabolic engineer by shifting flux through the lipid metabolic network in unexpected ways.
Sunday, August 7, Afternoon

Symposium II: Integrated Omics: Technology and Application

Chairs: Mark Berhow and Lloyd Sumner

Oliver Fiehn has pioneered developments and applications in metabolomics with over 180 publications to date, starting in 1998 as postdoctoral scholar and from 2000 onwards as group leader at the Max-Planck Institute for Molecular Plant Physiology in Potsdam, Germany. Since 2004 he is Professor at the UC Davis Genome Center, overseeing his research laboratory and the satellite core service laboratory in metabolomics research. Since 2012, he is Director of the NIH West Coast Metabolomics Center, supervising 35 staff operating 15 mass spectrometers and coordinating activities with four UC Davis satellite labs, including efforts for combined interpretation of genomics and metabolomics data. Prof. Fiehn specifically aims at integrating new approaches or technologies, including pathway-based mapping and statistical and data processing tasks. Prof. Fiehn specifically focuses on establishing metabolomic methods, databases and libraries, for example the MassBank of North America that hosts over 190,000 public metabolite mass spectra.

[S2-1] Using metabolomics to address biochemical diversity and function in plants

Oliver Fiehn, Dominique Ardura, Yan Ma, Arpana Vaniya, Sajjan Mehta, Gert Wohlgemuth
Genome Center, University of California at Davis, Davis, CA, USA

Plants are characterized by very large metabolic diversity and flexibility. Using metabolomics over the past 15 years, we have seen huge differences even in very well studied plants, such as Arabidopsis hybrids. We often have more questions than answers, but still, those questions are important to move forward in research. We first started with simple questions in primary metabolism, for example in metabolic long-distance transport. How can we find Arabidopsis hybrids, a sucrose producer and sucrose transporting plant, with virtually absent sucrose in its leaves? How can we understand the importance of raffinose in hybrid vigour in Arabidopsis? We then moved these questions to raffinose-family transporting plants, and identified multiple compounds in phloem that had never been identified before. How can we address functions for those small molecules?

More recently, we focused on specific (‘secondary’) metabolism, starting with chemotaxonomic investigations of plant families and then continuing with a systematic approach to catalogue both known and unknown plant compounds through mass spectrometry databases. At this point, we have analyzed over 1,000 genuine natural products in MS^n fragmentation trees, with the idea that these ion trees will guide us to new rules in MS-fragmentation, which ultimately will be helpful to identify the plethora of unknown compounds that we see in LC-accurate mass MS screens. For example, for simple grape-based mixtures, we record over 1,500 compounds within 5 minutes, of which we identify over 250 compounds (including more than 150 polyphenols). Similar efforts have been completed for volatiles and primary metabolites. Yet, the overall genetic and metabolic diversity in plants remains daunting. In addition to our update in tools and databases, we also present success in identifying novel metabolic functions in plant (and microbial) genes, which we call “metabolite repair”. Anything that can break, will break: this is not only true in the laboratory, but also in the cell. We propose that many genes of unknown function actually serve in repairing mishaps in metabolism, and give examples from our past findings.
Dan Kliebenstein’s laboratory utilizes a two-prong approach to understand how plant defense metabolites evolve to maximize plant fitness in a complex environment. First is to study how the Arabidopsis glucosinolate defense network functions in real environments undergoing complex combinations of stimuli. The second is to study how the broad host range fungal pathogen, Botrytis cinerea, can infect nearly every plant tested.

[S2-2] Adaptive metabolites solve the fluctuating environment via genetic variation and large regulatory networks

Dan Kliebenstein
University of California at Davis, Davis, CA, USA

A central goal of systems biology is modelling how complex systems are regulated. One of the most complex systems in multi-cellular biology is metabolism which exists as a large non-cell autonomous network involving reactions in diverse cell types connected by the vascular system. This multi-cellular aspect complicates the ability to take regulatory models built on cell-autonomous systems and apply them to understand how metabolic pathways are controlled. We apply diverse genomics tools from genome wide association mapping to high-throughput Yeast-1-hybrid (Y1H) to expand our understanding of how plant metabolism is regulated.

A fundamental question we are addressing using secondary metabolites is to ask how many transcription factors can actually influence a metabolic pathway. Using a high-throughput Y1H approach, we have found that the aliphatic glucosinolate pathway likely has >100 TFs that control the accumulation of glucosinolates at a level consistent with dramatic field fitness consequences. These TFs predict key biotic and abiotic influences expected to modulate glucosinolate content in a complex environment. These inputs were also found using a novel genome wide association mapping approach. This is generating unexpected observations about how “secondary” metabolic pathways are as central in regulatory networks as primary systems.

One conundrum of modern systems biology theory is that it is built on an unstable hierarchical regulatory model that requires stabilization. In engineering, this stabilization is usually provided by feed-back regulation coming from the actual output. We are directly testing if defense compounds, aliphatic glucosinolates, have unidentified regulatory roles. Work will be presented showing that the end products of this pathway are actually key regulators of core physiological and transcriptional processes and that the plant must have a complement of structurally specific glucosinolate sensors.

Finally, we are directly testing if the aliphatic glucosinolates and associated genetic variation is adaptive in the field using multi-year field trials. This analysis is showing that this pathway is adaptive in all environments tested but that the optimal alleles change from year to year and location to location. This suggests that there is a high level of fluctuating selection and little local adaptation. This actually establishes a system in which maintaining genetic variation is actually the most fit solution.
Omics insights into regulation of isoflavonoid biosynthesis in Soybean

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¹London Research and Development Centre, Agriculture and Agri-Food Canada, London, Ontario, Canada
²Department of Biology, University of Western Ontario, London, Ontario, Canada

Soybean (Glycine max [L.] Merr) is one of the largest grain legumes grown in Canada, yielding approximately 5.5 million acres, and producing $2.4 billion in profit per year. However, there is a significantly large yield loss due to root and stem rot disease caused by Phytophthora sojae. Many strategies have been implemented throughout the years to combat the pathogen such as the use of pesticides and certain agricultural practices. An alternative approach to this problem is to select a trait naturally found in soybean, such as glyceollin, an isoflavonoid compound, that can increase innate resistance. Glyceollins act as antimicrobial agents that are synthesized from the isoflavonoid branch of general phenylpropanoid pathway. Isoflavonoids are the actors in symbiosis with nitrogen-fixing bacteria, and are also noted for their human health benefits. By transcriptomic analysis, we identified CHS7 and CHS8 as genes that are critical for isoflavonoid synthesis in soybean seeds. Expressions of these CHS genes are regulated by GmMYB176 transcription factor. A systematic study of GmMYB176 using RNAseq, proteome and metabolome analyses have provided insights into the mechanism of isoflavonoid biosynthesis and regulation in soybean.

In-vivo and in-vitro antidiabetic effect of Achillea setacea Waldst. & Kit. and detection of major phenolic compounds by LC-MS/MS and NMR techniques

Sanem Hosbas Coskun¹,², Aaron Urbas¹, Mustafa Aslan², Benjamin Place¹, Didem Deliorman Orhan²
¹National Institute of Standards and Technology, Gaithersburg, Maryland, USA
²Gazi University Faculty of Pharmacy, Ankara, Turkey

Achillea species are widely used in traditional medicine in Turkey. A. setacea Waldst. & Kit. is used in diabetes around Konya region. In the present study, the hypoglycemic effects of aqueous and ethanol extracts of A. setacea were investigated in normal, glucose loaded hyperglycemic and streptozocin (STZ)-induced diabetic rats. More effective ethanol extract has been fractionized by BAGF technique. Isolation and chemical structure analysis has been carried out on the significantly active antidiabetic fractions and sub-fractions. Dicaffeoylquinic acid and dicaffeoylquinic acid methyl ester derivatives have been isolated as the major compounds for the first time from fractions of A.setacea by using LC/MS-MS and NMR techniques. These fractions showed higher activities than tolbutamide. Furthermore, luteolin-7-O-glucoside, apigenin-7-O-glucoside, vitexin, isovitexin, isoorientin and rutin compounds shown to have antidiabetic activities in different studies are found to be present essentially in the active extract fraction. Mechanism of the antidiabetic activity of the extract was evaluated by in vitro α-glucosidase enzyme inhibition assay.
Integrated metabolomics, genome-wide association studies (GWAS), correlated gene expression analyses and gene regulatory network (GRN) associations were used to identify the first 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyron-4-one (DDMP)-transferase known in any species and associated with triterpene saponin biosynthesis in *Medicago truncatula* (MtDPT1). Additional metabolomics analyses of overexpressing hairy roots and Tnt1 mutant knockdowns, combined with complementation in mutant hairy roots further validate the functional characterization of this novel enzyme. In addition a novel DDMP-sugar compound and a series of DDMP conjugate soyasapogenol B (soyB) compounds were also discovered in *M. truncatula*. The DDMP conjugated soyB compounds were characterized using mass spectrometry and nuclear magnetic resonance spectroscopy, and they were found to be naturally occurring in two *M. truncatula* ecotypes. The discovery of DDMPtransferase provides legume breeders the opportunity to produce seeds with higher levels of beneficial soyasapogenol B compounds.

### [S2-6] Rapid analysis of the chemical composition in plant products: Correlating rapid spectral analysis with comprehensive phytochemical composition

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Plants produce renewable resources used for almost everything we humans need and use: food, fuel, drugs, shelter, pest control, and much more. Plant materials need to be assessed for their complete chemical composition in order to further utilize their products and by-products effectively, as well as being able to detect and measure harmful and poisonous constituents and contaminants. With modern analytical separation and spectrometry methods we are close to being able to do this fairly accurately. However, translating this analysis quickly, accurately and comprehensively into modern agricultural harvesting, storage and processing requires indirect measuring methodology such as near-infrared spectrophotometry (NIRS) can provide. Modern computer technology and multivariate mathematical analysis methods have enabled NIRS to be used to measure levels of individual chemical components in seconds in intact samples. Determining the economically important constituents and developing accurate NIRS measurements for these components is now on the horizon in agricultural commodities. Using soy as an example the process of characterizing chemical composition, choosing and evaluating the economically chemical components, and developing usable NIRS calibrations that can be implemented at all levels of agricultural product evaluation.
[S2-7] Identifying and localizing plant secondary metabolites in specific cell types in situ: Integrated MALDI imaging, ion mobility separation and collision-induced dissociation approaches

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While MALDI imaging is providing many interesting findings as regards metabolite localization in phytochemical factories in specialized cell types in situ, one major problem is in ensuring that localization data can distinguish between isobaric (same molecular weight) species. Accordingly, we considered that by applying ion mobility mass spectrometry analyses together with collision-induced fragmentation as well, we should be able to unambiguously identify key metabolites of interest and determine their localization in specific cell types.

Ion mobility mass spectrometry separates ionized molecules based on their charge, size and shape, thereby providing an additional dimension for ion separation. Thus, in this study, we demonstrated unambiguous identification of various metabolites in various plant species using MALDI imaging coupled with ion mobility and collision induced dissociation. This was used to detect the anti-chagas natural product (−)-grandisin, as well as (+)-conocarpan, and the anti-cancer scaffold (−)-podophyllotoxin in *Piper solmsianum*, *Piper regnellii* and *Podophyllum peltatum* species, respectively. (−)-Grandisin and other phenylpropanoids isoelemicin, myristicin, and apiol were found to be co-localized in epidermis, collenchyma and vascular bundles in *P. solmsianum* petiole tissue, whereas (+)-conocarpan was in epidermis and collenchyma of *P. regnellii* stem tissue. Periderm and cortical parenchyma, by contrast, were the major sites of accumulation for (−)-podophyllotoxin in *P. peltatum* rhizome tissue. These data thus provide important insights into possible sites of biosynthetic and storage processes. The results are also discussed in the context of potentially interfering isobaric compounds present in these plant systems.

[S2-8] Genetic mapping of a new anthocyanin phenotype in Maize

Michael Paulsmeyer, John Juvik, Patrick Brown
University of Illinois, Urbana, IL, USA

In a previous study analyzing the diversity of anthocyanin content in diverse maize accessions, a unique phenotype was discovered that had previously never been characterized in maize. Some aleurone pigmented accessions produced markedly less acylated anthocyanin pigments than normal accessions. In these accessions, cyanidin 3-glucoside was the major pigment, whereas acylated cyanidin 3-(6”-malonyl) glucoside is typically the most abundant pigment. It is hypothesized that this phenotype is due to a loss-of-function acyltransferase. To map the location of the mutant in the maize genome, a mapping population was developed from a mutant phenotype line crossed to B73, the reference genome. After HPLC analysis of kernels from 129 F2 lines, the trait was determined to be controlled at a single locus. A genotyping-by-sequencing approach was used to discover SNPs to map the locus. The percentage of anthocyanins modified by acylation was used as the response to model the most significant SNP markers. This study concludes that the most significant locus is at the end of chromosome 1. A single acyltransferase candidate sequence resides at this locus and will be investigated further to determine its functionality.
[S2-9] Structure elucidation of procyanidin oligomers by MALDI TOF-TOF – characteristic ions for identifying A and B type procyanidins from MS² data

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Procyanidins are a class of anthocyanins consisting of oligomers of catechins and/or epicatechins. This class of compounds occurs in a wide variety of food plants and botanical dietary supplements including apples, cinnamon, cocoa beans, grape skins, peanut skins, and more. These oligomers have antioxidant properties via radical oxygen scavenging. As this class of compounds is often encountered when characterizing new plants and occurs as a variety of oligomers of varying length, there is need for a rapid method to characterize and identify procyanidins. One critical component of the identification is determining the type of bonding between each unit in the oligomeric chain: A or B type procyanidins.

We present MALDI TOF-TOF high resolution data of 22 procyanidins collected on a Bruker AutoFlex. In this set, we have 13 high purity procyanidins ranging from catechin to tetramers identified by NMR. By analysing the MSMS data of A and B type monomers, dimers, trimers and tetramers of mixed A and B type procyanidins, we developed a decision tree in order to identify the lengths and the bonding compositions of unknown procyanidins. Using this decision tree, we could identify the number of A and B bonding in 9 unknown procyanidin oligomers. Procyanidin fragmentation pathways have been reported in the literature up to trimers – the decision tree developed from this work was used to identify unknown procyanidins from trimers to pentamers. In addition, much of the literature analyses this procyanidins in positive mode – this work described a method to identify procyanidins through negative mode. This decision tree can possibly be used to characterize longer oligomers.
**Monday, August 8, Morning**

**Symposium III: Phytochemical Metabolism**

*Chairs: Xiao-Ya Chen and Deyu Xie*

Ian Graham is Head of the Department of Biology (www.york.ac.uk/biology) and holds the Weston Chair of Biochemical Genetics at the University of York. His research team is based in the Biology Department’s Centre for Novel Agricultural Products (www.cnap.org.uk). During his career Ian has made major contributions to our understanding of plant metabolism and seed biology. His research has shed new light on the production of small molecule natural products from plants such as the anti-cancer compound noscapine, morphinan-based analgesics such as codeine and morphine and the antimalarial drug artemisinin. He led the way in the genetic dissection of lipid mobilization in Arabidopsis oilseeds and most recently has discovered a role for oxylipins in controlling seed germination. Ian has recently been awarded the 2017 Heatley Medal and Prize by the UK’s Biochemical Society (http://www.york.ac.uk/news-and-events/news/2016/quality/biochemical-society-award/) and was elected a Fellow of the Royal Society and a member of EMBO in 2016.

[S3-1] **Molecular breeding of medicinal crops and discoveries along the way**

Ian Graham  
University of York, York, UK

Opium poppy (*Papaver somniferum*) remains one of the most important medicinal plants in the world. The discovery of a 10 gene cluster responsible for the production of the anti-cancer compound noscapine in opium poppy provided the tools for molecular breeding of new commercial varieties (Winzer et al., 2012, Science, 336:1704-8). The discovery of a novel P450 – oxidoreductase gene fusion described the last unknown step in synthesis of the painkiller drugs morphine and codeine (Winzer et al., 2015, Science, 349: 309-312), proving a valuable tool for development of bespoke, high yielding poppy varieties. The Chinese medicinal plant *Artemisia annua* (Sweet Wormwood or Qing Hao) is the primary source of the leading anti-malarial drug artemisinin. Characterisation and genetic mapping of traits responsible for production of artemisinin (Graham et al., 2010, Science, 327:328-31) has enabled development of F1 hybrid seed that can deliver a robust source of this vital anti-malarial drug for the developing world. Many other plant species also produce valuable bioactive molecules but in amounts that are not commercially viable. For example the Euphorbiaceae or spurge family produce a diverse range of diterpenoids, many of which have pharmacological activity. We are elucidating diterpenoid biosynthetic pathways from the spurge family (King et al., 2014, Plant Cell, 26:3286-98; King et al., 2016, ChemBiochem, doi:10.1002/cbic.201600316) and developing new production platforms for their synthesis. This talk will reflect on the different production routes for high value chemicals from plants.
Guodong Wang is a principle investigator at the Institute of Genetic and Developmental Biology, Chinese Academy of Sciences. He gained the bachelor degree in chemistry from Nankai University and a Ph.D. degree in molecular biology from Chinese Academy of Sciences. The overall interest within his laboratory is to study how plant synthesizes diverse terpenoids, the key enzymes potentially involved in these biosynthesis pathways and the mechanism of the enzymatic reactions. Currently he focuses on the sesterterpenoid pathway in Brassicaceae plants, soyasaponin (triterpenoid) pathway in soybean (Glycine max) and the terpenophenolics pathway in hops (Humulus lupulus). Meanwhile, he also tries to translate the pathway knowledge into microbial system to produce these value-added terpenoids in a large scale.

[S3-2] Genome mining for sesterterpenoid discovery in Brassicaceae plants

Qingwen Chen¹, Chengyuan Wang², Haili Liu², Zhixi Liu¹, Juan He¹, Yuwei Chang¹, Peng Zhang², Yong Wang², Guodong Wang¹
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Terpenoids represent the largest and most diverse class of plant specialized metabolites, with diverse physiological functions during plant development. In the biosynthesis of terpenoids, short-chain isoprenyl diphosphate synthases (SC-IDS) account for the majority biosynthesis of linear isoprenyl pyrophosphates, which are the direct precursors for terpenoids. Arabidopsis thaliana possess a group of SC-IDSs (GGPPS-like proteins, which were supposed to mainly produce geranylgeranyl diphosphate) whose functions are not clearly known. In this study, we found the neo-functionalization of Arabidopsis GGPPS-like gene family: GGPPS6, 7, 9 and 10 have GFPPS activities (the predominant product is geranylfarnesyl diphosphate [C25]) and GGPPS8 produces even longer chain isoprenyl diphosphate [>C25]. By solving the crystal structures of GGPPS7 [C25], GGPPS8 [>C25] and GGPPS11 [C20], we disclose the product chain-length determination mechanism and describe it as a “three floor” model. Using this model, we discovered the novel GFPPS in various Brassicaceae species. Genome mining further reveal that these Brassicaceae GFPPS genes are usually linked to terpene synthase gene and P450 genes. The GFPPS-TPS-P450 gene clusters for sesterterpenoids biosynthesis were functional identified and the potential biological functions of these sesterterpenoids in Brassicaceae plants are also discussed.
The structures, biosynthesis and genetics of wheat wax polyketides

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²Weizmann Institute of Science, Rehovot, Israel

To prevent uncontrolled water loss, above-ground organs of terrestrial plants are coated by cuticular wax, a complex mixture of very-long-chain fatty acid derivatives. The species-specific wax profiles result from biosynthetic pathways that are fairly well understood, mainly due to extensive studies in the Arabidopsis thaliana model. Further valuable insight into wax chemical diversity, biosynthesis and physiological function can now be expected from detailed investigation of other model species, combining chemical analyses with molecular genetics.

Bread wheat (Triticum aestivum) is rapidly becoming a new model species for wax biosynthesis studies, due to its importance as a major staple crop world-wide and its susceptibility to drought, combined with the recognized role of cuticular waxes in conferring drought resistance in this species. Here, we have performed a comprehensive GC-MS analysis of the wax mixtures covering the flag leaf blade and peduncle of T. aestivum cv. Bethlehem. We quantified various fatty acid derivatives similar to those in Arabidopsis wax, together with compounds characteristic of Poaceae waxes such as β-diketones, hydroxy-β-diketones, alkylresorcinols, methyl alkylresorcinols and 2-alkanol esters. In further studies we identified 13 classes of novel wax components, mostly with secondary oxygen functionalities. Several of these novel compounds are likely formed by P450-catalyzed oxidation of common wax precursors, while others were recognized as polyketides with structures similar to the prevalent β-diketones.

Based on the newly identified structures, the currently unknown β-diketone biosynthesis pathway and the underlying molecular genetics could be revisited. In this context, we discovered a metabolic gene cluster responsible for β-diketone biosynthesis. The cluster region was identified in both genetic and physical maps of wild-type and diketone-deficient wheat, and in planta gene silencing together with heterologous expression in bacteria revealed novel pathway intermediates. Taking all our chemical, biochemical and genetic data together, we propose a model for a novel β-diketone biosynthesis pathway involving a hydrolase, a P450 and a type-III polyketide synthase enzyme.
[S3-4] Functional characterization of a tea (Camellia sinensis) R2R3-MYB suppressor that downregulates the phenylpropanoid and shikimate pathways

Mingzhuo Li, Lili Guo, Yanzhi Li, Niandi Gong, Yongzhen Pang, Wenbo Jiang, Yajun Liu, Xiaolan Jiang, Lei Zhao, Yunsheng Wang, Liping Gao, Tao Xia
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Green tea (Camellia sinensis, Cs) contains an abundant level of numerous phenylpropanoid compounds. How phenylpropanoid biosynthesis in tea plants is regulated, however, is largely unknown. In the present study, an R2R3-MYB transcription factor (CsMYB4a) was isolated from leaf tissues. Amino acid sequence alignment and phylogenetic analyses indicated that CsMYB4a is a member of the MYB4-subgroup. Ectopic expression of CsMYB4a in tobacco plants resulted in dwarf, shrinking and yellowish leaves, and early senescence phenotypes. Genome-wide transcriptomic analysis revealed significant downregulation of multiple genes involved in the shikimate and phenylpropanoid pathways which regulate the biosynthesis of phenylalanine (Phe), lignin, and other compounds. Multiple genes involved in the tricarboxylic acid (TCA) cycle, glycolysis, and sugar metabolism were also downregulated. Metabolic profiling revealed that there was a reduction of lignin in transgenic plants, as well as other phenylpropanoids, Phe and other amino acids, and sugars. The promoter sequences of 45 genes from tobacco and the coding region of 5 genes from tea plants were isolated. Four types of AC-elements were identified as binding sites. Both EMSA and Dual-luciferase analyses demonstrated that CsMYB4a can bind to the promoters of the identified, down-regulated genes. The collective data indicate that CsMYB4a is a repressor of the phenylpropanoid and shikimate pathways in tea and has pleiotropic effects on plant metabolism.

[S3-5] De-mystifying chemical diversity in the Thunder God Vine - new avenues for the production of triptolide, a diterpene epoxide for treating kidney disease and cancer

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Washington State University, Pullman, WA, USA

Tripterygium wilfordii Hook. f. (also known as "Thunder God Vine"), has a long history of use in traditional Chinese Medicine. The genus Tripterygium (Celastraceae) is known to be a rich source of specialized metabolites. Root extracts have been evaluated as a medication for rheumatoid arthritis, cancer, hepatitis, nephritis, ankylosing spondylitis, polycystic kidney disease, and obesity. The most promising results in clinical trials, however, have been obtained with minnelide, a water soluble pro-drug analogue of triptolide, a structurally complex diterpene epoxide of Tripterygium roots. One of the critical challenges for further clinical evaluations is a shortage of triptolide and other diterpenoids, which are still extracted from Tripterygium roots. The yields of diterpenoids extractable from Tripterygium roots are accordingly poor and alternative, sustainable, production methods need to be developed. Tissue culture represents a promising alternative for the production of high value plant metabolites.

We have evaluated the entire known space of chemical diversity in Tripterygium, and have developed new methods to quantitatively assess the accumulation of all major root constituents. Furthermore, we have localized the principal roots metabolites to specific cell types. We then generated adventitious root cultures that accumulate triptolide at unprecedented levels. Transcriptome profiling allowed the selection of candidate genes involved in diterpenoid biosynthesis in Tripterygium and their functional characterization will be presented. In summary, we have developed vital resources for research on one of the most exciting medicinal plants under investigation today, and demonstrate the power of plant tissue culture for scaling up production of target metabolites.
An aromatic amino acid, tyrosine, is essential for protein synthesis in all orders of life and also serves as a precursor to numerous specialized metabolites crucial for plant and human health (e.g., vitamin E, isoquinoline alkaloids, and plastoquinone). Most plants synthesize tyrosine via arogenate dehydrogenase (ADH/TyrAa) that is strongly feedback inhibited by tyrosine. However, we recently found that soybean and *Medicago truncatula* have, besides ADH, prephenate dehydrogenase (PDH/TyrAp) enzymes, which are completely insensitive to tyrosine inhibition. In this study, we further identified non-canonical ADHs (ncADHs), which are phylogenetically more similar to PDHs than ADHs, but prefer arogenate substrate and are partially inhibited by tyrosine. ncADHs were present in many eudicot lineages, while PDHs were uniquely present in legumes through a recent gene duplication of ncADH. Utilizing these PDH and ncADH homologs that are similar in sequence but have distinct biochemical properties, we conducted a phylogeny-guided, structure-function analysis of legume PDH enzymes to identify key residue(s) responsible for their regulation and substrate specificity. An amino acid alignment of the PDH and ncADH sequences identified ten residues uniquely conserved in PDHs but not in ncADH enzymes. In parallel, the x-ray crystal structure of soybean PDH (GmPDH) was solved and refined at 1.7 Å. Combining both phylogenetic and structural data, two varying residues were found within the active site. Site-directed mutagenesis of the identified residues showed that Asn222, which corresponds to an aspartate in soybean ncADH (GmncADH) resulted in loss of PDH and gain of ADH activity. Interestingly, the N222D mutation also introduced tyrosine sensitivity into the insensitive GmPDH enzyme. Finally, the reciprocal mutation, D218N, on GmncADH introduced PDH activity and relieved its tyrosine sensitivity. Thus, Asn222 of GmPDH and Asp218 of GmncADH simultaneously alter substrate specificity and tyrosine sensitivity. This study highlights the use of phylogeny-guided enzymatic characterization coupled with x-ray structural analysis for identification of a key residue responsible for unique biochemical properties. The identified residue can be altered in various plants to modify tyrosine biosynthesis directing more carbon flow to tyrosine and its derived plant natural products.
Carotenoid and anthocyanin biosynthesis and accumulation in fleshy fruits of *Lycium* L.

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South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong Province, China

*Lycium* L. is a genus of Solanaceae containing about 80 species distributed in the temperate and subtropical zones. *Lycium* species are mostly found in dry, semi-saline environments. Chinese Pharmacopoeia (2010) recorded Lycii Fructus (*Gouqizi*, dry red fruit [RF] of *L. barbarum*), and Lycii Cortex (*Digupi*, dry root bark of *L. chinense* and *L. barbarum*). Black fruits (BF) of *L. ruthenicum* have been used as folk medicine, especially in Tibetan and Mongolian medicine. Therefore, wolfberry (or Goji, Gouqi) nowadays in China refers to the products prepared from *L. chinense*, *L. barbarum*, and *L. ruthenicum*, which is one of the most famous anti-aging herbs. Transcriptome sequencing and organelle development have been investigated for identification of key genes involved in the biosynthesis and accumulation of carotenoids, especially zeaxanthin dipalmitate, and anthocyanin. The failure of the chromoplast development in BF causes low carotenoid biosynthesis levels and continuous carotenoid degradation, which ultimately leads to undetectable carotenoid levels in ripe BF. The abundant zeaxanthin accumulation in RF is primarily determined via the high levels of carotenoid biosynthesis, transportation, and storage, as well as the lack of carotenoid degradation, which are regulated at the transcriptional level. High level of anthocyanin in BF and undetectable level of anthocyanin in RF are also regulated at the transcriptional level. Function of several key genes will be characterized and reported.
Nudicaulins - a unique group of flower pigments of Papaver nudicaule: Biosynthesis and potential ecological function

Anne-Christin Warskulat¹, Evangelos C. Tatsis¹, Bettina Dudek¹, Wolfgang Eisenreich¹,², Bernd Schneider¹
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Nudicaulins are unique indole alkaloids responsible for the flower color of the yellow variety of Papaver nudicaule (Iceland poppy). Phytochemical studies of the pigments of P. nudicaule petals started already in the 1930th.¹ Finally, the skeleton of the nudicaulins was identified as a polyphenol-indol hybrid structure.² The biosynthetic pathway was elucidated (1) by incubating the plants with ¹³CO₂ and MS- and NMR-based isotopologue analysis using an retrobiosynthetic approach,³ (2) by feeding experiments of petal material with specifically ¹³C-labeled precursors ⁴ and (3) by transcriptomic and proteomic studies.⁵

The data showed that nudicaulins are formed from indole and pelargonidin, which is the pigment of the red Iceland poppy variety. The genes and proteins of the biosynthetic pathway are expressed in the petals during the developmental stage when nudicaulins are formed. Indole also occurs in the petal tissue and was found as a scent component in the headspace of yellow flowers. Behavioural experiments with honeybees trained to the color and to the odor of different color varieties of P. nudicaule demonstrated the ability of the insect to discriminate colors of different polymorphs and also scent component to the insect, suggesting an ecological role in plant-pollinator interaction.⁶

Andrew L. Waterhouse is a third generation Californian, but moved frequently while growing up, including some years abroad. He attended the University of Notre Dame where he earned a Bachelor of Science in Chemistry, and then UC Berkeley for his PhD in organic chemistry. In 1991, he moved to the Department of Viticulture and Enology at UC Davis where his research program has delved into wine oxidation as well as various aspects of phenolics. Present in grape skins and seeds, these account for several aspects of flavor as well as antioxidant activity, which helps wines age and may reduce chronic disease in wine drinkers. His graduate students and post-docs are winemakers, researchers and professors across California and elsewhere around the globe. He is currently a Co-Editor in Chief of the Journal of the Science of Food and Agriculture has served as Chair of his department at UC Davis.

[S4-1] Gut metabolites of dietary phenolics

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Phenolic compounds are ubiquitous in plant foods, and have always been in human diets. However, the human metabolism of these compounds is the same as xenobiotics, with methylation and conjugation steps to reduce reactivity and to accelerate clearance, and no active absorption pathway. One result of this is that there are still good quantities of phenolics in the digestive tract when the digested food arrives in the large intestine. Here there are a plethora of bacteria that have a much larger metabolic toolkit. A major microbial pathway is that flavonoids are broken into fragments related to their biosynthetic precursors, yielding a series of compounds with decreasing numbers of carbons attached to the “A-ring” fragment (which arose from phenylalanine). The presence of the highly modified metabolites presents new challenges in assessing the impact of phenolics in the diet as many compounds end up as similar metabolites and it is not immediately clear which might have arisen from a specific dietary source. The phenolics and/or their metabolites also alter the gut microflora profile and constitutive metabolic pathways. Thus, a mechanistic explanation of how phenolics in the diet can alter health is fraught with many questions that will require disentangling interacting chemical, enzymatic and microbial systems for clear answers.
Thomas Prisinzano graduated from the University of Delaware (1995) and received a doctorate in Pharmaceutical Sciences from the School of Pharmacy at Virginia Commonwealth University (2000). From 2000 – 2003, he was an Intramural Research Training Award Fellow in the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, MD. Currently, Dr. Prisinzano is Chair of the Department of Medicinal Chemistry and Co-Director of the Graduate Certificate Program in Chemical Biology. He has received a number of awards including the D. John Faulkner Travel Award from the American Society of Pharmacognosy (2005), the Jack L. Beal Award from the Journal of Natural Products (2006), the Matt Suffness Award from the American Society of Pharmacognosy (2008), the Joseph Cochin Young Investigator Award from the College on Problems of Drug Dependence (2011), and the David W. Robertson Award for Excellence in Medicinal Chemistry from the American Chemical Society (2012).

[S4-2] Salvia divinorum: A unique CNS active plant

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The neoclerodane diterpene salvinorin A is the major active component of the hallucinogenic mint plant Salvia divinorum Epling & Játiva (Lamiaceae). Since the finding that salvinorin A exerts its potent psychotropic actions through the activation of opioid receptors, the site of action of morphine and related analogues, there has been much interest in elucidating the underlying mechanisms behind its effects. These effects are particularly remarkable, because (1) salvinorin A is the first reported non-nitrogenous opioid receptor agonist, and (2) its effects are not mediated by 5-HT2A receptors, the classical target of hallucinogens such as LSD and mescaline. This talk will outline our research program, illustrating a new direction to the development of tools to further elucidate the biological mechanisms of drug tolerance and dependence. Our multidisciplinary approach combines natural product isolation, synthetic medicinal chemistry, and behavioral pharmacology to better understand the actions of salvinorin A at opioid receptors with the goal of designing novel agents to treat pain, drug abuse, and other CNS disorders.
**[S4-3] Biotransformation of xanthohumol and related flavonoids by intestinal bacteria**

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Xanthohumol is the major prenylated chalcone found in hops and has been shown to exhibit various biological effects. Its metabolism is relatively well documented, however, only a few reports are available about the transformation capacity of the intestinal microbial community. We have identified a novel metabolite, α,β-dihydroxanthohumol, in fecal samples of mice treated with xanthohumol, leading us to think that its biotransformation is more complex than previously reported. In the present study, we are investigating the role of *Eubacterium ramulus*, a strict anaerobe from the human gastro-intestinal tract and a key organism for flavonoid degradation, in the biotransformation of xanthohumol. A possible transformation pathway of xanthohumol will also be postulated in this work.

**[S4-4] Studies of pharmacokinetic interactions between drugs and botanical dietary supplements used by menopausal women**

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The use of botanical dietary supplements has grown steadily over the last 20 years despite incomplete information regarding active constituents, mechanisms of action, efficacy, and safety. An important but under-investigated safety concern is the potential for popular botanical dietary supplements to interfere with the absorption, transport and/or metabolism of pharmaceutical agents. Due to cost and concern for human subjects, clinical trials of drug-botanical interactions are usually carried out only when indicated by unexpected consumer side effects or, preferably, by predictive in vitro studies. We evaluate why some in vitro models overestimate or sometimes underestimate the potential for drug-botanical interactions. Using examples of popular botanical dietary supplements used by menopausal women as alternatives to hormone therapy, we applied in vitro models that predicted potential drug-botanical interactions for black cohosh (*Actea racemose*), red clover (*Trifolium pratense*), hops (*Humulus lupulus*), and licorice (*Glycyrrhiza glabra*). Phase I studies of drug-botanical interactions showed minor inhibition of drug metabolism by black cohosh, and clinical trials of these other botanical dietary supplements are in progress.

Supported by grant P50 AT000155 from the NIH Office of Dietary Supplements and National Center for Complementary and Integrative Health.
Modulation of the brain metabolome by an aqueous extract of *Centella asiatica*

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*Centella asiatica* (CA), also known as gotu kola, has received considerable attention as a medicinal herb that exhibits neuroprotective properties, with possible applicability to the management and treatment of age-related cognitive impairment. Our previous work indicates that an aqueous extract of CA leaves impacts positively cognitive functions in healthy young and aged C57BL/6 mice, a recognized aging model. Aqueous extract of CA leaves are rich source of phenolic compounds. Here, we report on our comparative metabolomics studies designed to elucidate the mechanisms by which aqueous extracts of CA leaves may modulate brain function.

We used untargeted metabolomics workflows based on high-resolution mass spectrometry coupled to UPLC for metabolite detection and quantification. Assignment of metabolites was based on experimental accurate mass and fragment ions and comparison with entries from our in house compound library containing >600 metabolites and other publically available databases. In our initial studies we first looked at age-dependent metabolite alterations in hippocampus and cortex of C57BL/6 mice. Multi and univariate analysis revealed differences in metabolite composition dependent on age (3 months and 21 months), brain tissue and gender. Differential metabolites associated with brain aging included metabolites related to energy metabolism, cholesterol and endocannabinoid metabolisms, components of nicotinamide adenine dinucleotide metabolism and phosphatidylcholines. Subsequently, we determined metabolite changes associated with the administration of CA aqueous extracts. We detected and quantified 100+ metabolites and lipids in the hippocampal extracts. Metabolite changes in response to CAW administration were associated with the following pathways/biofunctions: purine metabolism/DNA synthesis; amino acid utilization/protein synthesis and turnover, energy utilization/mitochondrial function, polyamine metabolism/cell proliferation, glutathione synthesis/oxidative stress response and metabolites associated with neurotransmission. The metabolite alterations will be discussed with reference to gene expression changes observed in the mice in response to CAW administration.
Björn Hamberger received his PhD degree for work in the core phenylpropanoid pathway in Prof. Klaus Hahlbrock’s group at the Max-Planck Institute for Plant Breeding Research in Cologne (2003). Following postdoctoral fellowships at the University of British Columbia, in the Department of Botany (Prof. Carl Douglas) and the Michael Smith Laboratories (Prof. Jörg Bohlmann) where he became fascinated with chemical diversity of bioactive terpenoids, he was appointed Assoc. Prof. at the University of Copenhagen, Denmark (2010), and a two-year group-leadership in the Novo Nordisk Foundation Center for Biosustainability, Danish Technical University. In 2015 he gladly accepted an offer for a faculty position as Assistant Professor in Plant Biology and the MSU-DOE Plant Research Laboratory at the Department of Biochemistry and Molecular Biology at Michigan State University, where his newly founded team further develops approaches in Plant Synthetic Biology, combinatorial biochemistry and production of terpenoids.

[S5-1] Discovery of terpene biosynthetic pathways in plants: Synthetic biology tools for production

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With over 50,000+ known discrete structures, plant produced terpenoids represent the oldest and most diverse class of specialized (i.e. primary) metabolites. A considerable fraction of the chemical diversity of the C₂₀ derived plant diterpenoids is determined by two initial key conversions. Structurally complex diterpene scaffolds with a characteristic decalin core are cyclized by modules of paired diterpene synthases. Regio- and stereoselective oxidation, catalyzed by enzymes of the cytochromes P450 family, results in increased structural complexity and often additional chiral centers. Those resulting molecular handles can be further functionalized by diverse enzymes, introducing for example ester functions, or structural rearrangements. Integrating metabolomics and transcriptomics led to discovery and characterization of diTPS and P450s from plant species accumulating exceptional high-value diterpenoids yielded a functionally diverse biosynthetic toolbox. Beyond reconstruction of the target pathways, these parts allowed engineering of functional neo-natural pathways, opening stereoselective access to an increased range of diterpene scaffolds. The discoveries fuel engineering of both conventional microbial and recently emerging photosynthetic hosts, but also indicate novel strategies for biosustainable diterpene production.
John Dueber is an Associate Professor of Bioengineering at University of California, Berkeley. He earned his Ph.D. in Prof. Wendell Lim’s laboratory at University of California, San Francisco investigating the modular design of many metazoan signaling proteins though an engineering approach – building synthetic switch proteins by recombining differing arrangements of input domains to achieve different gated behaviors. As a QB3 Distinguished Fellow, mentored by Prof. Jay Keasling, he focused on the use of synthetic biology approaches for improved metabolic engineering performance. Modular protein-protein interaction domains were used to build synthetic scaffolds capable of co-localizing metabolic enzymes tagged with corresponding ligands for these protein-protein interaction domains. Starting in 2010 in the Bioengineering department at Berkeley, his lab has continued to develop strategies for introducing designable, modular control over living cells. In particular, the lab is interested in generating technologies for improving engineered metabolic pathway efficiency and directing flux to desired products. Applications include the development of biofuels through utilization of alternative feedstock sugars as well as the production of higher-value specialty chemicals and therapeutics via microbial fermentation. For this work, he has been awarded a NSF CAREER, DOE Early Career, and a Bakar Fellowship award.

[S5-2] Use of an enzyme-coupled biosensor to engineer a BIA fermentation pathway from glucose in \textit{Saccharomyces cerevisiae}

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The benzylisoquinoline alkaloids (BIAs) represent a large family of natural products rich in potential bioactivities including analgesics, antitumor, and antibiotic activities. There have been ample recent advances in engineering \textit{S. cerevisiae} strains that can convert fed intermediate reticuline into multiple products of interest, including morphine, codeine, noscapine, and dihydrosanguinarine in \textit{S. cerevisiae}. These pathways contain numerous P450 enzymes, a class of enzyme that frequently demonstrate superior expression in eukaryotic hosts. However, direct fermentation of reticuline from central metabolism in yeast requires a previously elusive tyrosine hydroxylase activity to produce L-DOPA. To identify an enzyme capable of catalyzing this activity, we constructed an enzyme-coupled biosensor: production of a colored, fluorescent metabolite in the presence of L-DOPA. Further, we used this screenable phenotype to isolate a mutant that preferentially performs the desired hydroxylation by lowering the undesired further oxidation of L-DOPA to the melanin biosynthetic pathway. Replacing the enzyme for biosensor metabolite production with the reticuline biosynthetic enzymes enabled the production of this major BIA branchpoint intermediate.
[S5-3] Transcriptome and metabolite analysis across xylem development in Sandalwood trees reveal the final step in sandalwood oil biosynthesis

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The xylem of Santalum album trees is known for accumulating one of the most valued oils in the fragrance industry. Sesquiterpene alcohols, namely the (Z) stereoisomers of α-, β-, and epi-β-santalol, and α-exo-bergamotol, are the key fragrance components of the oil. Recently, a santalene synthase and ten P450 members of the CYP76F subfamily were cloned and characterized to be responsible for the biosynthesis of santalols in vitro and in engineered yeast cells. However, the ratio between (Z) and (E) stereoisomers varied from the ratio in authentic sandalwood oil. We hypothesized that additional genes are involved in sandalwood oil biosynthesis in planta affecting the overall composition of enantiomers. To address this hypothesis, and to improve our understanding of heartwood formation and oil biosynthesis, we performed deep transcriptome sequencing of different stages of developing xylem from S. album trees. We analyzed the transcriptomes and metabolite profiles of the outer sapwood, inner heartwood (HW), and the intermediate transition zone. We found a HW-specific transcriptome signature with all mevalonate acid pathway genes, farnesyl diphosphate synthase, and santalene synthase being preferentially expressed in HW tissue. Several members of the P450 superfamily also showed a HW-specific signature, with a member of the CYP736A subfamily, SaCYP736A167, as the most highly expressed P450 in HW. Coexpression with SaSSy, SaFPPS and SaCPR2 in yeast cells revealed SaCYP736A167 as the enzyme responsible for the biosynthesis of fragrance-defining compounds: (Z)-α-santalol, (Z)-β-santalol, (Z)-epi-β-santalol, and (Z)-α-exo-bergamotol. Our results provide the necessary tools to engineer microbial systems for the production of sandalwood oil components, potentially offering an alternative source of oil for the fragrance industry. Our data also highlights the diversity of P450 metabolic functions within CYP subfamilies.

[S5-4] Enhancement of morphadiene pathway for antimalarial medicines in Artemisia annua

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We have developed a novel self-pollinated Artemisia annua for metabolic engineering of arteisinin, the most effective medicine for treatment of cerebral and malignant malaria infected by Plasmodium falciparum. It is formed from the morphadiene pathway in A. annua. The pathway to morphadiene is a constitutive pathway in above-ground tissues of self-pollinated A. annua. Here we report increase a terpenoid pathway flux from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). The sequence of a type I IPP isomerase is optimized to direct an active enzyme in the cytosol. Its overexpression leads to 4% (g/g, dry weight) arteannuin B, 0.17-0.25% (g/g, dry weight) arteisinin, and 1-1.2% (g/g, dry weight) arteisinic acid in 1-2 months old transgenic seedlings. In addition, the production of isoprene, a direct product of DMAPP, is significantly decreased. This study opens a new opportunity to improve limitation of arteisinin supply for fighting against malaria.
Molecular evidence for protein complex in natural rubber biosynthesis in lettuce (*Lactuca sativa*)

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Natural rubber (NR; *cis*-1,4-polyisoprene) is an irreplaceable biopolymer in material industry. NR is known to be synthesized in hundreds of plant species. However, despite the frequent occurrence of NR in the plant kingdom, the Brazilian rubber tree (or Para-rubber tree) is a single species that currently supply NR, while other plant species produce either economically low quantity or much shorter NR polymers. As the Brazilian rubber tree is a tropical, perennial woody species, it is difficult to apply modern molecular genetics tools to this plant species to elucidate NR-biosynthetic mechanism. To overcome this, an annual, genetically amenable lettuce (*Lactuca sativa*), known to produce NR of 1.2 million Dalton in average size, has been studied to unravel NR biosynthetic mechanism. A proteomics study of lettuce latex identified an unusual *cis*-prenyltransferase-like (CPTL) enzyme, which does not possess the conserved *cis*-prenyltransferase catalytic residues. The CPTL is exclusively expressed in the lettuce latex. Importantly, silencing the CPTL transcript markedly decreased the NR content in multiple lettuce transgenic lines, suggesting that CPTL is a necessary gene for NR biosynthesis in lettuce. CPTL interacts with a traditional *cis*-prenyltransferase (CPT) and the CPTL tethers CPT enzyme on the endoplasmic reticulum, as supported by yeast two-hybrid and subcellular localization studies in yeast and tobacco. However, microsomes isolated from the yeast expressing the latex-specific CPT and CPTL could not synthesize high molecular weight NR observed in lettuce latex. Intriguingly, a separate, homologous CPTL/CPT protein pair, which is ubiquitously present in all cell-types showed a potent catalytic activity to synthesize small *cis*-1,4-polyisoprenes, known as primary metabolic product, dolichols, *in vitro*. However, neither CPTL nor CPT recombinant enzyme alone can catalyse the dolichol-biosynthetic reactions. Collectively, these results suggest the necessity for the formation of CPT/CPTL protein complex in NR and dolichol biosynthesis in lettuce. Based on these results, we proposed a new model which involves both CPT and its binding protein CPTL on the endoplasmic reticulum in plant. Similarity of this model to eukaryotic lipid-droplets will be further discussed.
The seed-transmissible fungal endophyte, *Epichloë coenophiala*, is an obligate, mutualistic symbiont with tall fescue, a forage and pasture grass that is especially popular due to its high productivity, stand longevity, stress tolerance and pest resistance; all characteristics that are significantly enhanced by the presence of the endophyte. However, commonly occurring strains of *E. coenophiala* also synthesize ergot alkaloids, such as the particularly toxic ergovaline, which cause substantial losses to beef and other livestock industries. The ergot alkaloids are one of four classes of alkaloids produced by strains of *E. coenophiala*; others include peramine and loline alkaloids, which are nontoxic to livestock but protective against invertebrate pests, and indole-diterpenes, which vary in activities against pests and livestock. *Epichloë coenophiala* is a hybrid of three parental species, two of which contributed homologous ergot alkaloid gene clusters, designated *EAS*1 and *EAS*2. The genome sequence of *E. coenophiala* strain e19 (ATCC 90664) revealed that both clusters are telomeric, and that the *lpsB*2 gene in *EAS*2—which is required for ergovaline biosynthesis—has an inactivating frame shift mutation. We developed a novel technique for chromosome-end manipulation, and thereby removed more than 162 kb of *EAS*1 without any introduction of foreign genes. The genome sequence of two independently derived strains confirmed elimination of the *EAS*1 cluster and absence of any exogenous genes. The resulting strain produced no detectable ergovaline, but instead produced the early intermediate, chanoclavine I, and a spur product, ergotryptamine. Complementation with a functional *lpsB* gene restored ergovaline production.
Modification of lignin aromatic composition in *Oryza sativa* for biomass refinery

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Lignin has long been considered recalcitrant in polysaccharide-oriented biomass utilization processes such as pulping and bioethanol fermentation. Lignin is, however, a potential feedstock for aromatic products. Hence, structural modification to produce lignins that are suitable for lignin utilization as well as higher lignin production in biomass plant species are important breeding objectives. Previously, we produced transgenic rice plants with increased lignin content, and obtained transgenic rice T1 lines with about 1.5-fold lignin contents in culms compared to control wild-type plants, which was estimated to contribute about 7% increase of heating value. In addition, we produced a transgenic rice plant in which *Oryza sativa* ferulate 5-hydroxylase 1 (*OsCAld5H1* or *OsF5H1*) was downregulated by RNAi technique. Down-regulation of *OsCAld5H1* severely decreased syringyl (S) lignin content without significant change in the total lignin depositions in rice cell walls; reduced S lignin levels in the transgenic lines were compensated with increased guaiacyl (G) and *p*-hydroxyphenyl (H) lignin levels.

Here, we show that overexpression of *OsCAld5H1* and down-regulation of *Oryza sativa* *p*-coumaroyl ester 3-hydroxylase 1 (*OsC3H1*) in rice resulted in considerably increased S and H lignins, respectively, as determined by a series of cell wall structural analyses using 2D NMR and wet-chemical approaches. A higher G or H lignin content contributes an increase in biomass heating value, as those lignins have higher carbon contents than S lignin, whereas a higher S content could be beneficial for lignin polymer utilization. In addition, simplification of lignin aromatic composition could be beneficial in producing value-added aromatic chemicals from lignins. This strategy may be applicable to lignin upregulation and simplification of lignin aromatic composition in large-sized grass biomass plants, such as *Sorghum*, *Miscanthus* and *Erianthus*, which are suitable for solid fuel applications and aromatic chemical production.
Elucidation of diterpene synthases in *Salvia divinorum* toward novel plant-derived therapeutics

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Salvinorin A is a hallucinogenic clerodane-type diterpenoid naturally produced in the glandular trichomes of the medicinal plant *Salvia divinorum* (Lamiaceae). As the first known non-alkaloid κ-opioid receptor agonist, salvinorin A is currently undergoing clinical trials as a potential therapy for neuropsychiatric diseases and drug addictions. With the aim to identify and engineer the salvinorin A pathway, transcriptome analysis of *S. divinorum* trichomes was performed, revealing five candidate diterpene synthases (diTPSs), including two class II diTPSs (SdCPSL1&2) and three class I diTPSs (SdKSL1,2&3). The proposed salvinorin A pathway involves a pair of class II and class I diTPSs that catalyze a sequential reaction converting the central precursor, geranylgerranyl diphosphate (GGPP), into the key clerodane diterpene scaffold. Quantitative real-time PCR demonstrated a trichome-specific transcript abundance for SdCPSL2, SdKSL2 and SdKSL3, suggesting a role in salvinorin A biosynthesis. Functional characterization via *in vitro* assays and transient co-expression in *Nicotiana benthamiana* showed that the class II diTPS SdCPSL2 converts GGPP into the clerodane diterpenoid, (−)-kolavenyl diphosphate, as confirmed by nuclear magnetic resonance (NMR) analysis. Furthermore, homology modeling of SdCPSL2 followed by single amino acid substitutions identified active site residues that control product specificity towards generating prenyl diphosphate intermediates with a distinct stereochemistry and regio-specific oxygenation. Additional *in planta* co-expression of SdCPSL1 identified the enzyme as an ent-copalyl diphosphate synthase. Among the class I diTPSs, *in planta* co-expression of SdKSL1 with the identified class II enzymes showed no activity with SdCPSL2, while the coupled reaction of SdCPSL1 and SdKSL1 resulted in the formation of multiple products, including ent-kaurene and several related labdane diterpenes, indicating a possible role in both primary and secondary metabolism. These findings provide deeper insight into clerodane diterpenoid formation and the evolutionary diversification of class II diTPSs. Expression of SdCPSL2 in *E. coli* and *N. benthamiana* offers an opportunity to develop proof-of-concept salvinorin A production systems for its further development as a drug candidate.
**[S6-1] Volatile-mediated information transfer in Tall Goldenrod, *Solidago altissima*: Ecological consequences and evolutionary aspects**

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Plant secondary metabolites mediate a large diversity of interactions from the cell over the tissue and whole plant to the community levels. Thus, their most basic and common function is information transfer and chemical signaling and communication should underlie the same principles as other types of information, such as acoustic and optical. Plant volatile organic compound emissions include secondary metabolites with the seemingly most obvious functions as information vehicles and thus provide a nice example of how to apply information theory to chemical information transfer.

The application of information theory to plant secondary metabolite production allows deriving new questions and hypotheses about the ecological roles and functions of noise in chemical signaling and, in consequence, the evolution of plant chemical signaling in different information landscapes.

Here I use tall goldenrod, *Solidago altissima*, as a model to illustrate various aspects of information theory for plant secondary metabolite production. More specifically, I review induction characteristics and ecological functions of volatile-mediated plant-plant information transfer in this community ecology model species. The special environmental circumstances in *S. solidago* habitats as well as the importance of herbivore dominance patterns provide strong agents of natural selection on plant chemical traits as vehicles of information.
Elizabeth Sattely is an Assistant Professor in the Department of Chemical Engineering at Stanford and a Stanford ChEM-H Faculty Fellow. She completed her graduate training at Boston College in organic chemistry and her postdoctoral studies in biochemistry at Harvard Medical School where she worked on natural product biosynthesis in bacteria. Inspired by human reliance on plants and plant-derived molecules for food and medicine, Beth's laboratory is focused on the discovery and engineering of plant metabolic pathways that are important for both plant and human health. Her work has been recognized by an NIH Pathway to Independence Award, an NIH New Innovator Award, and a DOE Early Career Award.

[S6-2] A gene-centric approach for the discovery and engineering of plant chemistry

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Plants thrive in the face of virtually every environmental stress: low nutrient input, pathogen attack, drought, and high salinity. Small molecules play a key role in plant fitness, yet we know relatively little about how they are made and their specific mechanisms of action. A major goal in my lab is to use a gene-centric approach to systematically elucidate pathways in the model plant Arabidopsis and related Brassicas that are critical for disease resistance, plant-microbe dialogs, and nutrient acquisition. In this talk, I will highlight some of our recent findings, including a new branch of Trp metabolism required for pathogen defense and molecules linked to Fe acquisition. In addition, I will discuss new efforts in the lab to use metabolic engineering in order to quantify the role of these pathways in both plant and human health.
Nasturlexins and cyclonasturlexin, novel phytoalexins from *Nasturtium officinale*, originate from parallel biosynthetic pathways

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Plants protect themselves from pathogens using metabolic pathways that synthesize various secondary metabolites, some of which are produced only under stress conditions. Phytoalexins are antimicrobial secondary metabolites produced *de novo* by plants under stress. These metabolites play an important role in the defence response of plants, and are part of their defence mechanisms. Crucifer species (family Brassicaceae) are known to produce tryptophan-derived phytoalexins, with more than 45 metabolites discovered to date.

In this work the phytoalexins produced by the crucifer watercress (*Nasturtium officinale*) were investigated. Elicitation of leaves of watercress led to the production of several metabolites that were not detected in control plants. These metabolites were isolated and their structures were confirmed by synthesis. The metabolites were inhibitory against fungal pathogens, indicating them to be the novel phytoalexins nasturlexins and cyclonasturlexin.

Biosynthetic investigations indicated that cyclonasturlexin is derived from the tryptophan pathway, whereas nasturlexins are biosynthesized from phenylalanine via gluconasturtiin and phenylethyl isothiocyanate. The biosynthetic intermediates of these pathways will be presented and potential alternative pathways will be discussed. The discovery of new biosynthetic intermediates should facilitate the discovery of the corresponding biosynthetic enzymes and genes present in specific crucifer species.
[S6-4] Terpene synthase ZmTps21 is responsible for a previously undetected β-selinene derived antimicrobial phytoalexin in maize (Zea mays)

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As a dominant feature of the global agricultural landscape, maize (Zea mays) is protected by a diverse array of highly inducible terpene volatiles that function as indirect defenses and oxygenated non-volatile terpenoids that serve as directly antimicrobial phytoalexins. Unlike herbivore-induced terpene volatiles, the predominant terpenoid phytoalexins are just now being uncovered. As a rarely encountered maize sesquiterpene volatile, β-selinene was reported following fungal disease in developing ears yet its origin and function remain unknown. Analysis of diverse inbred maize lines revealed that only select germplasm originating from North American breeding programs produced β-selinene. Using natural biotic stress factors in the field, including root herbivory and fungal pathogens, we leveraged the intermated B73 × Mo17 (IBM) population to perform metabolite-based quantitative trait loci (QTL) mapping in effort to identify underlying genes. Genetic mapping with IBM recombinant inbred lines (RILs) and near-isogenic lines placed the locus on chromosome 9 within an interval containing two bacterial artificial chromosome (BAC) clones, AC213878 and AC204415. Examination of the respective maize B73 genome region revealed the presence of a pseudo terpene synthase (tps21), which lacks the conserved DDxxD and RxR motifs required for its activity. A corresponding functional Tps21 gene, encoding a 571-amino acid protein, was isolated from the inbred line Mo17. Biochemical characterization of the bacteria expressed Mo17 Tps21 confirmed that it cyclized farnesyl diphosphate into β-selinene as the main product with comparatively low yet detectable amounts of α-selinene and germacrene A. Similar to the previously characterized sesquiterpene synthases Tps6/11, Tps21 is induced by a wide range of fungal infections but not by wounding alone. Production of the oxygenated β-selinene derivative, β-costic acid, in maize tissues requires a functional Tps21 and contributes to significant antifungal activity against numerous phytopathogenic fungi at physiologically relevant concentrations. While not previously established in maize, specific field collected IBM roots were found to contain β-costic acid levels exceeding 100 µg g⁻¹ FW, which are equivalent to or exceed most known biological defenses established in maize. The relative restriction of functional Tps21 sequences in diverse maize lines suggests that the β-costic acid pathway is maintained by positive selection in North American breeding programs. Together these results demonstrate that ZmTps21, β-selinene and ultimately β-costic acid are major components of a previously undescribed maize terpenoid phytoalexin defense pathway.
Non-targeted and targeted metabolomic approaches reveal differences in legume chemistry before and after infestation with pea aphid host races

Carlos Sanchez-Arcos, Jonathan Gershenzon, Grit Kunert
Max Planck Institute for Chemical Ecology, Jena, Turingia, Germany

The pea aphid (*Acyrthosiphon pisum*), an insect that feeds on several host plants in the legume family (Fabaceae), is a complex of at least 15 genetically different host races. Each race prefers different host plant species and performs better on these plants than on other legumes, which makes it an attractive model insect for the study of ecological speciation. Pea aphids consume the sugar-rich phloem sap of plants through their sucking mouthparts. Phloem contains other metabolites besides sugars, and aphids contact other plant tissues to reach the phloem. Thus, aphids encounter many different plant chemicals. So far, the contribution of chemistry to the differential performance of the host races on legume plants is still largely unknown. Using a mass spectrometry-based non-targeted metabolomic approach, we found significant differences among the metabolic fingerprints of the host plants before aphid infestation and observed that each aphid host race altered the metabolome of the plants specifically. This analysis resulted in a list of candidate chemical compounds that could be responsible for the specialization of pea aphid host races on their host plants. Also, using a targeted metabolomic approach we collected information about the time course of plant hormone concentrations, including jasmonates, salicylic acid, and abscisic acid in response to pea aphid infestation. The results suggest that aphids were able to modulate the phytohormone levels on their native host plants to evade defensive responses. Thus, we conclude that pea aphid host races use active strategies to avoid the chemical defences of their food plants. This information opens new opportunities to understand how plant chemistry can influence aphid performance and vice-versa, as well as how plant chemistry can be modified to reduce the infestation of aphids on crop plants.
Wednesday, August 10, Morning

Symposium VII: Phytochemical Signaling

Chairs: Kent Chapman and Dhirendra Kumar

Giourage Howe received his PhD in Biology from the University of California at Los Angeles, where he worked on metalloenzyme biogenesis in Sabeeha Merchant’s lab. He did his postdoctoral research on the tomato wound response in Bud Ryan’s group at Washington State University. In 1998, he started his own research group in the Plant Research Laboratory (PRL) at Michigan State University. His group employed genetic approaches to dissect the role of jasmonate (JA) in induced resistance of tomato to herbivore attack. That work expanded to include Arabidopsis as a model system to study the mechanism of JA signaling. His lab has made contributions to understanding the biosynthesis and catabolism of JA, identification of the JA receptor, and characterization of JAZ transcriptional repressors. Recent research in his lab has focused on understanding the dual role of JA in promoting defense and inhibiting growth.

[S7-1] Phytochemical signaling in plant defense

Giourage Howe
Plant Research Lab, Michigan State University, East Lansing, MI, USA

Signal transduction is integral to understanding the spatial and temporal expression of phytochemicals that exert toxic effects on plant pathogens and herbivores. Ongoing research in our laboratory highlights different ways in which signaling is linked to the production of defensive plant metabolites. First, we are studying how the lipid-derived hormone jasmonate orchestrates the expression phytochemical traits. A fascinating but poorly understood aspect of this question is that JA-induced synthesis of defense compounds typically coincides with potent inhibition of plant growth. I will describe our recent efforts to genetically uncouple JA-mediated growth-defense tradeoffs in Arabidopsis and discuss how achieving this goal may be used to assemble phytochemical and developmental traits in new and potentially useful ways. In addition to hormone-based defense signaling, increasing evidence suggests that defensive phytochemicals may themselves possess signaling activity. I will discuss progress on a recently initiated project to understand how metabolic output from the flavonoid pathway is coordinated with the production of terpenoids in glandular trichomes of tomato. As a specialized metabolic “factory”, the study of glandular trichomes provides unique opportunities to integrate signaling with metabolism.
Dinesh-Kumar’s laboratory is pursuing basic research activities to understand the molecular mechanisms by which plant immune receptors recognize pathogens and initiate innate immune signaling. In addition, they are interested in inter-organelar communications during innate immunity and understanding the role of autophagy in innate immunity and programmed cell death (PCD). Towards these goals, they are taking multiple complementary approaches including new forward and reverse genetic screens, sophisticated biochemical and imaging approaches, innovative genomics and proteomics approaches, small-molecule screening, and structural biology approaches. More information on Dinesh-Kumar laboratory is available at: http://www-plb.ucdavis.edu/labs/dinesh-kumar/

[S7-2] Emerging perspectives on chemical modulators of autophagy for disease control

Savithramma Dinesh-Kumar, Jongchan Woo, Ugrappa Nagalakshmi, Eunsook Park, Neeraj Lal
University of California at Davis, Davis, CA, USA

Macroautophagy, hereafter referred to as autophagy, is a dynamic process that is conserved across eukaryotes and entails the engulfment of cellular components or cargoes in double membrane vesicles called autophagosomes. Autophagosomes are then targeted to the vacuole/lysosome for degradation or recycling. It has been well established that recycling of long-lived cellular proteins and organelles by autophagy is an important adaptive response to nutrient deprivation. However, recent studies have revealed that autophagy participates in other diverse biological processes including innate immunity and programmed cell death (PCD).

Our long-term goal is to understand the molecular mechanisms by which autophagy regulates different biological processes in plants. The core Autophagy (Atg) proteins first identified in yeast are conserved in plants. Analysis of number of Atg knockout mutants in the model plant Arabidopsis has established that plants contain an autophagy system similar to other eukaryotes. Accumulating evidence suggests that autophagy in plants play an important role in defense against pathogen infections. We will discuss emerging perspectives on plant autophagy, cell death, and innate immunity. In addition, we will discuss strategies to identify small-molecule regulators of autophagy for disease control.
[S7-3] Structural insights into the biochemical properties of Arabidopsis fatty acid amide hydrolase

Kent Chapman, Mina Aziz, Lionel Faure, Xiaoqiang Wang
University of North Texas, Department of Biological Sciences, BioDiscovery Institute, Denton, Texas, USA

Fatty acid amide hydrolase (FAAH) is a member of the amidase superfamily of proteins defined collectively by their “amidase signature” sequence. FAAH is a promiscuous serine hydrolase enzyme acting on a wide range of acyl amide or acyl ester bioactive substrates. FAAH-mediated hydrolysis of N-acylethanolamines (NAEs) is a feature of diverse eukaryotic organisms including higher plants, and in Arabidopsis, FAAH cooperates with lipoxygenases to metabolize endogenous NAEs during seedling establishment. Recent studies in Arabidopsis seedlings have suggested that FAAH may hydrolyze endogenous oxylipin derivatives of polyunsaturated NAEs, expanding the range of potential lipid substrates for this amidase. This has prompted us to examine in more detail the structural and biochemical properties that distinguish plant FAAHs from the FAAH protein in mammals. In support of this goal we have initiated X-ray crystallography studies to elucidate the three-dimensional structure of Arabidopsis FAAH. Here, we will present a first view of the structural organization of the only plant FAAH to be resolved to date. Functional implications of the structural features of the Arabidopsis FAAH protein, especially with respect to the catalytic site, oligomerization, and membrane association, will be discussed.

[S7-4] A reverse genetics approach to elucidating substrate specificity of a hydroxycinnamoyl-CoA hydroxycinnamoyl transferase using transgenic Alfalfa

Michael Sullivan
US Dairy Forage Research Center, ARS-USDA, Madison, WI, USA

Hydroxycinnamoyl adducts (amides or esters) accumulate in the tissues of many plant species and may serve roles in protection against biotic and abiotic stress. We are especially interested in caffeic acid derivatives whose oxidation by endogenous polyphenol oxidases can have positive effects on post-harvest quality in forage crops. We previously identified a red clover hydroxycinnamoyl-CoA:malate hydroxycinnamoyl transferase (HMT) responsible for phaselic acid (caffeoyl-malate) accumulation in red clover. Based on sequence identity, we identified a HMT candidate gene from bean (Phaseolus vulgaris). When the bean gene (HXT) was expressed in Escherichia coli, however, the resulting enzyme was incapable of transferring hydroxycinnamic acids from CoA to malic acid, suggesting an alternate acceptor substrate specificity. Transformation of the bean gene into alfalfa caused accumulation of several new compounds in its leaves. MS-TOF analysis of the compounds provided accurate mass information which, combined with UV spectral data, suggested the new compounds were hydroxycinnamoyl esters of tetrahydroxylated adipic acid isomers such as mucic or galacteric acid. We subsequently found that E. coli-expressed HXT was capable of transferring p-coumaroyl, caffeoyl, and feruloyl moieties from CoA to both mucic and galacteric acid acceptors and that some of the resulting esters were also present in bean leaves. No transfer was seen with other potential acceptors including malic, tartaric, shikimic or quinic acids, or glucose. Because it is often hard to predict hydroxycinnamoyl transferase function from primary sequence, the reverse genetic approach of expressing putative hydroxycinnamoyl transferase genes in alfalfa and characterizing the resulting products may be fruitful in characterizing this class of enzymes.
Neish Award Speaker

Clare Casteel completed her M.S. in entomology at the University of California - Riverside and her Ph.D. in plant biology at the University of Illinois. Since 2014 she has been an assistant professor in the Department of Plant Pathology at the University of California in Davis. Her research addressed the function of microbes in plant-insect interactions using genetic and biochemical approaches. Her current focus is on plant signaling and defenses in response to insect vectors and the pathogens they transmit. Concerned with the practical application of biology and ecology, she has examined impacts of global climate change, soil management and invasive pathogens on natural and agricultural ecosystems. In addition to her research, she is devoted to teaching and interested in fostering science literacy. She has taught courses in introductory biology, entomology, plant pathology and global disease biology.

[S7-5] The role of vector-borne viruses in altering host plant defenses

Clare Casteel, University of California, Davis, CA, USA
Aurélie Bak, Andrea Cheung, University of California, Davis, CA, USA
Chunling Yang, Steven A. Whitham, Iowa State University, Ames, IA, USA

Plants employ diverse responses to defend themselves against pathogens and herbivores. Previously, we demonstrated that infection with *Turnip mosaic virus*, a member of one of the largest families of plant-infecting viruses, increases vector attraction and reproduction on infected hosts through changes in plant chemistry. These changes were due to the expression of a single viral protein, Nla-Pro. Here, we show that Nla-Pro reversibly responds to the presence of the aphid vector during infection, relocating from the nucleus to the vacuole. Importantly, relocation is required for Nla-Pro’s ability to increase aphid reproduction on host plants and this phenomenon occurs for other potyviruses. Taken together, these results suggest that the virus must somehow “recognize” the presence of the vector and respond actively, promoting insect performance and transmission only when needed, a phenomenon that has not been previously demonstrated for any animal or plant virus.
[S7-6] SIP68 is a putative glucosyltransferase enzyme with a likely role in plant stress response

Dhirendra Kumar, Abdulkareem Odesina, Olivia Simo  
Department of Biological Sciences, East Tennessee State University, Johnson City, TN 37614, USA

SIP68, a tobacco protein was found to interact with SABP2 in a yeast two-hybrid screening. SABP2 is an important component of the salicylic acid-mediated pathogen response pathway in tobacco and other plants. In-silico analysis showed SIP68 to be a putative UDP-glucose: flavonoid glucosyltransferase, having a GT1_Gtf_like conserved domain as well as the 44-amino acid PSPG Box characteristic of Family 1 glycosyltransferases (UGTs). Glucosides are a ubiquitous class of secondary metabolites involved in roles ranging from the protection of plants against pathogens and herbivory to the physical appearance of plants, transportation of metals, symbiotic agents between plants and microorganisms, and acting as sexual hormones. Glucosyltransferases transfer glucose molecules from activated glucose donors like UDP-glucose to potential aglycones, producing the corresponding glucosides. The full-length SIP68 gene was cloned and expressed heterologously both in E. coli as well as in Pichia pastoris. Purified recombinant protein from Pichia was further used for biochemical analyses. A total of fourteen different substrates has been tested so far, including flavonoids and simple phenolics. The recombinant SIP68 showed relatively high activity with several flavonoids known to have a role in plant stress signaling. Biochemical analyses using simple phenolics as aglycones is in progress. Research is also ongoing to determine the effect of SIP68’s interaction with SABP2 on its biochemical activity. Earlier studies on the gene expression analyses of SIP68 showed that it is likely modulated upon viral pathogen infection. Studying SIP68 will help to improve our general understanding of how plants respond to pathogen attacks.

[S7-7] Development of JAZ-subtype selective agonist based on Coronatine

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¹Tohoku Univ., Sendai, Japan  
²RIKEN Quantitative Bio Center, Osaka, Japan

Coronatine (COR) is a phytotoxin and structural mimic of the plant hormone jasmonoyl-isoleucine (JA-Ile). COR functions as a ligand of the COI1-JAZ co-receptor, which is the exclusive receptor of JA-Ile. In this study, we succeeded in total synthesis of COR and its stereocchemical derivatives by using coronafacic acid (CFA) and coronamic acid (CMA), those are components of COR (reference: S. Egoshi et al., RSC Adv., 6, 19404-19412 (2016)). Moreover, toward qualitative analyses for COI1-JAZ agonists in vitro, we would like to introduce our original pull-down assay system by using recombinant COI1 and epitope-conjugated JAZ subtypes.

From the structure-activity relationship study by using these COR derivatives and our in vitro screening system, one of the COR derivatives was revealed as a lead molecule of subtype-selective COI1-JAZ agonist. Furthermore, combination of the total synthesis of COR with in silico screening studies, we succeeded in development of a selective agonist toward only two JAZ subtypes with COI1. This compound would be a useful chemical tool to elucidate the complexed JA signalling pathway.
Norman G. Lewis currently holds positions of Regents Professor and Eisig-Tode Distinguished Professor, Institute of Biological Chemistry, at Washington State University, as well as Affiliate Scientist at the New Mexico Consortium (NMC) and the National Center for Genome Resources (NCGR). He serves as Regional Editor of Phytochemistry, and has been on the Editorial Board since the early 1990’s.

Professor Lewis has received numerous forms of external recognition including elections to: Corresponding Fellow of the Royal Society of Edinburgh (FRSE), Scotland’s National Academy of Science and Letters; Fellow, American Society for Plant Biologists (ASPB); Fellow, American Association for the Advancement of Science (FAAAS); Life Member, Phytochemical Society for North America (PSNA), and Fellow, International Academy of Wood Science (IAWS). He also held a Fulbright Distinguished Professor Fellowship (Science without Borders) to Brazil for 2014/2015. He has held many leadership positions in various learned societies, such as President, PSNA, and President of the American Society of Gravitational and Space Biology (ASGSB) as well as responsibilities/offices with the American Chemical Society and other professional organizations. He serves on several editorial boards, federal and international grant review panels, and scientific advisory boards worldwide.

Dr. Lewis’ current research interests are largely in discovering/studying/modifying plant biochemical pathways, as well as with bioenergy/bioproducts and medicinal plant biosynthetic pathway research (e.g., using transcriptomics, metabolomics and tissue metabolite imaging). His laboratory discovered the “dirigent” proteins, the first example of control over radical-radical phenolic coupling in planta, and which lead to anticancer compounds such as podophyllotoxin and etoposide. He has published in excess of 220 scientific papers and patents, and personnel from his laboratory now hold academic positions in the U.S., Canada, Brazil, China, France, Japan, Korea, New Zealand, Thailand, and the United Kingdom.

His research program has largely been supported by the U.S. Department of Energy, National Aeronautics and Space Administration, National Institutes of Health, National Science Foundation, U.S. Department of Agriculture, as well as the Thomas G. and Anita Hargrove and Arthur M. and Katie Eisig-Tode Foundations.

Presentation at the Award Banquet

Reflecting on some favorite agricultural and medicinal plant biotechnological discoveries

Norman G. Lewis
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Plant medicinal and agricultural sciences have progressed at near breakneck speed. This includes: extension of dirigent protein involvement from lignan to terpenoid, lignin and stilbene biochemistries; multi-omics and synthetic biology approaches to unravel biosynthetic steps to complex medicinals and specialty chemicals, as well as progress in various imaging technologies in situ.
Poster Abstracts

Poster session I
Odd number posters (e.g. P1, P3, P5...)
Sunday, August 7, 5:30-7:30 pm

Poster session II
Even number posters (e.g. P2, P4, P6...)
Monday, August 8, 5:30-7:30 pm

[P1] iTreeLib: Development of the MS^n Mass Spectral Tree Library of Plant Natural Products

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In metabolomics, compound identification of small molecules such as natural products is a challenge due to the large number of diverse molecules. Without identification, biological context and significance is often ambiguous. Matching MS/MS spectra will often not suffice for identification of isomers or compounds that share similar fragment ions. Here we set the foundation for the development of iTreeLib; a mass spectral tree library of natural products. MS^n data was generated by multi-stage mass spectrometry to aid in the process of identification and structural elucidation in untargeted metabolomics experiments. iTreeLib provides substructure information, analysis of fragmentation pathway, and expansion of both chemical space and fragmentation data. Expansion of the chemical space beyond the tested natural products; for example, is where MS^3 to MS^4 spectrum should be identical as if we had acquired an MS/MS spectrum of the authentic structure from that MS^3 level. We have validated this idea by comparing flavonoid glycosides and their aglycones, showing that the MS^3 of a flavonoid glycoside is the same to that of the MS/MS spectrum of the aglycone. Expansion of fragmentation data is due to the fact that any two stages of mass spectral analysis can be regarded as another tandem mass experiment. MS^n data was acquired on the Thermo LTQ in (+) and (-) electrospray ionization. Natural product standards were selected for MS^n experiments based on Tanimoto structural similarity. Breadth and depth of MS^n experiments was ensured by the number of triggered ions at each stage and the value of n going from 2 to 5. Substructure annotation was carried out using in silico fragmentation with Mass Frontier 7.0 and CFM-ID. Both provided more complete annotations in positive mode data compared to negative mode data. In positive mode, Mass Frontier 7.0 correctly annotated 44% of all observed fragment ions whereas CFM-ID annotated about 54% of all fragments. To date we have acquired data for more than 1,200 natural product standards which resulted in over 5,000 mass spectral trees. As a result, mass spectra for over 800 standards have also been uploaded into MassBank of Northern America (MoNA); a novel public mass spectral database for public access. We have used iTreeLib for screening for polyphenols and flavonoids in food and plant samples.
Integrated metabolomics is a revolutionary systems biology tool for understanding plant metabolism and elucidating gene function. Although the vast utility of metabolomics is well documented in the literature, its full scientific promise has not yet been realized due to multiple technical challenges. The number one, grand challenge of metabolomics is the large-scale confident chemical identification of metabolites. To address this challenge, we have developed tandem mass spectral libraries, powerful custom software entitled Plant Metabolite Annotation Toolbox’ (PlantMAT) and a sophisticated ensemble composed of Ultra high pressure liquid chromatography coupled to mass spectrometry coupled to automated solid-phase extraction and NMR (UHPLC-MS-SPE-NMR) for the large-scale systematic and biological directed annotation of plant metabolomes.

The initial step in annotation involves dereplication, or the identification of known metabolites by comparing the mass spectra of analytes in the sample with the mass spectra of known compounds. Thus, MS and MS/MS libraries were constructed using a UHPLC coupled to a Bruker Impact HD QToFMS/MS and containing spectra from 222 authentic compounds. These libraries were used to identify approximately 100 metabolites in the M. truncatula extracts. The utility of these libraries to identify metabolites in plant extracts analysed on similar systems was also tested and it was found that over 80% of metabolites could be identified using the custom libraries on similar UHPLC-QToFMS/MS instruments. Unfortunately, authentic compounds are not available for all metabolites; especially plant natural products, and a large number of the Medicago truncatula detected peaks could not be identified using spectral matching. Thus, UHPLC-MS/MS data were imported into custom PlantMAT software and structures for approximately 100 saponins and polyphenolic glycosides were predicted. The accuracy of these predictions were then validated by NMR. Targeted compounds were isolated, purified and concentrated by mass directed UHPLC-MS-SPE, and 1D and 2D NMR spectra acquired. The NMR spectral data confirmed a 100% accuracy in the PlantMAT predicted structures. The cumulative computational and empirical platforms allowed for higher-throughput and high confidence metabolite identifications necessary for metabolome ‘sequencing’.
[P3] Themes and variations in homogentisate biosynthesis: Knowledge needed for a herbicide trait

Daniel Siehl
DuPont Pioneer, Hayward, CA, USA

The aromatic pathway is chloroplastic (theme) except for the cytosolic isozyme of chorismate mutase (variation). Most plants exclusively use arogenate dehydrogenase and arogenate dehydratase to make tyrosine and phenylalanine, respectively, each enzyme being inhibited by its reaction product (theme). New evidence shows that legumes have phephenate dehydrogenase that is located in the cytosol and that it is not feedback-inhibited (three counts of variation). Lastly, we show that HPPD is located in the chloroplast in monocots (theme), but that in legumes, dual localization in chloroplast and cytosol is attained from one gene that has two transcription start sites (variation). Thus, legumes appear to have a cytosolic mini-pathway consisting of chorismate mutase, phephenate dehydrogenase and HPPD, through which homogentisate can be produced in the cytosol. We used that information to design an expression construct consisting of a modified soybean HPPD promoter and the N-terminal 86 amino acids of soybean HPPD fused to an evolved maize HPPD desensitized to HPPD-inhibiting herbicides. Transformed soybean plants have broad-spectrum HPPD inhibitor tolerance without phenotypic abnormalities.

[P4] Biochemical characterization of a Cp-3-O-GT mutant P145T and study of the tags effect on GT activity

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Glucosyltransferases catalyze glucosylation by transferring glucose from UDP-activated sugar donor to the acceptor substrates. This research is focused on the study of the effect of a single point mutation on enzyme activity, characterization of a flavonol specific 3-O-glucosyltransferase (Cp-3-O-GT) mutant- P145T, and further modification of the clone to cleave off tags from recombinant wild type and P145T mutant proteins in order to crystallize the proteins. Multiple sequence alignment and homology modeling was done to identify candidate residues for mutation. Cp-3-O-GT was modeled with a flavonoid 3-O-GT from Vitis vinifera (VvGT) that can glucosylate both flavonols and anthocyanidins. We identified a proline residue at position 145 of Cp-3-O-GT that corresponded to a threonine residue in VvGT and designed a Cp-3-O-GT- P145T mutant to test the hypothesis that that mutation of proline by threonine in Cp-3-O-GT could alter substrate or regiospecificity of Cp-3-O-GT. While the mutant P145T enzyme did not glucosylate anthocyanidins, it did glucosylate flavanones and flavones in addition to flavonols. This is significant because flavanones and flavones do not contain a 3-OH group. HPLC was performed to identify the reaction products. Early results indicated that the mutant protein glucosylates naringenin at the 7-OH position forming prunin. Results are being used to revisit and refine the structure model. In other related work, a thrombin cleavage site was inserted into wild type and recombinant P145T enzyme and we are currently working on transformation into yeast for recombinant protein expression. Cleaving off tags is a pre-requisite to future efforts to crystallize the proteins. Solving the crystal structures will make a significant contribution to the structural and functional study of plant flavonoid GTs in general and Cp-3-O-GT in particular.
Enzymatic Synthesis of 1-Tuliposide A Using Tuliposide-Converting Enzyme, a Lactone-Forming Carboxylesterase Discovered in Tulip

Taiji Nomura, Yasuo Kato
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6-Tuliposides A (6-PosA) and B (6-PosB), the glucose esters having 4'-hydroxy-2'-methylenebutanoyl and (3'S)-3',4'-dihydroxy-2'-methylenebutanoyl side chains, respectively, at C6 position of D-glucose, are well-known major secondary metabolites in tulip (Tulipa gesneriana). 6-PosA and 6-PosB serve as precursors of the antimicrobial lactonized aglycons, tulipalins A (PaA) and B (PaB), respectively. We recently identified PosA-converting enzyme (TgTCEA: EC 4.2.99.22) and PosB-converting enzyme (TgTCEB: EC 4.2.99.23), which preferentially catalyze 6-PosA conversion to PaA and 6-PosB conversion to PaB, respectively, as the first reported members of the lactone-forming carboxylesterases. 1-Tuliposide A (1-PosA), one of the minor tuliposides having 4'-hydroxy-2'-methylenebutanoyl side chain at C1 position of D-glucose, has been identified in some wild tulip species. However, its biological activity has not yet been investigated in detail due to limited availability from plants and to difficulty in chemical synthesis. Here, we aimed to establish the enzymatic process for the synthesis of 1-PosA utilizing TgTCEs as catalysts from naturally occurring 1,6-diacyl type of tuliposides, PosD and PosF. Firstly, we investigated the possibility that TgTCEs accept PosD and PosF as substrates and catalyze the formation of 1-PosA and Pa by specifically acting on the 6-acyl group of the substrates. As a result, TgTCEA and TgTCEB preferentially reacted with PosD and PosF, respectively, to form 1-PosA and corresponding Pa. Secondly, following the optimization of the reaction conditions, we performed the isolation of 1-PosA. The enzyme reaction was carried out in a reaction mixture, containing 50 mM KPi buffer (pH 6.5), 300 mg (0.77 mmol) of PosF and 1,440 U of recombinant TgTCEB expressed in E. coli, in a total volume of 77 mL. The reaction was completed in 10 min at room temperature, and, after stopping the reaction by adding 1/10 volume of 1 N HCl, the reaction mixture was subjected to activated charcoal column chromatography and subsequently the preparative RP-HPLC, which resulted in the isolation of 160 mg (0.58 mmol) of 1-PosA (yield 75%) whose authenticity was confirmed by spectroscopic analyses. This facile process dramatically improves the accessibility to 1-PosA, which allows exploration of its detailed biological activities.
Identification of a diterpene synthase and a cytochrome P450 monooxygenase that govern a novel specialized diterpenoid pathway involved in maize stress resistance

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Terpenoid phytoalexins play an essential role in maize (Zea mays) defense responses against biotic and abiotic stressors both in above- and below-ground tissues. Recently, sesqui- and diterpenoid phytoalexins, termed zealexins and kauralexins, were identified in maize. In addition to the characteristic antimicrobial functions of phytoalexins, these metabolites were shown to be highly induced under water and salt stress. Here, we report the discovery and biochemical characterization of a class I diterpene synthase (ZmKSL4) and a cytochrome P450 monooxygenase (CYP71Zxx) that together catalyze a previously unidentified diterpenoid pathway branch in maize. In vivo functional characterization of ZmKSL4 shows that it converts ent-copalyl diphosphate (CPP) into dolabradiene as verified by NMR analysis. In addition, ZmKSL4 shows substrate promiscuity also converting (+)-CPP and syn-CPP to form an array of specialized diterpenes previously observed in rice and wheat. Dolabradiene is further converted by ZmCYP71Zxx to afford distinct hydroxylated downstream products. The formation of dolabradiene adds a novel function to the portfolio monofunctional class I diterpene synthases and terpene-biosynthetic cytochrome P450s of the CYP71 family. Although the biological role of the dolabradiene pathway branch remains to be deciphered, collaborative analysis of dolabradiene accumulation in roots of Zea mays under copper sulfate (CuSO4) stress suggests a function in mediating responses to abiotic stress factors in maize roots.

Evolution of cytosolic indole synthase of Polygonum tinctorium from tryptophan synthase α-subunit through gene duplication and splicing

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2College of Horticulture and Gardening, Yangtze University, Jingzhou, Hubei, China

Indigo is an old natural blue dye produced by plants such as Polygonum tinctorium. Key step in plant indigoid biosynthesis is production of indole by indole-3-glycerol phosphate lyase (IGL). Two tryptophan synthase α-subunit (TSA) homologs, PtIGL-short and -long, were isolated by RACE PCR from P. tinctorium. The two genes were transcribed from separate genomic sequences. The short and the long forms respectively encoded 273 and 316 amino acid residue-long proteins. The short form complemented E. coli ΔtnaA ΔtrpA mutant on tryptophan-depleted agar plate signifying production of free indole, and thus was named indole synthase gene (PtINS). The long form did not complement the mutant and tentatively named PtTSA. PtTSA was delivered into chloroplast as predicted by 43-residue-long targeting sequence, whereas PtINS was localized in cytosol. Genomic structure analysis suggested that acquisition of alternative splicing sites by a PtTSA duplicate resulted in deletion of the targeting sequence during the evolution of PtINS. PtINS had about two to five folds higher transcript level than that of PtTSA, and treatment of 2,1,3-benzothiadiazole caused the relative transcript level of PtINS over PtTSA was significantly enhanced in the plant. The results indicate participation of PtINS in indigoid production.

Acknowledgement: This work was supported by Biogreen 21 Program administered by Systems and Synthetic Agrobiotech Center, Kyeongsang National University (SSAC Project No. PJ1105501).
The enzyme polyphenol oxidase (PPO) catalyzes the hydroxylation of monophenols to o-diphenols and the subsequent oxidation of o-diphenols to quinones. PPO activity is most commonly observed in damaged plant tissues, in which PPO-generated quinones polymerize to form dark-colored phytomelanins (postharvest browning). However, the native physiological function of PPO in undamaged plant cells is not well understood. *Juglans regia* (English walnut) produces a wide range of phenolic compounds and has a single gene encoding a PPO (*jrPPO1*), making it an ideal model to study PPO function. We generated a series of PPO-silenced transgenic walnut lines displaying >95% reduction in leaf PPO activity, all of which displayed spontaneous necrotic lesion development. In order to characterize the metabolic alterations occurring in PPO-silenced lines that are potentially associated with this cell death phenotype, transcriptome profiling and metabolite profiling were performed. Results revealed substantial alterations in the metabolism of tyrosine (a monophenol) in the leaves of PPO-silenced lines. Namely, tyrosine-derived tocopherols and tyramine were highly elevated in PPO-silenced lines, while the levels of 5,6 dihydroxyindole and dopamine, which are derived from an alternative pathway of tyrosine catabolism, were greatly decreased. These results suggest that PPO catalyzes the hydroxylation of tyrosine and/or tyramine *in vivo*, leading to the production of dopamine. The o-hydroxylation activity of PPO using tyramine and tyrosine as substrates was verified *in vitro*. Previous studies have shown that tyramine can be phytotoxic at high concentrations, and we demonstrated that tyramine-treated wild-type walnut leaves spontaneously develop necrotic lesions. This suggests that tyramine hyperaccumulation may be the key trigger that activates cell death in PPO-silenced lines. To investigate whether PPOs are involved in tyrosine metabolism in other plants, we are currently studying how the synthesis of tyrosine-derived benzylisoquinoline alkaloids (BIAs) is affected in California poppy (*Eschscholzia californica*) cell cultures treated with kojic acid, a specific inhibitor of PPO. Our preliminary results suggest that kojic acid significantly reduces levels of allocryptopine (BIA) secretion by poppy cells, potentially tying PPO to the biosynthesis of BIAs, which are of considerable biomedical importance.
Plants produce various tyrosine (Tyr)-derived compounds that are of pharmaceutical or nutritional importance to human. Tyrosine aminotransferase (TAT) catalyzes the reversible transamination between Tyr and 4-hydroxyphenylpyruvate (HPP), the entry reaction to the biosynthesis of many Tyr-derived plant natural products. Here we used model plant Arabidopsis to investigate the role of TATs in Tyr metabolism. Phylogenetic analysis showed that Arabidopsis has at least two TAT enzymes, At5g53970 (TAT1) and At5g36160 (TAT2). Recombinant TAT1 enzyme exhibited the highest activity towards Tyr, while TAT2 had a broad substrate specificity for both amino and keto substrates. Also, TAT1 favors the direction of Tyr deamination to HPP, while TAT2 favors the transamination of HPP to Tyr. The tat1 mutants of Arabidopsis showed elevated and decreased levels of Tyr and tocopherols, respectively, as previously reported. The tat2 mutation had no effects by itself but, together with the tat1 mutation, had additive effects on the Tyr accumulation, and the decreased level of tocopherols under highlight treatment. Global metabolite analysis showed lower levels of TCA cycle intermediates, citrate and malate, in tat mutants than wild type upon dark treatments. Also, during 13C-labeled Tyr feeding in the dark, tat1 exhibited much slower 13C incorporation than WT and tat2 into tocopherols as well as fumarate and other TCA cycle intermediates, indicating that TAT1 is the key enzyme of Tyr degradation, besides tocopherol biosynthesis. Subcellular localization analysis using green fluorescent protein (GFP)-fusion proteins and confocal microscopy showed that TAT1 and TAT2 are both localized in the cytosol. The study revealed TAT1 plays the major role in tocopherol biosynthesis and Tyr degradation, while TAT2 has a minor role in tocopherol biosynthesis under stress. The results also suggest that TAT1 is a potential target for metabolic engineering to enhance the production of HPP and Tyr-derived phytochemicals in plants.
Effect of environmental growth conditions on co-regulation of flavonoid and terpenoid biosynthesis in glandular trichomes of tomato

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Glandular trichomes are hair-like epidermal structures that are implicated as physical and chemical defensive barriers to attack by insect herbivores. The abundant type VI glandular trichome of cultivated tomato produces and stores high concentrations of both flavonoids and terpenoids. We previously showed that the classical anthocyanin free (af) mutation abolishes the function of a gene encoding the flavonoid biosynthetic enzyme chalcone isomerase (CHI1). The lack of CHI1 activity in type VI glandular cells not only impairs the biosynthesis of flavonoids but, interestingly, also causes a severe deficiency in terpenoid production. Genome-wide transcriptome and proteome analysis of purified trichome glands showed that the af mutation globally suppresses the expression of genes involved in terpenoid biosynthesis. These observations raise the possibility that CHI1 or a CHI1-related metabolite controls terpenoid production at the transcriptional level, perhaps as a means of co-regulating the terpenoid and flavonoid metabolic pathways in this specialized cell type. To test this hypothesis, we measured the metabolic composition of trichome glands from plants grown under different light intensities. The results revealed a strong positive correlation between light intensity and levels of major end products of both the terpenid and flavonoid pathways. A particularly strong correlation was observed between light intensity and levels of β-phellandrene (an abundant monoterpenoid) and glycosylated derivatives of the flavonol quercitin. These findings suggest that a flavonoid-related metabolite may be involved in transcriptional control of terpenoid production. The latest progress in understanding cross-regulation between the flavonoid and terpenoid pathways in trichome glands will be reported.

Monoterpene Synthases from Artemisia annua

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The Asteraceae family plant Artemisia annua has been used in Traditional Chinese Medicine for the treatment of fever. It produces not only the sesquiterpene lactone artemisinin, an active ingredient against malaria, but also a wealth of volatile monoterpenes and sesquiterpenes. Previously, we characterized several monoterpene synthases from A. annua, including QH1 and QH5 that catalyze the formation of (3R)-linalool from geranyl pyrophosphate (GPP) in vitro, and QH6 encoding β-pinene synthase. Recently, by mining the A. annua glandular trichome cDNA library, three monoterpene synthase genes, named AaTPS2, AaTPS5, and AaTPS6, were isolated. The recombinant AaTPS2 protein generated a single product, β-myrcene, from the substrate GPP, whereas AaTPS5 and AaTPS6 produced multiple monoterpenes with camphene and 1,8-cineole as the major component, respectively. Catalytic activities of all three monoterpene synthases could be supported by both Mg2+ and Mn2+, which resulted in different product spectra for AaTPS2 and AaTPS5. In aerial tissues and root of A. annua, more than 20 monoterpenes were detected by gas chromatography–mass spectrometry (GC-MS), of which more than 1/3 could be produced by the three enzymes. The three monoterpene synthase genes were expressed throughout the plant, but their transcript levels varied greatly among organs, and could be induced by mechanical wounding. When the plants were treated with phytohormones of methyl jasmonate, salicylic acid, and gibberellin, expression levels of AaTPS5 and AaTPS6 were elevated, implying their roles in plant-environment interactions.
Structure and biosynthesis of branched wax compounds from the cuticular waxes of *Arabidopsis thaliana*

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Aerial plant surfaces are covered with a thin layer of cuticular wax that forms an effective protective transpiration barrier. Diverse cuticular wax mixtures are generated by differential expression of genes encoding parallel wax biosynthesis pathways, suggesting that wax composition is optimized for local environmental variables. Linking composition to function has been exceedingly difficult and relies on fully characterized wax biosynthesis pathways and mutant lines. Though many wax biosynthesis pathways are well characterized, those leading to wax compounds with branched aliphatic chains are virtually unstudied, even though such compounds comprise major portions of the wax mixtures found on diverse species. Thus, the goal of this study was to fully characterize branched cuticular wax compounds in *Arabidopsis thaliana* waxes and determine which wax biosynthesis genes are involved in their production.

Arabidopsis waxes were systematically searched for homologous series of branched wax compounds. Branched alkanes and branched alcohols were found with characteristic chain length profiles in flower and leaf waxes, respectively. The low abundance of these compounds and difficulties associated with isolating even microgram quantities of a single homolog precluded NMR studies. However, a novel multi-step microscale derivatization and mass spectrometry approach indicated that these compounds were *iso*-branched, and synthetic standards confirmed these structural assignments. Next, Arabidopsis wax biosynthesis mutant lines were screened to test which wax biosynthesis genes are involved in the formation of branched wax compounds. The *cer1*, *cer3*, *cer6*, and *cer16* lines accumulated far fewer *iso*-alkanes than the corresponding wild-type plants, while *cer3*, *cer6*, and *cer16* accumulated fewer *iso*-alcohols. Overall, the wax mutant profiles indicated that the biosynthesis of *iso*-branched wax compounds is largely carried out by the same enzymes that handle unbranched wax compound biosynthesis, except that CER16 plays an important role specific to the biosynthesis of *iso*-branched wax compounds.
Plants produce a vast array of secondary metabolites. The phenolic compounds flavonoids are ubiquitous among plants and are known to aid in processes such as plant reproduction, UV defense, pigmentation and development. In relation to human health, flavonoids have also been found to possess anti-inflammatory, anti-cancer, and anti-oxidant properties. Flavonoids ability to participate in so many interactions is due in part to their subclass variation and further chemical modification. One such modification is glucosylation, where a glucose molecule is added to the flavonoid substrate, reactions catalyzed by glucosyltransferases. *Citrus paradisi* contains a glucosyltransferase that is specific to the 3-O position of flavonols. To further understand the reactions it catalyzes, Cp3-O-GT structure was modelled against an anthocyanidin/flavonol 3 GT found in *Vitis vinifera* to identify candidate amino acids for mutations. Mutants were then created using site-directed mutagenesis, and one mutant, D344P, was constructed by an aspartate being replaced with a proline based off of the sequence comparison of the original enzymes. Biochemically characterizing the mutant D344P protein will determine whether the mutation has an effect on the regio and/or stereospecificity of Cp3-O-GT. An initial screening assay has been performed using radioactive UDP-glucose as a sugar donor. Early results indicated that the mutant D344P has particular affinity for flavonols and for diosmetin, a flavone. Kinetic assays are being performed to confirm these results. Studies of time course, enzyme concentration, HPLC product analysis, pH optimum and reaction kinetics will be performed to further complete D344P protein characterization.

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Glycyrrhiza uralensis (licorice) is one of the important medicinal plants belonging to the Fabaceae. An oleanane-type triterpenoid saponin produced by licorice plants, glycyrrhizin, is widely used as medicine and natural sweetener. Although glycyrrhizin is the most prominent triterpenoid in licorice plants, they also produce other types of triterpenoids such as oleanolic acid, betulinic acid and soyasaponins. Several cytochrome P450 monooxygenases (P450s) regarding to the biosynthesis of glycyrrhizin and soyasaponins have been reported from licorice, however, P450s for the biosynthesis of oleanolic acid and betulinic acid are unknown. Here, we performed RNA-seq of tissue cultured stolons of licorice and identified a novel P450, CYP716A179, that could not be identified in our previous transcriptome data obtained from intact roots, stolons, or leaves. Enzymatic activities of CYP716A179 towards three triterpene skeletons, β-amyrin, α-amyrin, and lupeol were analyzed in engineered yeast, and the production of oleanolic acid, ursolic acid, and betulinic acid was confirmed respectively. Comparative analysis of intact roots and tissue cultured stolons has revealed that gene transcript level of CYP716A179 in tissue cultured stolons was more than 100 times higher than that in intact roots. Furthermore, oleanolic acid and betulinic acid were detected only in tissue cultured stolons. These results provide a better understanding of the molecular mechanisms of tissue-type specific biosynthesis of triterpenoids in licorice.

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Suberin is a complex biopolymer that serves to prevent water loss and microbial infection in specific plant tissues. The suberin macromolecule comprises two covalently-linked domains: the poly(phenolic) domain, which is assembled from hydroxycinnamic acids, hydroxycinnamoyl amides and monolignols, and the poly(aliphatic) domain, which consists of ester-linked ω-hydroxy acids, α,ω-dioic acids, fatty acids, 1-alkanols and glycerol. In *Solanum tuberosum*, the deposition of each domain is coordinately regulated, where the deposition of phenolics precedes that of the aliphatic domain. *De novo* synthesis of the hormone abscisic acid (ABA) after wounding plays a regulatory role in wound-induced suberization. In the present work, potato tubers that were wounded and treated with the ABA biosynthesis inhibitor fluridone (FD) demonstrated an attenuation of phenolic product biosynthesis and a pronounced reduction in aliphatic suberin monomers, relative to tubers treated with water or with FD and exogenous ABA. Gene expression analyses (RT-qPCR) across a 6 day wound-healing time course demonstrated earlier transcript accumulation for genes involved in phenolic suberin biosynthesis (*PAL1, CCR, C4H, THT*) than the majority of aliphatic metabolism genes (*FAR3, KCS6, FAAwH1, CYP86B1*) and genes involved in linkage and deposition (*GPAT5, GPAT6, FHT, ABCG1*), which accumulated at highest levels 3-4 days after wounding. While FD treatment did not have a pronounced effect on phenolic-related genes, FD treatment delayed wound-induction of aliphatic and linkage pathway genes and led to lower expression levels in these two subsets of genes across wound-healing, relative to controls. Treatment with ABA restored normal gene expression patterns. These findings support a role for ABA in the regulation and expression of genes involved in aliphatic suberin metabolism, but not phenolic suberin biosynthesis. The roles of putative MYB and WRKY family transcription factors that may orchestrate the differential timing of suberin biosynthetic pathway induction are currently being explored. This work aims to elucidate the regulatory link between phytohormone signaling and the downstream induction of stress-responsive genes required for suberin production.
[P17] Unraveling stilbene biosynthesis in *Theobroma cacao*

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Stilbene compounds, including resveratrol and pterostilbene, have garnered much attention for their roles in plant defense against pathogens and also for their beneficial effects on human health. Stilbene compounds have been detected in cocoa products, raising questions of their biosynthesis in cacao and their role in cacao development and pathogen defense. Stilbene synthase has been identified in other species (grape, peanut, etc.) as the enzyme responsible for the biosynthesis of resveratrol, the first compound in the stilbene pathway. Using a translational biology approach and taking advantage of the cacao genome sequence, we have identified 9 candidate genes for the stilbene synthase homolog in cacao. We have cloned and sequenced these 9 genes' open reading frames from the 'Scavina 6' variety. We're currently using a model species, *Nicotiana benthamiana*, to transiently express these genes and functionally characterize them for stilbene synthase activity. Stilbenes have not yet been detected in *Nicotiana benthamiana*, so this species provides a clean background to detect resveratrol formation after expression of the cacao stilbene synthase homolog. HPLC analysis of transiently transformed *Nicotiana benthamiana* tissue is currently underway. We are also working to identify the resveratrol O-methyltransferase in cacao that converts resveratrol into pterostilbene, a potent phytoalexin. Four candidate genes have been identified. We have begun cloning and biochemical characterization of these candidates.

[P18] Localization of diterpenoids in *Salvia divinorum* by MALDI-FTICR-MS imaging

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*Salvia divinorum* (Lamiaceae) is a powerful hallucinogenic plant traditionally used in psycho-spiritual and healing ceremonies by the Mazatecs of Oaxaca in Mexico. Its psychoactivity is largely due to the diterpenoid salvinorin A, a highly selective kappa-opioid receptor agonist. *S. divinorum* is the only natural source of salvinorin A known to date. However, its biosynthesis remains virtually unknown. An understanding of localization of proposed pathway intermediates in tissues and cells may help better define the salvinorin A biosynthetic pathway in *S. divinorum*. MALDI-FTICR-MSI was used to directly profile and image salvinorins, divinatorins and other compounds in trichomes on leaves of *S. divinorum*. Fresh leaves were attached to a glass slide using double-sided conductive tape and 2,5-dihydroxybenzoic acid was applied by a TM-sprayer (HTX Technologies). MALDI-MSI of the leaf surface was carried out using a solariX 9.4T MALDI-FTICR mass spectrometer (Bruker Daltonics). The raw data were then processed and ion maps were visualized in flexImaging 4.1 (Bruker Daltonics). Under the conditions employed, major intermediates and end products were localized to trichomes in veins and margins. Moreover, there were gradients of accumulation of compounds, particularly earlier pathway intermediates, in trichomes from one end of the leaf to another, which matched what is known about trichome development and biosynthetic capacity. These results demonstrate the localization of salvinorins and divinatorins directly from a single leaf. Mass spectrometry imaging is a powerful tool to better understand the organization and localization of biosynthetic pathways in plant tissues.
Oats produce a group of natural products termed avenanthramides. These compounds are produced in both the vegetative tissue and the grain. In leaf tissue they are produced in response to crown rust infection and likely other environmental stresses or by treatment with chemical plant defense activators. Grain avenanthramide production tends to be constitutive but concentrations are highly variable and strongly influenced by environmental conditions. Here we report the effect of a plant defense activator (Benzothiadiazole (BTH)) on the temporal expression and tissue distribution of avenanthramides in the leaf, stem, root, panicle stem, glumes, lemma/palea and filling/mature grain in the oat plant. HHT, the final enzyme in the biosynthetic pathway to the avenanthramides, activity is also determined in these tissues as well as the relative expression ratios of HHT mRNA resulting from benzothiadiazole treatment. Evidence for phloem transport of the avenanthramides is also presented. In summary, following BTH treatment, leaf tissue is the predominate location for avenanthramide biosynthesis. However, significant amounts are also found in the upper and lower stems, roots, panicle stems and glumes. The lemma/palea and filling grain contained demonstrable, but substantially lower amounts of the avenanthramides. Avenanthramides were also detected in the phloem sap, indicating source to sink transport of these metabolites following BTH treatment. Interestingly, only in the leaf tissue, glumes and developing seed is the expression of HHT mRNA significantly up-regulated relative to the untreated controls but, paradoxically, Ct values for HHT mRNA are approximately 3-5 cycles lower in the stem and root tissue.

Identification of phenolic phytoalexins from UV-treated rice leaves

Phytoalexins are inducible secondary metabolites possessing antimicrobial activity against phytopathogens. Rice produces a wide array of phytoalexins in response to pathogen attacks and environmental stresses. Except the flavonoid sakuranetin, most phytoalexins identified in rice are diterpenoid compounds. Phytochemical analysis of UV-treated rice leaves showed that several phenolic compounds in addition to sakuranetin accumulated remarkably in rice leaves. Four phenolic compounds were isolated from UV-treated rice leaves and identified as N-trans-cinnamoyltyramine, N-benzoyltryptamine, p-coumaroylserotonin and N-trans-cinnamoyltryptamine by NMR and MS analyses. To unravel the role of UV-induced phenylamides as phytoalexins, we examined their antimicrobial activity against rice fungal and bacterial pathogens. N-trans-cinnamoyltryptamine inhibited the growth of rice brown spot fungus (Bipolaris oryzae). In addition to the known antifungal activity to the blast fungus, sakuranetin had antimicrobial activity to B. oryzae and Rhizoctonia solani (rice sheath blight fungus). UV-induced phenylamides and sakuranetin also had antimicrobial activity against rice bacterial pathogens for grain rot (Burkholderia glumae), blight (Xanthomonas oryzae pv. oryzae) and leaf streak (X. oryzae pv. oryzicola) diseases. These findings suggested that the UV-induced phenylamides are rice phenolic phytoalexins against a diverse array of pathogens.
Biochemical analysis of stress-responsive CCR genes in rice

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Phenylpropanoid pathway is a main biosynthetic route of the cell wall component lignin and defensive phenolic compounds, which participates in plant growth, development and defense. Cinnamoyl-CoA reductase (CCR) is a key enzyme in the monolignol biosynthetic branch of the phenylpropanoid pathway, which converts phenolic acid-CoAs to phenolic aldehydes such as coumaroyl, coniferyl and sinapyl aldehydes. In rice genome, 33 genes are annotated as CCR and thirty CCR-like genes. To identify rice CCRs involved in stress-response, we performed in silico expression analysis of public microarray data obtained from various biotic and abiotic stresses. Some CCRs were substantially expressed throughout the growth period of rice, which might be involved in the lignin synthesis for secondary cell wall formation. While, expression of several CCRs was induced in response to external stresses such as UV and salt. These stress-responsive CCRs were expressed in E. coli and biochemical characteristics of the recombinant CCRs were examined. Biochemical analysis showed that some CCRs have reductase activity to phenolic acid-CoAs, including coniferyl-, feruloyl- and sinapyl-CoAs. These findings demonstrated the functional rice CCR isozymes involved in normal growth and/or defense responses.

Subcellular Localization of UGT84 Family Glycosyltransferases Responsible for Hydrolyzable Tannin Biosynthesis

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Hydrolyzable tannins (HTs) and other galloylated metabolites have a unique distribution among the land plants and play important roles in plant ecology as well as the promotion of human and animal health. The formation of β-glucogallin by a UDP-glucosyltransferase (UGT) activity is critical for the biosynthesis of these galloylated metabolites. Although enzymes capable of producing β-glucogallin have previously been cloned and characterized from several plant species, the subcellular localization of these UGTs has not been clearly defined. Determining the site of UGT action in relation to the acyltransferase activity necessary for the formation of downstream products has important implications for the regulation of these pathways. Recently, we demonstrated that two UGTs from pomegranate, PgUGT84A23 and PgUGT84A24, produce β-glucogallin in vitro and are responsible for HT production in pomegranate hairy roots. We will present our findings on the localization of these UGTs using bioinformatics, subcellular fractionation, immunogold labeling, and transient expression in a heterologous system. The results paint an emerging picture of the coordination of the activity of these UGTs with other biochemical pathways in the metabolic network.
[P23] Alpha amylase inhibition of fractions from the methanol extract of the leaves of *Tragia benthamii* (Euphorbiaceae)

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The aim of this present study is to provide some scientific proof for the folkloric use of *Tragia benthamii* (TB) in African traditional medicine for the treatment of diabetes. Phytochemical and alpha amylase inhibitory properties of the solvent fractionated extract of *Tragia benthamii* leaves were investigated. Acarbose was used as a positive control. The methanol was fractionated using solvents of increasing order of polarity (n-hexane, chloroform, ethylacetate, butanol methanol and water). Phytochemical screening of the methanol extract showed the presence of bioactive compounds like flavonoids, alkaloids, saponins, reducing sugars and cardiac glycosides. The methanol, n-hexane, chloroform and acarbose exhibited potent activities as alpha amylase with IC<sub>50</sub> values of 63.52, 193.70, 913.19 and 455.3 µg/ml respectively. The ethylacetate, n-butanol and water extracts were not active. The study showed that the methanol and the n-hexane fractions were seven times and twice as potent compared to Acarbose respectively. The chloroform fraction was half as potent acarbose.

[P24] Biological activities and chemical composition of an aboriginal herbal medicine, *Piperaceae kadsura* (Choisy) Ohwi, in Taiwan

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Chinese herbal medicine has been wildly used as a traditional medicine in East due to its clinical and theoretical fundament. In this study, a Taiwanese aboriginal plant, *Piper kadsura* (Choisy) Ohwi, was extracted with 95% ethanol, and the crude extract was partition extracted with different organic solvent based on the polarity. The chemical compositions of the active extract were separated by column chromatography. The antioxidant activity was analysed by the methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability, Trolox equivalent antioxidant capacity and Reductant capacity. In addition, the total phenolic and flavonoids contents were determined. For the antibacterial activity, the methods of disc diffusion, minimum inhibition concentration (MIC), minimal bactericidal concentration (MBC), and time killing curve were used for the analysis. The test strains were clinical antibiotic resistant isolates, included *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The results of this study showed that the ethyl ether fraction possess significant antimicrobial and antioxidant activities among all of the various extracts. According to the results of antibacterial analysis, the ethyl ether fraction of *Piper kadsura* (Choisy) Ohwi revealed a broad antibacterial spectrum against all of the test strains by disc diffusion test; the time killing curve indicated the extract was able to completely kill the pathogens within 2 hours; the MIC of the extracts was ranged between 0.5-1.0 mg/mL. In the antioxidant analysis, the ethyl ether fraction showed higher DPPH free radical scavenging ability (IC<sub>50</sub> 9.47 ppm.) than the commercial antioxidant, butylated hydroxytoluene (BHT, IC<sub>50</sub> 31.59 ppm). Moreover, the ethyl ether fraction also revealed higher reductant capacity (109.6 abs / 10<sup>-3</sup> ppm) than BHT (2.9 abs / 10<sup>-3</sup> ppm) and natural antioxidant, Vitamin C (21.9 abs / 10<sup>-3</sup> ppm). The determination of total phenolic (125.66 g Gallic acid /100 g DW) and flavonoids contents (1.044 g Quercetin / 100 g DW) suggested that the polyphenol ingredients in the ethyl ether fraction might provide the biological activities for *Piper kadsura* (Choisy) Ohwi. In conclusion, the ethyl ether extract obtained from *Piper kadsura* (Choisy) Ohwi has the potential to be developed as a natural antimicrobial or antioxidant agent.
Chemical composition and biological activity of a Taiwan aboriginal herb, *Oxalis corniculata*

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Over the few years, many studies have found that herbs contain a variety of antioxidant substances which can reduce free radical oxidation and incidence of some diseases. Therefore, herbs have become an effective source for natural product extraction. In this study, a Taiwan aboriginal herb, *Oxalis corniculata*, was used for the investigation of antioxidant and antimicrobial activity. *Oxalis corniculata* has been wildly used as a traditional medicine in the treatments of urinary tract infections, burns, snake bites, athlete's foot and eczema. However, the medical properties and biological compositions have not been deeply investigated. In this study, the crude extracts of *Oxalis corniculata* were obtained by 95% ethanol extraction. The crude extracts were then further partition extraction by organic solvents (Hexane, chloroform, ethyl acetate) based on their polarity. All of the various extracts were analysed for their antioxidant and antibacterial activities. The methods of antioxidant activity analysis included: DPPH radical scavenging ability, trolox equivalent antioxidant capacity, reductant capacity, total phenolic content and flavonoids content. The antibacterial activity tests included: disc diffusion, minimum inhibition concentration, time-killing curve, and synergy effect with antibiotics. In addition, the chemical compositions of the active extracts were determined by gas chromatography–mass spectrometry. The results showed that the ethyl acetate fraction of *Oxalis corniculata* revealed significant antioxidant and antibacterial activities among all of the test extracts. In conclusion, the current study indicated that the ethyl acetate extracts of *Oxalis corniculata* are valuable to be developed as natural antioxidant or antimicrobial agents. The results of this study provide a scientific evidence for the traditional medicine using, and even may help for the investigation of the antioxidant and antibacterial mechanisms in the future.

In-vitro cytotoxicity activity of *Eclipta alba* against A-549, HEp-2 and HeLa cell lines

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The present study was aimed to evaluate the *in-vitro* cytotoxicity activity of ethanolic extract of *Eclipta alba* Linn aerial parts (EaE) against three human cancer cell lines namely lung (A- 549), liver (Hep-2) and Cervix (HeLa) cell lines. The cytotoxicity potential of EaE was evaluated by SRB and MTT assays and the percentage viability of the cell lines was carried out using Trypan blue dye exclusion method. The EaE extract showed the maximum percentage of growth inhibition against A-549 cell line; whereas in the case of Hep-2 cell lines no cytotoxicity activity was observed. In concentration range of 1000 to 31.25 (µg/ml), EaE significantly produced cytotoxicity effect on A-549 and HeLa cell lines with CTC₅₀ values 44.60 and 51.30 in SRB and MTT assays, respectively. Therefore, the results of present study scientifically validate the use of *Eclipta alba* as an anticancer agent in the traditional system of medicine and provide potential information for its further *in-vivo* and phytochemical studies.
A Comparative Study of Polysaccharides Isolated from Two Prominent Ginseng Species (*Panax ginseng* and *Panax quinquefolius*) and their Immunomodulatory Activities

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Botanical polysaccharides have attracted a great deal of attention due to their immunomodulatory activities with low toxicity. Extensive research has shown that polysaccharides play an important role in the broad spectrum therapeutic properties of the two prominent ginseng species *Panax ginseng* and *Panax quinquefolius*. Although, there are reports differentiating the important secondary metabolite (ginsenosides) profiles of the two species, there is no known direct comparative study of their polysaccharide profiles. In the present research, crude polysaccharides from the two species were fractionated using a sequential method of ion-exchange (DEAE-Sephadex column) and size-exclusion chromatography (Superdex-200 column). The two tier purification process yielded 6 fractions in both the ginseng species. Purity, molecular weight and monosaccharide composition analysis of the isolated fractions were carried out using a novel method utilizing high performance liquid chromatography-charged aerosol detector (HPLC-CAD). The analyses revealed that the fractions had a diverse range of molecular weight and heterogenic monosaccharide composition. The effect of ginseng polysaccharides on differentiated human THP-1 cells indicated a stimulation of multiple pro and anti-inflammatory cytokines and chemokines. Furthermore, a lymphocyte proliferation assay was conducted to assess the role of these compounds in adaptive immunity.

Effects of Sutherlandia on triglyceride levels of rats fed high-fat diets

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*Sutherlandia frutescens* (L.) (Sutherlandia) is a South African medicinal plant that is widely used as in traditional remedies and in dietary supplements for Type 2 Diabetes (T2DM). Intake of high-fat (HF) diets results in insulin resistance (IR) and chronic inflammatory responses, resulting in the development of T2DM. Animal studies in South Africa indicate that consumption of Sutherlandia aqueous extracts increases glucose uptake in muscle and adipose tissue and reduces plasma free fatty acid levels of rats fed a high fat (HF) diet, suggesting that mitochondrial activity and lipid synthesis are altered by Sutherlandia. My research investigates these pathways and the role of the NF-κB inflammatory signaling pathway and downstream targets in these effects. Male Wistar rats were fed a HF diet for 12 weeks with or without Sutherlandia aqueous extracts. Sutherlandia significantly reduced levels of triglycerides in the liver ($p = 0.01$) and white adipose tissues ($p = 0.02$), but not muscle tissues of rats fed a HF diet compared to those fed the same HF diet with the water control. No significant changes were observed in plasma glucose, insulin and triglyceride levels, suggesting Sutherlandia presents metabolic effects in a tissue-specific manner. Quantitative real-time PCR and Western blots will be used to measure expression levels of genes and proteins related to triglyceride production, insulin signaling and inflammation in order to test the hypothesis that Sutherlandia is able to improve insulin function by reducing pro-inflammatory signaling induced by consumption of HF diets.
Evaluation of the Anti-malarial Activity of *Callichilia stenopetala* Stapf (Apocynaceae) Root Bark in Mice

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The challenge of not having prompt access to healthcare facility and poor financial status have directly and indirectly encouraged the currently observed increase in the use of herbal or plant products as an alternative means of healthcare in developing countries. In Nigeria, malaria constitutes a major cause of health insecurity, particularly for pregnant women, children and under aged pregnant girls. This is aggravated by the increasing spread of drug-resistant and death causing *Plasmodium falciparum* strains. The plant *Callichilia stenopetala* Stapf (family Apocynaceae) is commonly used by traditional practitioners in South-East part of Nigeria as a remedy for the treatment of malaria, “re-current” fever and other ailments. The methanolic crude extract of the plant was suspended in water and partitioned between hexane, chloroform and ethyl acetate successively to obtain various fractions. Antimalarial effects of the crude extract and the active fractions against early (chemosuppressive) and established (curative) infections were evaluated in chloroquine sensitive *Plasmodium berghei berghei* NK-65 infected mice. Phytochemical constituents and oral acute toxicity in mice were also studied. Activity reside more in the non-polar fractions in chemosuppressive test. The crude extract, the hexane and chloroform fractions demonstrated intrinsic chemosuppressive properties (80.3, 91.9, 71.4% respectively at 500 mg kg⁻¹) that were dose-dependent compared with the standard drugs, chloroquine 5 mg kg⁻¹ and artesunate 10 mg kg⁻¹ (82.22 and 73.8% respectively). The tested fractions, produced significant (p<0.05), dose dependent activity against the parasite in the Rane curative test compared with the standard drug chloroquine. Results of the phytochemical analysis test showed relative abundance of alkaloid and other secondary metabolites. At a dose of 8000 mg kg⁻¹, no mortalities or evidence of adverse effects was observed in acute toxicity test. Thus, the plant could be considered as a potential source of new antimalarial lead, thereby improving on health status of the society.

Key words: Malaria, *Plasmodium falciparum*, *Callichilia stenopetala*, chemosuppressive, Rane curative test
Enrichment and purification of flavonoids from the alcoholic extract extract of *Althaea officinalis* roots

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*Althaea officinalis* L. Family: Malvaceae, commonly known as Marshmallow, is a perennial that grows to 5 feet in salt marshes and moist regions throughout Europe, western and northern Asia, and the eastern US. Its root is best known for its source of valuable mucilage, which has been used for more than 2 millennia to treat topical wounds and as a remedy for sore throats, coughs, and stomach ailments. The mucilage is incorporated into ointments to soothe the chapped skin and is added to foods in small quantities to provide bulk and texture. The aqueous and alcoholic extracts of the roots, leaves & flowers of Marshmallow have been demonstrated to possess antioxidant, protection against indirect UVA-induced oxidative stress, antibacterial, and antifungal, activities. It was also reported that mucilage and flavonoids have the property of covering and protecting gastric mucosa, thereby reducing the incidence of gastric ulcer.

In the present study, the performance and separation capabilities of several normal and reversed phase open column chromatography packing materials and resins for the enrichment and purification of flavonoids were evaluated. According to our results, a brominated polystyrene resin (SP-207) was found to be offering higher adsorption and desorption capacities. The method delivers simple, scalable and environmentally friendly means of isolation and purification of extracts enriched in flavonoids and phenolic compounds in general. This method can also be used for routine rapid and selective sample preparation of phenolic compounds prior to qualitative and quantitative analytical chromatography. The results of these studies including the extraction, isolation and optimization of the technical parameters of the separation process of the compounds in question will be presented.

Natural products as a source of anti-inflammatory agents for treating inflammatory bowel disease

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In attempt to discover novel bioactive plant natural products for the potential treatment of inflammatory bowel disease (IBD), we examined commonly available natural products that have been used traditionally to treat IBD. Specifically, we examined Silymarin fractions that could have significant potential for containing an anti-inflammatory drug lead. The anti-inflammatory effects of these compounds isolated from selected plants were tested for blocking cytokine production induced by lipopolysaccharide (LPS) on RAW 264.7 cells and tumor necrosis factor (TNF-α) on HT-29 cells.

The murine macrophages were stimulated with Escherichia coli LPS (1 μg/ml) in the presence and absence of non-cytotoxic concentrations of isolated compounds. TNF-α was measured from these stimulated macrophages. The effect of TNF-α (10 ng/ml) on chemokine (IL-8) production in the HT-29 colonic epithelial cell line was illustrated. Cytokine determinations were conducted after 4 hours of cellular stimulation. Selected compounds were also evaluated in a co-culture system utilizing RAW 264.7 and Caco-2 cells. The presentation will focus on the biological activities in three different cell culture systems of Silymarin, a complex of four major classes of flavonolignans silibinin, silychristin, silydianin and isosilibinin, obtained from *Silybum marianum* (Milk thistle) seeds. An ongoing study will involve measurement of the cytokine production and NF-κB activation from colonic strips of mice with colitis with an ex-vivo system.
Collision-induced dissociation MS/MS of cimitrypazepines, a new class of alkaloids from black cohosh (*Actaea racemosa*)

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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, and menopausal symptoms such as hot flashes. We recently discovered that black cohosh produces a large diversity of alkaloids some of which contain novel structural scaffolds. One such novel class of compounds is the cimitrypazepines which contain an azepine ring and can be regarded as Pictet-Spengler cyclization products of *N*-methylserotonin and various aldehydes. In this study, a detailed investigation was carried out of the collision-induced dissociation MS/MS product ion fragmentation patterns of cimitrypazepines.

Test azepines were synthesized by reacting *N*-methylserotonin with the corresponding aldehyde under basic conditions (Pictet-Spengler condensation). Analogues containing both the aliphatic and aromatic side chains were prepared. Tandem mass spectra were acquired on a Waters SYNAPT quadrupole/time-of-flight mass spectrometer operated in positive ion electrospray using argon as collision gas.

The main fragmentation pathways observed corresponded to the loss of methylamine, [MH-CH$_3$NH$_2$]$^+$ and loss of methylene imine, [MH-CH$_2$=NH$_2$]$^+$. These fragment ions were characteristic for this structural class and served to identify many analogues present in the plant extract. Since a nitrogen atom is a part of the azepine ring, elimination of methyl amine involves ring opening followed by hydrogen transfer. An interesting fragmentation pathway corresponding to the methyl group transfer was observed during fragmentation of aromatic analogues. This pathway was determined to correspond to the intramolecular electrophilic attack of the methyl cation onto the aromatic side chain. Origins of other important fragments observed in the spectra are discussed in more detail.

Overall, our data provide a template for interpretation of tandem mass spectra of this class of molecules and will be useful for future metabolomics studies of not only black cohosh but other plants as well.
All plants possess a lipophilic interface with the environment. In above-ground tissues this is comprised of a waxy cuticle. Below-ground storage organs (tubers or tuberous roots) are protected with a lipid heteropolymer, suberin, and associated waxes that are embedded within the periderm or skin. These waxes, along with suberin, act as a barrier to prevent water loss and defend plants from pathogen attack. A phytochemical study on the periderm wax composition of sweet potato (*Ipomoea batatas* L.) resulted in the isolation and identification of a novel phytosterol of the cycloartane type. The resulting phytosterol is produced in large quantities within the periderm of the *Beauregard* sweet potato (*Ipomoea batatas* L.) cultivar, comprising 66.0% of the total periderm wax extract by mole percent. The structure and relative configuration was determined by mass spectrometry and spectroscopic techniques, including 2D NMR and IR as well as chemical transformation. Total wax extracts from sweet potato periderm and the isolated sterol show promising anticancer potential including inhibited growth and proliferation of Triple-Negative Breast Cancer (TNBC) MDA-MB-231 human mammary carcinoma. Ongoing research is focused on titrating periderm extracts to determine optimal antineoplastic effective doses and possible mechanisms, with preliminary data demonstrating a dose-dependent response in all extracts. Future studies will focus on analyzing wax extracts from other cultivated sweet potato varieties to better understand the diversity and biosynthesis of phytosterols in sweet potatoes.

**[P34] Comparative Transcriptome Analysis of Genes Involved in Polysaccharides Biosynthesis in Dendrobium officinale**

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*Dendrobium officinale* is one of the most valuable Chinese medicinal and edible herbs, the stem of which is used as a crude drug named “Fengdou”. Glucomannan (GM) is the main active polysaccharides in *D. officinale*, which has important biological functions, e.g. immunomodulation, antitumor, and cardioprotection, etc. Previous studies showed that the content of polysaccharides in samples collected in May was significantly higher than those collected in February in Zhejiang, China. Furthermore, the content of polysaccharides in leaves was about a quarter in stems. To identify the genes involved in GM biosynthesis, transcriptome analysis of leaf and stem tissues of adult *D. officinale* harvested in two growth stages (February and May) was performed. Illumina sequencing for the four samples generated 36 million high quality reads that were assembled into 32,691 unigenes totally. Among them, 8 identified unigenes of *cellulose synthase* (*CESA*) superfamily expressed differently, included *cellulose synthase* (*CESA*) genes and *cellulose synthase-like* (*CSL*) genes. Moreover, 13 glycosyltransferase genes were revealed as differential expression genes (DEGs). This study enabled us to further understand the molecular mechanisms regulating polysaccharide biosynthesis in *D. officinale* and set the foundation for the genetic and quality improvement of this important Chinese orchid herb.
[P35] Assessing the effects of autoclave and steam treatments on the ginsenoside profiles and immunomodulatory properties of North American Ginseng

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The North American species of ginseng (*Panax quinquefolius* L.) is cultivated in southern Ontario for sale to international markets, and is primarily sold as a fresh product or as low temperature dried ginseng known as white ginseng. The Asian species of ginseng (*Panax ginseng* C.A.Mey.), is sold in a wide variety of forms, including red ginseng, a boiled and/or steamed ginseng product, and black ginseng, a ginseng product which has typically undergone multiple rounds of high temperature steam treatments. Red and black versions of North American ginseng are not widely available, and little research has investigated the effect of these processing methods on the phytochemical and pharmacological properties of *P. quinquefolius*. Western Phytoceutica, a natural health product start-up based in Sarnia, has recognized the need for high quality processed North American ginseng products on the market. In this study, the effects of various autoclave temperatures and times on aqueous and alcoholic *P. quinquefolius* extracts, as well as the effects of various steam treatment temperature and times, were investigated. The pro- and anti-inflammatory effects of differentially treated ginseng samples were evaluated in RAW macrophages by measuring the production of various mediators of inflammation (TNF, IL-1β, IL-6, IL-10, and NO). Total polysaccharide content and the content of seven ginsenosides (Rg1, Re, Rb1, Rc, Rb2, Rd, and Rg3) was also measured. In general, longer and higher temperature treatments resulted in a conversion of the more polar ginsenosides to less polar ginsenosides. While the various treatments did not have a major impact on the total polysaccharide content, there was an increase in the pro-inflammatory activity of ginseng samples subjected to moderate temperature treatments. However, this increase in the pro-inflammatory activity was lost in samples that we subjected to the longest and highest temperature treatments. Taken together, these results are an important step in the optimization of processing methods to produce high quality North American ginseng products.


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Ginger (*Zingiber officinale* Roscoe) rhizome has a wide range of usage traditionally, from nausea to treatment of respiratory disorders. There is also numerous scientific literature on the bioactivities of this plant, such as antibacterial, anti-inflammatory, antidiabetic, antiemetic, hepatoprotective etc properties. Phenolic compounds like gingerols and shogaols are known to be the active constituents. The National Institute of Standards and Technology (NIST), in collaboration with the National Institutes of Health-Office of Dietary Supplements (NIH-ODS) is producing two ginger reference materials to support the determination of gingerols, shogaols, and toxic elements. An analytical method developed based on liquid chromatography with absorbance detection at 281 nm using pentafluorophenyl (PFP) column. A variety of approaches will be reported for the extraction.
[P37] Diversity in anthocyanin composition of Apache Red corn germplasm

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Increasing consumer demand for food and beverage products free of synthetic dyes has stimulated interest in finding an economical yet highly concentrated and stable source of natural phytopigments. Maize kernels are an abundant source of anthocyanins, the orange to blue pigments most frequently used to replace synthetic dyes. Most of the diversity and research in anthocyanin-containing maize is comprised of pigmented aleurone lines; however, pigmented pericarp lines are capable of producing concentrations an order of magnitude greater than aleurone lines. Here we investigate diversity in anthocyanin composition and the corresponding genetics in Apache Red, a landrace that produces kernels with highly pigmented pericarp. Principal component analysis and hierarchical cluster analysis of 267 Apache Red derived lines revealed several unique anthocyanin compositional clusters. Anthocyanin extracts, which contained primarily cyanidin, pelargonidin, and peonidin based anthocyanins, ranged in concentration from 4 to 1211 µg per ml, and varied in hue according to anthocyanin profile. Many lines contained flavanol-anthocyanin condensed forms, with some containing high percentages of a previously unidentified species. As a pilot study, 174 lines were genotyped using Illumina sequencing, and association mapping was performed to identify candidate genes associated with anthocyanin profiles. The Apache Red lines created and analyzed herein will serve as a valuable source of diversity both for understanding the genetics underlying anthocyanin biosynthesis in maize pericarp and for breeding lines capable of replacing synthetic dyes.

[P38] Antinociceptive effects of the essential oil of Lippia oaxacana

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Lippia oaxacana Rob. & Greenm. (Verbenaceae) is an aromatic herb widely used in mexican folk medicine. Since the essential oil of this herb has many pharmacological activities, including antispasmodic effects. The aim of the present study is to investigate the antinociceptive activity of essential oil from the aerial part of L. oaxacana from community of Santiago Huauclilla, Oaxaca, México. All experiments were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals. Male mice CD1 (25-30 g) were used. The number of animals in each control and experimental group was 6. Oral treatment with the essential oil of L. oaxacana (vehicle: 0.1% Tween 80 in water), elicited inhibitory activity on acetic acid effect at 1, 10 and 30 mg/kg (36.4 ± 28.7, 26 ± 11.2 and 9.5 ± 6.6, abdominal writhes respectively, as compared with the control group (105.5 ± 3.9). Ketorolac (1mg/kg) was used as pharmacological reference. These results show a relationship between increase dose of essential oil and antinociceptive activity as dose-dependent effect. The GC-MS analysis of essential oil allowed the identification of three major compounds of L. oaxacana as a limonene (27.92 %), camphor (4.15%) and camphene (2.1%). The presence of these metabolites of L. oaxacana promotes antinociceptive activity of essential oil, therefore the use of this plant is reinforced against pain in folk medicine of Santiago Huauclilla, Oaxaca, Mexico.
[P39] Investigation of an American Indian Herb, Amorpha canescens Pursh, for Menopause and other Women’s Health Ailments

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Currently, women are the top consumers of botanical dietary supplements to treat ailments that are gender specific, such as menstrual symptoms, urinary tract infections, and menopause. Specifically for menopause, the leading botanicals that women take are black cohosh and red clover, which were also traditionally used by American Indian women. While these two plants have been investigated extensively, there are still numerous American Indian plants that lack scientific studies on their safety and efficacy for menopause and other women’s health ailments.

The purpose of this project was to investigate an American Indian herb, Amorpha canescens Pursh (Fabaceae), also known as leadplant, for its potential estrogenic, chemopreventive and anti-inflammatory activity. We are using bioassay guided fractionation (BGF) and de-replication techniques to identify the active compounds of leadplant. Using BGF, we identified that leadplant's methanol extract has dose-dependent anti-estrogenic and anti-inflammatory activity in the Ishikawa and Griess cell-based assays. Also, leadplant demonstrated low activity in the NQO1 induction assay for chemopreventive potential. After partitioning the methanol extract, the chloroform partition was the most active in the Ishikawa and Griess assays. For our de-replication approach, we are using either Pulsed Ultrafiltration (PUF) or Magnetic Microbead Affinity Selection Screening (MagMass) with the estrogen receptor (ER- anti/estrogenic), 15- lipoxygenase (15-LOX- anti-inflammation), and retinoid X receptor (RXR- chemopreventive and anti-inflammation) to quickly identify the ligands that may be responsible for the observed activity. For the RXR and 15-LOX receptors we did not identify any ligands from leadplant's methanol extract. Thus, the anti-inflammatory and chemopreventive activity of leadplant may be contributed by another mechanism of action other than the RXR or 15-LOX pathways.

Currently, we are optimizing the PUF assay with the ER receptor and continuing the bioassay guided-fractionation with leadplant’s chloroform partition to ascertain the active constituents for the anti-estrogenic and anti-inflammatory activity.


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L-Ascorbic acid (AsA, vitamin C) is a key antioxidant and enzyme cofactor in plants. Ascorbate controls cell division, affects cell expansion, and plays an important role in modulating plant senescence. It protects plants against reactive oxygen species that are produced in response to abiotic and biotic stresses. Manual phenotyping indicated that Arabidopsis lines over-expressing enzymes in the myo-inositol pathway have elevated AsA, accumulate more biomass of both aerial and root tissues and are tolerant to abiotic stresses including salt, cold, heat, and environmental pollutants. However, manual phenotyping is time consuming, low throughput, subjective, and limited to the resolution of the human eye. In contrast, high throughput phenotyping technologies are accurate, non-destructive, and more sensitive, allowing the detection of subtle phenotypes. Therefore, we used a Scanalyzer HTS system to phenotype our high AsA Arabidopsis lines with visible, fluorescence, and near infrared cameras. Based on this approach, high AsA lines grew faster, accumulated more biomass, and displayed healthier chlorophyll fluorescence and water content profiles than controls. By studying abiotic stress in a high throughput fashion using optimized protocols, we have also shown that these high AsA lines are tolerant to salt and water limitation stresses. In addition, we developed new modules based on the PlantCV (Plant Computer Vision) suite to analyze images, and by comparing results obtained with the LemnaGrid software against our algorithms, here we show that our method achieved good accuracy for all phenotypic parameters of interest including projected leaf area, rosette diameter (caliper length), compactness, and color classification.

[P41] Enhancing the accumulation of β-carotene in tetraploid wheat grains by TILLING

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Vitamin A is an essential micronutrient for human, which must be obtained from dietary sources, such as provitamin A carotenoids with β-carotene being the most effective one. Wheat grains do not accumulate β-carotene while the primary carotenoid in tetraploid wheat grains is lutein, a non-provitamin A carotenoid competing with β-carotene for the common biosynthetic precursor. To enhance β-carotene accumulation in tetraploid wheat grains, it is necessary to block the competing reactions (catalyzed by lycopene ε-ring cyclase/LCYe) and the catabolism of β-carotene (catalyzed by β-carotene hydroxylases/HYDs and carotenoid cleavage dioxygenases/CCDs). Mutants with lesions in target genes were isolated from tetraploid wheat Targeting Induced Local Lesions in Genomes (TILLING) pool. Mutated gene alleles were cloned and functionally characterized. Spatial gene expression in developing grains indicated that LCYeA, HYD-A2, HYD-B2 and CCD-A1 are relatively highly expressed in the endosperm. Phenotypic analysis along with spatial gene expression results enabled us to select single mutants to generate specific higher level mutant combinations which will lead to increased β-carotene accumulation in tetraploid grains. Information obtained from this study will not only be directly applied to improving β-carotene accumulation in tetraploid wheat grains through breeding, but also facilitate future provitamin A biofortification in hexaploid wheat.
[P42] Elucidating an Insect Terpenoid Pheromone Pathway to Engineer Trap Crops for Pest Management

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Insect pheromones are commonly used in agricultural pest management. However, they usually require chemical synthesis and repeated application in insect lures. Plants are promising alternative platforms for a highly specific and continuing production of pheromones to develop trap crops in area wide pest management. Our objective is to engineer a trap crop that can be applied for managing the harlequin bug, *Murgantia histrionica*, a specialist pest of cole crops. The harlequin bug aggregation pheromone, murgantiol, a sesquiterpene alcohol epoxide, is formed in two or three main steps: a terpene synthase converts farnesyl diphosphate to the sesquiterpene alcohol, sesquipiperitol, which is then converted to murgantiol via isomerization and epoxidation either directly or through zingiberenol as a possible intermediate. We are currently performing feeding assays to elucidate the sequence of these possible conversion steps. Moreover, we are in the process of identifying cytochrome P450 enzyme candidates involved in these steps by comparative gene expression analysis of different sexes, developmental stages, and tissues. The identified gene tools will be assembled for the metabolic engineering of murgantiol in different plant production systems.

[P43] Identification of Cytochrome P450 Monooxygenases Involved in Marrubiin Biosynthesis

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Marrubiin is a furanic labdane diterpene produced as a major constituent in the aerial parts of white horehound (*Marrubium vulgare*, Lamiaceae). While its biological function remains elusive, pre-clinical studies on marrubiin demonstrated potent antidiabetic and analgesic properties. In light of the rapid progression of diabetes affecting more than 300 million people worldwide, marrubiin and related natural products could provide new solutions in the prevention and treatment of the disease. However, further production and development of marrubiin and related metabolites as potential therapeutics requires a deeper knowledge of the genes and enzymes involved in their biosynthesis. To this end, we recently identified two diterpene synthases (dITPSs), *MvCPS1* (class II) and *MvELS* (class I) which in a sequential reaction convert the universal diterpene precursor, geranylgeranyl diphosphate, into a unique diterpene intermediate, 9,13-epoxy-labd-14-ene. This diterpene exhibits the characteristic 9,13-spiro ether function found in marrubiin and related compounds, suggesting that *MvCPS1* and *MvELS* catalyze the committed pathway steps. Building on this foundation, tissue-specific transcriptome analysis, phylogenetic studies and gene expression analysis revealed two previously unidentified members (*Mv1270* and *Mv233*) of the CYP71 clan of cytochrome P450 monooxygenases (CYPs) with possible functions in marrubiin biosynthesis. Co-expression of these candidate CYPs with *MvCPS1* and *MvELS* in engineered microbial (*Saccharomyces cerevisiae*) and plant (*Nicotiana benthamiana*) platforms showed the formation of novel hydroxylated diterpenoid intermediates that may expand our capacity for producing marrubiin and related natural products as potential antidiabetic therapeutics.
Understanding the biosynthesis of bioactive secondary metabolites from *Penicillium chrysogenum*

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The symbiotic association of endophytic fungi with plants generates a rich population of secondary metabolites with structural features that represents a promising source of new chemical entities. Some plants such as liverworts can host bioactive soil microbes as epiphytes and/or endophytes and have historically been known to be one of the most fertile sources for new natural products. As part of our systematic research centered on finding novel antiproliferative (against human breast adenocarcinoma (MCF-7) and the human colorectal adenocarcinoma (HT-29) cell lines) compounds from endophytes of liverworts, we established a library of microbial extracts and obtained cytotoxic activities with ED₅₀ values ranging from 0.07 to >20 µg/mL for both cell lines in cell based assays. In the present study, we selected a *Trichoclea tomentella* (Trichocoleaceae) endophytic fungus *Penicillium chrysogenum* for further investigation due to its significant cytotoxicity (ED₅₀ values of 2.2 µg/mL against MCF-7). Bioassay guided fractionation and isolation of an ethyl acetate extract of the *P. chrysogenum* cultured on rice medium yielded one new chlorinated xanthone: 3, 8-dihydroxy-6-chloro-1-methylxanthone together with fifteen known compounds. Among the isolated compounds, epiepoxydon was the most cytotoxic compound for MCF-7 while the chlorinated compound: griseofulvin was the most potent against HT-29 cell line. In addition, our results demonstrated that halogenated scaffolds from *P. chrysogenum* were more active than their non-halogenated counterparts. This selectivity incited us to isolate more halogenated compounds via induced halogenation. We subsequently manipulated the fermentation by supplementing halide salts to the rice medium to generate new brominated and new and known chlorinated compounds. One of the isolated new halogenated compounds: gentisyl alcohol bromide, displayed a stronger cytotoxic activity against MCF-7 (IC₅₀= 8.4 µM) compared to the non-brominated analog gentisyl alcohol (IC₅₀= 35 µM). During this study we could also demonstrate that some of the isolated halogen-containing compounds have been produced by non-enzymatic pathways. Studies on biosynthetic and non-biosynthetic pathways of the halogenated secondary metabolites of *Penicillium* will be discussed.
The thunder god vine (genus *Tripterygium*) is a Chinese medicinal plant that has been used traditionally to treat fever, swelling, and different forms of inflammations. Root extracts from the plant and isolated diterpenoids (most prominently the abietane tricyclic triptolide) have recently been investigated as treatments for autoimmune diseases and certain types of cancer. This study is focused on characterizing the functions of diterpene synthase genes in *Tripterygium regelii*. We developed an adventitious root culture (ARC) system in which diterpenes, which naturally only occur at very low concentrations, are accumulated at high levels. Transcriptome data sets were obtained from this ARC using RNA Illumina sequencing. Filtering of a total of 50,811 contigs led to the identification of five gene candidates for diterpene synthases. Expression in heterologous hosts - *Escherichia coli* and *Nicotiana benthamiana* – and functional evaluation revealed that TrCPS1 converts geranylgeranyl diphosphate (GGPP) to ent-copalyl diphosphate (CPP), while TrCPS2 converts GGPP to (+)-CPP. Both TrKSL1 and TrKSL2 further convert ent-CPP ~ 95% ent-kaurene and ~ 5% ent-isokaurene. Kauranes derived from these intermediates have previously been characterized as bioactive *Tripterygium* metabolites. TrKSL1 also converts (+)-CPP to sandaracopimaradiene and isopimaradiene, and syn-CPP to syn-pimaradiene and syn-stemodene. TrKSL3 converts ent-CPP, (+)-CPP and syn-CPP to a mixture of diterpenoid products. The peptide sequences of TrKSL1, TrKSL2 and TrKSL3 cluster with ent-kaurene synthases involved in gibberellin biosynthesis in other plants (members of the TPS-e/f subfamily). Our results indicate that a limited number of diterpene synthases generate significant chemical diversity. The implications of these findings for terpenoid pathway evolution and function will be discussed.
Differential transcriptome analysis of leaves of tea plant (*Camellia sinensis*) provides comprehensive insights into the defense responses to *Ectropis oblique* attack using RNA-Seq

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Tea is a very popular and healthy non-alcoholic beverage worldwide. As an evergreen woody plant, the cultivation of tea plants (*Camellia sinensis*) is challenged by biotic stresses, and one of which is feeding of *Ectropis oblique*. In China, *E. oblique* infestation causes serious damages in many tea cultivation areas. Tea plants have evolved sophisticated strategies to cope with attack by *E. oblique*. To elucidate the molecular mechanisms of the response to *E. oblique* in tea plants, the differential gene expression profiles between the *E. oblique* damage-induced tea plants and undamaged control using RNA sequencing (RNA-Seq) were obtained.

In total, 1859 differentially expressed genes were identified, including 949 up-regulated and 910 down-regulated genes. Overall, 90 signal transduction genes, 100 anti-insect responsive transcription factors, 50 genes related to phenylpropanoid biosynthesis, 41 unigenes related to herbivore-induced plant volatiles (HIPVs) biosynthesis, and 8 caffeine biosynthesis genes were found to be differentially regulated. Metabolic pathway analysis indicated that plant secondary metabolites and the signaling pathways may play an important role in defense against insects, and a closer examination at the expression of some crucial genes revealed differential expression patterns after feeding by *E. oblique*. Furthermore, quantitative RT-PCR (qRT-PCR) analysis further confirmed the results of RNA-Seq. Our dataset provides the most comprehensive sequence resource available for studying the resistance to *E. oblique* in tea, which will benefit our understanding of the overall mechanisms underlying inducible defenses responses, and may be useful to create novel prevention measures against insects to reduce pesticide usage in eco-friendly tea farming.

Diketopiperazines from the phytopathogenic fungus *Alternaria dauci*

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Seven diketopiperazines were isolated from culture filtrates of *Alternaria dauci*, a necrotrophic fungus that causes leaf blight in carrots. The phytopathogen was cultured in Czapek-Dox medium, under shake conditions (100 rpm), at room temperature (25 °C), for 96 hours. Chromatographic separation (Silica gel, Hex-CH2Cl2-MeOH) of the ethyl acetate extract yielded twelve semipurified fractions (A1 to A12). HPLC purification of fractions A6 to A9 and A12 resulted in the isolation and identification of diketopiperazines cyclo(pro-leu) (1), cyclo(pro-phe) (2), cyclo(pro-val) (3), cyclo(val-leu) (4), cyclo(val-phe) (5), cyclo(leuphe) (6), and cyclo(leu-tyr) (7). The chemical structures were determined by Mass Spectrometry (LREIMS) and 1H-NMR analysis, and by comparing the spectroscopic data with those reported in the literature.
Synthesis of deuterated precursors for biosynthetic investigation of the cruciferous phytoalexins nasturlexins

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In response to biotic and abiotic stress, plants produce phytoalexins, a group of secondary metabolites that are biosynthesized de novo from primary precursors. Possessing antifungal activity against a variety of plant pathogens, phytoalexins play an important role in the chemical defence of plants.

Crucifers (family Brassicaceae) are known to produce tryptophan-derived phytoalexins, with more than 45 metabolites discovered to date. Recently, nasturlexins, a group of phenylalanine-derived phytoalexins have been isolated from watercress (Nasturtium officinale), upland cress (Barbarea verna), and winter cress (B. vulgaris). The occurrence of another biosynthetic pathway in addition to the previously known tryptophan-derived pathway poses an opportunity to explore this intriguing discovery.

In this work, the biosynthesis of nasturlexins is proposed, starting from homophenylalanine via the glucosinolate gluconasturtiin. To probe this pathway, a variety of precursors labelled with deuteria have been synthesized, including gluconasturtiins, isothiocyanates, and dithiocarbamates. Current work is in progress to administer these labelled precursors into watercress and upland cress to determine biosynthetic intermediates, necessary to discover the corresponding biosynthetic enzymes and genes present in crucifer species.
Specific inhibition of monolaurin and monobehenin on biofilm formation of three bacterial strains, *Streptococcus mutans*, *Xanthomonas oryzae*, and *Yersinia enterocolitica*

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Monoacylglycerols are emulsifiers in food and recognized as safe. In this study, the inhibitory activity of monoacylglycerols on bacterial biofilm formation was systematically evaluated. Biofilm provides a bacterial hiding place by forming a barrier and causing physiological changes in cells. The elimination of biofilm is the main goal of hygiene. Monoacylglycerols with two specific lengths of fatty acid moiety, monolaurin and monobehenin, were found to have strong inhibitory activity on specifically bacterial biofilm formation. This result suggested that bacterial biofilm formation was not inhibited by the detergent characteristics of monoacylglycerols. It was supported by the inhibitory action of monolaurin on biofilm development but not by the initial cell attachment in flow cytometric observation. The results of this study suggested the existence of two strain-specific inhibitory response systems in bacteria.

The effect of ethanol concentration on antimicrobial and anti-biofilm activity of licorice extracts against *Streptococcus mutans*

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*Streptococcus mutans* is an important bacteria in oral hygiene because it makes the biofilm called plaque. This biofilm provides resistance to cells from various stresses which are processes which remove microbes from the mouth. Therefore, the existence of *S. mutans* in the biofilm causes persistent infection and diseases in the mouth. In previous studies, we found that licorice strongly inhibited the cell growth and the biofilm formation of *S. mutans*. In this study, the ethanol concentration for licorice extraction was investigated to maximize the antibacterial and anti-biofilm activity against *S. mutans*. After extraction, ethanol was removed by evaporation and the remaining extracts were dissolved in water to mimic the aqueous condition of the mouth. Interestingly, the extracts made with 60 ~ 70% ethanol showed the strongest antibacterial and anti-biofilm activity against *S. mutans* but the extracts made with pure water and pure ethanol had no activity. Because the difference in physical and chemical characteristics between 60% ethanol and water and between 60% ethanol and ethanol is minor, this result suggested that a well-controlled extraction process was required for antimicrobial and anti-biofilm activity of licorice.
Early detection of *Ganoderma* disease on oil palm seedlings via *Ganoderma boninense* microbial volatile organic compounds (MVOCs) biomarkers

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*Ganoderma boninense* is a white rot fungus that has been the major phytopathogen causing *Ganoderma* disease to oil palm trees in Malaysia. Thus causing tremendous yield decrease resulting in devastating economic losses to the country’s oil palm industry yearly. The disease was previously identified as basal stem rot disease and it progresses via root infection. However, an early detection method could sustain the economic life span of the palm. Therefore, the objectives of this study were i) to profile the microbial volatile organic compounds (MVOCs) of *G. boninense* and ii) to detect the potential MVOC markers at an early stage of infection in oil palm seedlings. Metabolite extraction using methanol and Gas Chromatography-Mass Spectrometry (GC-MS) were employed to achieve the first objective. Fifty-nine compounds were detected and identified from pure dikaryotic culture of the pathogenic *G. boninense* isolated from its basidiocarp. The compounds comprised of alkaloids, fatty acids, terpenoids, phenolics, sterols, flavonoid, carboxylic acids, ascorbic acids, and citric acids. Additionally, to achieve the second objective, 4 months old oil palm commercial variety (Dura × Pisifera) seedlings was inoculated with *G. boninense* and incubated in a *in vivo* nursery trial for 28 days and the total roots harvested were subjected to methanolic extraction prior to GC-MS analysis. After identification of the volatile compounds, univariate and multivariate data analyses, the potential MVOC biomarkers specific for *G. boninense* were highlighted being kojic acid and pyrimidine. This study suggest, the identified MVOC biomarkers ought to be applied in detecting *G. boninense* inoculums present in the oil palm fields and on healthy palms adjacent to the diseased palms via electric nose device or GC-MS.

Adding Value to Flex Crops: Engineering of Terpene Specialized Metabolism

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Terpenes are the largest class of specialized metabolites in plants, with diverse roles including plant defense and adaptation. Commercially relevant industrial targets have broad applications as nutraceuticals, pigments, flavors and fragrance molecules, polymers, and drugs. However, access is limited due to cumbersome purification from the plant source, or complicated chemical synthesis. In addition to emerging microbial platforms, engineering plants using Synthetic Biology approaches for bio-production of terpenes holds the potential for autotrophic industrial production. In plants, terpenes are generally synthesized and stored in specialized cell types or structures, such as glandular trichomes, resin ducts or laticifer cells. It has recently been found that accumulation of forskolin, an important medicinal diterpenoid, is associated with oil-bodies in the root cork cells of *Coleus forskohlii*.1 It was also shown, that the lipid biosynthesis can be upregulated in engineered lines of the model grass *Brachypodium distachyon*.2 The aim of our work, co-engineering of plants with pathways for both lipids and terpenoids, may result in improved capacity of production of terpenoids in the lipid environment and pave the way for generating flex crops.

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