PLANT SYNTHETIC BBB

Biology+Bioengineering+Biotechnology

2nd International Conference on Plant Synthetic Biology, Bioengineering, and Biotechnology

NOVEMBER 29 - DECEMBER 1, 2018

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TIPS FOR A SUCCESSFUL MEETING

Say **hello** to everyone.
You might make someone's day.

Introduce yourself to people you don't know.
They may be your next good friends.

Stop and **smile**.
You will brighten the room considerably.

Be **understanding**.
Everybody makes mistakes.

Help those with less experience.
We were all novices at some point.

Respect others.
We all have something valuable to contribute.

Value staff and volunteers.
They are here for you.

Be **kind**.
You will never like everybody, but you can be cordial to all.

Enjoy the meeting!
You can have fun while sharing,learning and networking.

Abstracts appear as submitted by their authors. Neither the American Institute of Chemical Engineers (AIChE) and its entities, nor the employers affiliated with the authors or presenting speakers, are responsible for the content of the abstracts.

GREETINGS!

We are very pleased to welcome all of you to the 2nd International Conference on Plant Synthetic Biology, Bioengineering, and Biotechnology (PSBBB) Conference in the beautiful Clearwater Beach, FL November 29th – December 1st, 2018.

The field of Plant Synthetic Biology has emerged into an important topic that has changed society as a whole with biofuels research, biopharmaceuticals, and medically effective secondary metabolites such as vaccines, antibodies, and metabolites. These topics will be discussed, as well as the following: Tissue Culture and Transformation of Recalcitrant Species, Transgenic Approaches for Improving Traits and Performance of Crops, Gene-Editing Tools, Engineering of the Chloroplast Genome, and Synthetic Biology Tools.

We are excited to host this conference with many great speakers. You will have the opportunity to hear from our keynote speakers: Poul Erik Jensen (University of Copenhagen) as well as Johnathan Napier (Rothamsted Research), and Christopher Voigt (Massachusetts Institute of Technology). A series of 11 invited speakers will certainly make this conference a success. Invited speakers include Scott Betts, Kim Boutillier, Henry Daniell, Pal Maliga, Bill Gordon-Kamm, June Medford, Donald Ort, Christian Rogers, John Shanklin, Patrick Shih, Qiudeng Que.

In addition to the plenary speaker talks, we hope that the reception and poster session will provide you with an opportunity to network and collaborate with new peers.

Finally, we would like to thank you for attending the conference, and hope that the program will be pleasant, educational, and inspiring.

Sincerely,

Fredy Altpeter
Conference Chair

Henrik Scheller
Conference Chair

CONFERENCE ORGANIZERS

Conference Co-Chairs

Fredy Altpeter, *University of Florida*

Henrik Scheller, *Joint BioEnergy Institute*

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Wayne Parrott, *University of Georgia*

June Medford, *Colorado State University*

Birger Lindberg Møller, *University of Copenhagen*

Nicola Patron, *Earlham Institute*

Neal Stewart, *University of Tennessee*

Yinong Yang, *Penn State University*

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THURSDAY, NOVEMBER 29

7:00 AM - 12:00 PM	Registration
8:00 AM - 8:15 AM	Welcoming Remarks: **Henrik V. Scheller**, *Joint BioEnergy Institute*, **Fredy Altpeter**, *University of Florida*
8:15 AM - 9:00 AM	KEYNOTE SPEAKER: Combining Parts: Coupling Photosynthetic Electron Transport to Metabolic Engineering **Poul Erik Jensen**, *University of Copenhagen*
9:00 AM - 9:30 AM	Coffee Break
9:30 AM - 10:45 AM	**SESSION 1: TISSUE CULTURE AND TRANSFORMATION OF RECALCITRANT SPECIES**
9:30 AM - 10:00 AM	INVITED SPEAKER: New Tools for an Old Problem **Kim Boutilier**, *Wageningen University & Research*
10:00 AM - 10:30 AM	INVITED SPEAKER: Maize Transformation At Pioneer – Meeting Future Demands For Genome Modification **Bill Gordon-Kamm**, *Corteva*
10:30 AM - 10:45 AM	Identifying the Genetic Basis of Transformation Efficiency in Barley **Beata Orman-Ligeza**, *NIAB*
10:45 AM - 4:30 PM	**SESSION 2: SYNTHETIC BIOLOGY: TOOLS AND APPLICATIONS**
10:45 AM - 11:15 AM	INVITED SPEAKER: Quantitative and Predictive Parts for Plant Synthetic Biology **June Medford**, *Colorado State University*
11:15 AM - 11:30 AM	Robotic plant protoplast manipulation **Neal Stewart**, *University of Tennessee*
11:30 AM - 11:45 PM	Improved Plant Synthetic Biology Using a Versatile and Robust Agrobacterium-Based Gene Stacking System **Roger Thilmony**, *USDA-ARS*
11:45 AM - 12:00 PM	Reprogramming Regulation **Nicola Patron**, *Earlham Institute*
12:00 PM - 1:30 PM	**LUNCH**
1:30 PM - 1:45 PM	Engineering Root Architecture with New Tools for Controlling Gene Expression in Plants **Jennifer A.N. Brophy**, *Carnegie Institution of Washington, Stanford, CA*
1:45 PM - 2:00 PM	Applying Digital Signatures to DNA for Advanced Quality Control and Security **Jenna Gallegos**, *Colorado State University*
2:00 PM - 2:15 PM	Reprogramming Plants Using Synthetic Signalling Systems **Arjun Khakhar**, *University of Minnesota*
2:15 PM - 2:30 PM	Development of Novel Optogenetic Tools By Continuous Directed Evolution of Plant Blue-Light-Dependent Interactions **Huachun Liu**, *University of California, Los Angeles*
2:30 PM - 3:00 PM	**BREAK**
3:00 PM - 3:30 PM	INVITED SPEAKER: Engineering a nitrogen-fixing symbiosis to create self-fertilizing cereals **Christian Rogers**, *Sainsbury Laboratory, University of Cambridge*
3:30 PM - 3:45 PM	In silico Model for Mining the Cis-Regulatory Determinants of Tissue-Specific Gene Expression **Molly Megraw**, *Oregon State*
3:45 PM - 4:00 PM	Transcriptional Rewiring: Editing Plant Stress Response Networks **Oliver Windram**, *London, United Kingdom*
4:00 PM - 4:15 PM	Parts-Prospecting for the Methanol Economy: Yhbo and Formaldehyde Resilience **Jenelle A. Patterson**, *University of Florida*
4:15 PM - 6:00 PM	**Networking Reception and Poster Session**
FREE EVENING	FREE EVENING

FRIDAY, NOVEMBER 30

7:00 AM - 12:00 PM	Registration
8:15 AM - 9:00 AM	KEYNOTE SPEAKER: Reverse Engineering Of The Omega-3 Lc-Pufa Pathway In Transgenic Plants - How To Make A More Nutritious Vegetable Oil **Johnathan Napier**, *Rothamsted Research*
9:00 AM - 9:30 AM	Coffee Break
9:30 AM - 12:00 PM	**SESSION 3: PLANT BIOFOUNDRY AND METABOLIC ENGINEERING**
9:30 AM - 10:00 AM	INVITED SPEAKER: Engineering the Composition and Content of Plant Oils **John Shanklin**, *Brookhaven National Laboratory*
10:00 AM - 10:15 AM	Synthetic Design of New Plant Oil Traits for Oilseed Crops As Sustainable and High-Quality Feedstock **Haejin Kim**, *University of Nebraska*
10:15 AM - 10:45 AM	INVITED SPEAKER: Production of the platform chemical muconic acid in plant biomass **Aymerick Eudes**, *Joint BioEnergy Institute*
10:45 AM - 11:00 AM	Model-Guided Metabolic Engineering of Increased 2-Phenylethanol Production in Plants **John A. Morgan**, *Purdue University*
11:00 AM - 11:30 AM	INVITED SPEAKER: Redesigning plant phytoalexin biosynthesis **Patrick Shih**, *University of California, Davis*
11:30 AM - 11:45 AM	Molecular Farming of Spider Silk Analogues for Regenerative Medicine **Congyue A. Peng**, *Clemson University*
11:45 AM - 12:15 PM	INVITED SPEAKER: From Discovery to Market: Fermentation for Ingredients and Natural Products **Matthew Mattozzi**, *Conagen, Inc.*
12:15 PM - 1:45 PM	**LUNCH**
1:45 PM - 3:00 PM	**SESSION 4: GENE-EDITING: FROM REFINED TOOLS TO CROP IMPROVEMENT**
1:45 PM - 2:15 PM	INVITED SPEAKER: Multi-allelic Precision Editing of the Complex Sugarcane Genome by Homology Directed Repair of CRISPR/Cas9 Induced DNA Breaks **Tufan Oz**, *University of Florida*
2:15 PM - 2:45 PM	INVITED SPEAKER: Gene Editing Across Wide Genetic Background via Haploid Induction **Qiudeng Que**, *Syngenta*
2:45 PM - 3:00 PM	Enhancing Photosynthesis, Vitamin E Production and Tolerance to Herbicides By Targeting Homogentisate Catabolism Using CRISPR/Cas9 Genome Editing System in Soybean **Cuong Nguyen**, *University of Missouri*
3:00 PM - 3:30 PM	**BREAK**
3:30 PM - 4:45 PM	**SESSION 5: ENGINERING OF THE CHLOROPLAST GENOME**
3:30 PM - 4:00 PM	INVITED SPEAKER: Advances in Synthetic Biology Approaches for Production of High Value Products in Chloroplasts **Henry Daniell**, *University of Pennsylvania*
4:00 PM - 4:30 PM	INVITED SPEAKER: Engineered RNA-Binding Protein for Gene Regulation in Non-Green Plastids **Pal Maliga**, *Rutgers University*
4:30 PM - 4:45 PM	Engineering the Chloroplast Genome with Synthetic Biology Tools for Microcompartment Construction **Vishalsingh Chaudhari**, *Cornell University*
FREE EVENING	FREE EVENING

SATURDAY, DECEMBER 1

7:00 AM - 12:00 PM	Registration
8:30 AM - 9:15 AM	KEYNOTE SPEAKER: Sensing and Computing in Plant-Associated Microbes **Chris Voigt**, *Massachusetts Institute of Technology*
9:15 AM - 9:45 AM	**Coffee Break**
9:45 AM - 12:00 PM	**SESSION 6: TRANSGENIC APPROACHES FOR IMPROVING TRAITS AND PERFORMANCE OF CROPS**
9:45 AM - 10:15 AM	INVITED SPEAKER: Optimizing Photorespiration for Improved Crop Productivity **Donald Ort**, *University of Illinois*
10:15 AM - 10:45 AM	INVITED SPEAKER: Consistent Transgene Expression with Minor Position Effects in Maize and Soybean **Scott Betts**, *Corteva*
10:45 AM - 11:00 AM	Directed Evolution of Energy-Efficient Non-Suicidal THi4s - a Next-Gen Synbio Strategy **Jaya Joshi**, *University of Florida*
11:00 AM - 11:15 AM	The GRAIN Platform: Identification of Gene Targets for Improving Crop Yield **Frank A. Skraly**, *Yield 10*
11:00 AM - 11:15 AM	Genetic Engineering for Enhancing Abiotic Stress Tolerance in Canola (Brassica napus) **Prem Bhalla**, *The University of Melbourne*
11:15 AM - 12:00 PM	Poster Awards & Closing Remarks

1. **Functional Analyses of Ovate-Interacting Trms in Tomato**
   Biyao Zhang[1], *Qiang Li*[2], *Neda Keyhaninejad*[2], *and Esther van der Knaap*[2]
   (1)Horticulure, University of Georgia, Athens, GA, (2) University of Georgia, Athens, GA

2. **Increasing Gene Editing Efficiency in Citrus through Transient Expression of CRISPR-Cas9 Proteins and Heat Treatments**
   Saroj Parajuli[1], *Huo Heqiang*[2], *and Zhanao Deng*[1]
   (1)IFAS-GCREC, University of Florida, Wimauma, FL, (2)IFAS-MFREC, University of Florida, Apopka, FL

3. **Identifying the Genetic Basis of Transformation Efficiency in Barley**
   Beata Orman-Ligeza[1], *Nicole Schatlowski*[1], *Wendy Harwood*[2], *Alison Hinchliffe*[2], *Cong Tan*[3], *Malcolm Macaulay*[3], *Pete Hedley*[3], *and Kay Trafford*[1]
   (1)Genetics and Breeding, NIAB, Cambridge, United Kingdom, (2)The John Innes Centre, Norwich, United Kingdom, (3)The James Hutton Institute, Invergowrie, United Kingdom

4. **Editing SWEET Genes for Understanding Their Roles in Citrus Susceptibility to Huanglongbing Disease**
   Saroj Parajuli[1], *Huo Heqiang*[2], *Yi Li*[3], *Fred G. Gmitter*[4], *and Zhanao Deng*[1]
   (1)IFAS-GCREC, University of Florida, Wimauma, FL, (2)IFAS-MFREC, University of Florida, Apopka, FL, (3)Department of Plant Science, University of Connecticut, Storrs, CT, (4) IFAS-CREC, University of Florida, Lake Alfred, FL

5. **Metabolic Engineering for Production of Lipids in Vegetative Sugarcane Biomass**
   Saroj Parajuli[1], *Baskaran Kannan*[2,3], *Ratna Karan*[3], *Hui Liu*[4,5], *Eva Garcia-Ruiz*[6], *Deepak Kumar*[7], *Vijay Singh*[7,8], *Huimin Zhao*[6,8], *Steve Long*[8,9], *John Shanklin*[4,5], *and Fredy Altpeter*[2,3]
   (1)IFAS-GCREC, University of Florida - IFAS, Wimauma, FL, (2)DOE Center for Advanced Bioenergy and Bioproducts Innovation, Gainesville, FL, (3)Agronomy Department, University of Florida - IFAS, Gainesville, FL, (4)Biosciences Department, Brookhaven National Lab, Upton, NY, (5) DOE Center for Advanced Bioenergy and Bioproducts Innovation, Upton, NY, (6)Department of Chemical and Biomolecular Engineering, University of Illinois, Urbana, IL, (7)Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, (8) DOE Center for Advanced Bioenergy and Bioproducts Innovation, Urbana, IL, (9)Institute for Genomic Biology, University of Illinois, Urbana-Champaign, Urbana, IL

6. **Engineering Plant-Microbe Communication for Environmental Sensors**
   Tyler Toth, *Biological Engineering, MIT, Cambridge, MA*

7. **Overexpression of Rice *Cullin* Gene Confers Salt Tolerance in the Salt-Sensitive Arabidopsis Mutant Lacking *cul3a***
   Chakkree Lekklar[1], *Toshiro Ito*[2], *Supachitra Chadchawan*[3], *and* ***Teerapong Buaboocha***[1]
   (1)Dept. of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, (2)GraduateSchool of Biological Science, Nara Institute of Science and Technology, Nara, Japan, (3)Dept. of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

8. **Rapid Prototyping of Constructs in a Chloroplast Cell-Free System for Plastid Engineering**
   Eszter Majer[1], *Lauren G. Clark*[2], *Michael C. Jewett*[3], *and Christopher A. Voigt*[4]
   (1)Biological Engineering, MIT, Cambridge, MA, (2) Department of Chemical and Biological Engineering, Northwestern University, (3)Chemical and Biological Engineering, Northwestern University, Evanston, IL, (4) Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA

9. **Synthetic Promoter Development for Drought Stress Response in Hybrid Poplar**
   Yongil Yang[1,2], *Junhyung Lee*[2], *Yuanhua Shao*[2], *Jake Massingill*[1], *and Neal Stewart*[1,2]
   (1)Center for Agricultural Synthetic Biology, Knoxville, TN, (2)Department of Plant Sciences, The University of Tennessee, Knoxville, Knoxville, TN

10. **Quantitative and Genome-Wide Analysis of a Transcription Factor BZR1 Interactions with Its Partner Transcription Factors and Target Genes**
    Jiaying Zhu[1] *and Zhiyong Wang*[2]
    (1)Plant Biology, Carnegie Institution for Science, Stanford, CA, (2)Plant Biology, Carnegie institution for Science, Stanford, CA

11. **Processing Genome Edited, Lignin-Modified Sugarcane Biomass with a Xylose Fermenting Yeast Strain Drastically Elevates Biofuel Production**
    Baskaran Kannan[1,2], *Je Hyeong Jung*[3], *Ja Kyong Ko*[4], *Ha Eun Kim*[5], *Kyoung Heon Kim*[5], *Hal Alper*[6], *Youngsoon Um*[4], *Sun-Mi Lee*[4,7], *and Fredy Altpeter*[1,2]
    (1)Agronomy Department, University of Florida - IFAS, Gainesville, FL, (2)DOE Center for Advanced Bioenergy and Bioproducts Innovation, Gainesville, FL, (3)Center for Natural Products Convergence Research, Korea Institute of Science and Technology, Gangneung, Korea, Republic of (South), (4)Clean Energy Research Center, Korea Institute of Science and Technology, Seoul, Korea, Republic of (South), (5)Department of Biotechnology, Korea University Graduate School, Seoul, Korea, Republic of (South), (6)Department of Chemical Engineering, The University of Texas at Austin, Austin, TX, (7)Clean Energy and Chemical Engineering, Korea University of Science and Technology, Daejeon, Korea, Republic of (South)

12. **Altering Chloroplast Size in Sugarcane By RNAi Suppression of FtsZ, Is Bigger Better?**
Baskaran Kannan*[1,2]*, *Aleel Grennan*[3,4] *Xiaoguo Zhang*[1]*, Jae Yoon Kim*[5] *Donald Ort*[3,6]*, and Fredy Altpeter*[1,2]
(1)Agronomy Department, University of Florida - IFAS, Gainesville, FL, (2)DOE Center for Advanced Bioenergy and Bioproducts Innovation, Gainesville, FL, (3)Institute for Genomic Biology, University of Illinois, Urbana, IL, (4)Dept. of Biology, Worcester State University, Worcester, MA, (5) Division of Biotechnology, Korea University, Seongbuk-Gu, Korea, Republic of (South), (6)DOE Center for Advanced Bioenergy and Bioproducts Innovation, Urbana, IL

13. **Overexpression of *OsTF1L*, a Rice HD-Zip Transcription Factor, Promotes Lignin Biosynthesis and Stomatal Closure That Improves Drought Tolerance**
Ju-Kon Kim
International Agricultural Technology, Seoul National University, Pyeongchang, Korea, Republic of (South)

14. **Ethephon Induced Alkaloid Production in Two Varieties of the Medicinal Plant *Catharanthus Roseus***
Valerie N. Fraser[1,2]*, Benjamin J. Philmus*[3]*, and Molly Megraw*[1]
(1)Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, (2)Molecular and Cellular Biology Graduate Program, Oregon State University, Corvallis, OR, (3) College of Pharmacy, Oregon State University, Corvallis, OR

15. **Particle Bombardment for Transient Gene Expression in Citrus Plant Cells Using the Helios® Gene Gun**
Yosvanis Acanda Artiga*, Chunxia Wang, and Amit Levy*
Plant Pathology, University of Florida, Lake Alfred, FL

16. ***LOC_Os01g68450* gene Increases Salt Tolerance By Photosynthesis Process Enhancement**
Panita Chutimanukul[1]*, Teerapong Buaboocha*[2]*, Aleel Grennan*[3]*, and* ***Supachitra Chadchawan***[4]
(1)Dept. of Botany, Chulalongkorn University, Bangkok, Thailand, (2)Dept. of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, (3)Dept. of Biology, Worcester State University, Worcester, MA, (4)Dept. of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

KEYNOTE SPEAKER BIOGRAPHIES

Poul Erik Jensen
University of Copenhagen, Center for Synthetic Biology

Poul Erik Jensen's group has a strong expertise within photosynthesis research focusing on photosystem I (PSI) and chloroplast biology. The ability to manipulate photosynthetic chloroplasts in order to tap into the light harvesting system of plants and to utilize this power for the production of valuable bioactive natural products is of great interest both for academia and industry due to the potential of an efficient biosynthetic production of e.g. anti-cancer pharmaceuticals.

His group identified genes for subunits of the PSI holocomplex and has elucidated the function of these subunits at the molecular and physiological level. Transgenic plants lacking individual subunits constitute important experimental tools. Current topics include structure and function of the photosynthetic complexes and their response to abiotic stresses such as nutrient limitation. Besides nuclear and chloroplast transformation and a wealth of biochemical tools, optic methods to measure photosynthetic partial reactions have been established.

One of his main focus points is manipulation of the photosynthetic chloroplasts which provides a platform to engineer new pathways and establish bio-factories which synthesize desired industry products for pharmaceuticals, fine chemicals, or fuels. Especially terpenoids are of interest due to their pharmacological properties, including anti-cancer activity. However, their availability is generally very limited and organic synthesis is often not feasible. By re-routing biosynthetic pathways and optimizing the chain of energy transfer we can overcome the inherent limitations in plants to channel photosynthetic fixed carbon and the light-excited electrons directly into production of such desired bioactive natural products.

Johnathan Napier
Rothamsted Research

Johnathan grew up in rural County Down, Northern Ireland. He studied Agricultural Sciences at the University of Nottingham and obtained his PhD from King's College, London. He carried out post-doctoral research at the University of Cambridge and then took a position at Long Ashton Research Station in Bristol. He relocated to Rothamsted Research in 2003 where he is currently Associate Director. Johnathan is also an Affiliated Lecturer at the University of Cambridge and Visiting Professor at the University of Nottingham, who also awarded him a DSc in 2006. He has published over 150 peer-reviewed papers, and is the inventor on multiple patents relating to the biotechnology of lipid metabolism. He is currently running the only GM field trial in the UK, evaluating the performance of plants engineered to accumulate omega-3 fish oils.

Christopher Voigt
Massachusetts Institute of Technology

Professor Voigt obtained his Bachelor's degree in Chemical Engineering at the University of Michigan, Ann Arbor and a PhD in Biochemistry and Biophysics at the California Institute of Technology. He continued his postdoctoral research in Bioengineering at the University of California, Berkeley. His academic career commenced as an Assistant and Associate Professor at the Department of Pharmaceutical Chemistry at the University of California-San Francisco. Professor Voigt joined the Department of Biological Engineering at Massacusetts Institute of Technology as Associate Professor in 2011 and is now the Daniel I. C. Wang Professor of Advanced Biotechnology.

INVITED SPEAKER BIOGRAPHIES

Scott Betts
Corteva Agriscience

Scott is a research program leader for genome modification at Corteva Agriscience™, Agriculture Division of DowDuPont™. He received his BA from Reed College and his PhD in Cell and Molecular Biology from The University of Michigan. After completing postdoctoral research in the Department of Biology at MIT, Scott joined Syngenta Biotechnology in 1999 serving as a research scientist, team leader, and project leader in Enzyme Traits and Agronomic Traits. While at Syngenta, he led teams that contributed to the development of Quantum Phytase™ and Enogen™ corn. Between Syngenta and joining DuPont Pioneer in 2012, Scott was a senior scientist and senior manager in the Agriculture Division of Intrexon and co-founded Benson Hill Biosystems.

Kim Boutilier
Wageningen University & Research

Kim Boutilier is a scientist in the Plant Developmental Systems group at Wageningen University and Research. The research in her group is focused on understanding the molecular basis of cell totipotency in plant tissue culture. The group uses a wide range of techniques, including high-throughput genomics, chemical biology, cell biology, and molecular-genetic analysis, with an emphasis on transcription factor function, to identify the signal transduction pathways that instruct differentiated cells to develop into embryos in vitro. This fundamental knowledge is also used to find practical solutions for the major bottlenecks encountered during plant propagation through tissue culture. The current topics in the group include the intersection between auxin and stress during in vitro embryo induction, transient transcription factor-mediated regeneration, and BABY BOOM signalling pathways.

KEYNOTE + INVITED SPEAKER **BIOGRAPHIES**

Henry Daniell
University of Pennsylvania

Dr. Henry Daniell is Professor and Director of Translational Research at the University of Pennsylvania. He is the Fellow of the American Association for the Advancement of Science and a foreign member of the Italian National Academy of Sciences (14th American to be inducted in the past 240 years) and the Editor in Chief of the Plant Biotechnology Journal, Oxford, UK. He is recipient of several awards including the American Diabetes Association Award, American Heart Association, Bayer Hemophilia global award and Bill and Melinda Gates Foundation Award for his outstanding contributions. He pioneered chloroplast genetic engineering as a new platform to produce and orally deliver low cost vaccines and biopharmaceuticals bioencapsulated in plant cells. Ranked by Nature Biotechnology among the top ten inventions of the past decade and among Biomed Central's Hot 100 authors in the globe, he has >100 published or granted global patents and >250 scientific publications.

Aymerick Eudes
Lawrence Berkeley National Laboratory

Aymerick Eudes, PhD, is a Research Scientist at the Lawrence Berkeley National Laboratory (LBNL), and Deputy Director of Plant Cell Wall Biology & Engineering at the Joint BioEnergy Institute (JBEI). He received his PhD from the University of Paris XI, France, where he worked on identifying genes involved in lignin biosynthesis. He was previously a postdoc with Prof. Andrew Hanson (University of Florida), studying folate metabolism in plants, and with Dominique Loque (JBEI), developing new engineering approaches for improving biomass for bioenergy applications. At JBEI, Dr Eudes is currently developing strategies for lignin manipulation and biomass valorization.

Bill Gordon-Kamm
DuPont Pioneer

Bill has worked in industrial biotechnology since 1987, first at DeKalb/Pfizer Genetics from which he and his colleagues reported on the successful production of fertile transgenic maize plants in 1990. After moving to Pioneer Hi-Bred in 1993 his lab has continued to focus on improving transformation of recalcitrant maize inbreds, first in the area of cell cycle stimulation to provide a positive growth advantage, and later working with morphogenic genes such as LEC1, WUS and BBM. As part of a growing team of researchers, recent progress with morphogenic genes has resulted in greatly increasing the efficiency and speed in the transformation process, extending transformation methods to many previously difficult-to-transform monocot crop varieties, and opening up new cell or tissue explants such as leaves for monocot transformation.

Pal Maliga
Rutgers University

Pal Maliga, Ph.D. received a MA degree (Genetics and Microbiology, 1969) from Eotvos Lorand University, Budapest, and a PhD (Genetics, Microbiology, 1972) from Jozsef Attila University, Szeged, Hungary. From 1971 to 1982 Dr. Maliga held appointments at the Biological Research Center, Szeged, Hungary, where his research group pioneered organellar genetics in cultured tobacco cells. Dr. Maliga spent the year of 1982 at Washington University, St Louis, MO, and between 1983-1989 served as Research Director for Advanced Genetics Sciences, a biotech start-up in Manhattan, KS, and Oakland, CA. Since joining Rutgers in 1989, Prof. Maliga developed the technology of plastid transformation in the tobacco model system and pioneered applications to basic research on plastid function and to chloroplast biotechnology. He was Chair of the Plant Cell and Tissue Culture Gordon Research Conference (1995) and Founding Chair of the Chloroplast Biotechnology Gordon Research Conference (2015). He is the recipient of the Thomas Alva Edison Patent Award, The Research and Development Council of New Jersey (1999) and was elected Inventor of the Year by The New Jersey Inventors Hall of Fame (2011). Dr. Maliga is External Member of the Hungarian Academy of Sciences (2001) and Doctor Honoris Causa, University of Debrecen, Debrecen, Hungary (2006). His seminal contributions to creating the field of chloroplast biotechnology was recognized by the Lawrence Bogorad Award for Excellence in Plant Biology (2016), American Society of Plant Biologists, and his election Fellow of The American Association for the Advancement of Science (AAAS) (2016).

Matthew Mattozzi
Conagen, Inc.

Matthew Mattozzi, Ph.D. is the Manager of Scientific Operations at Conagen Inc., a research, development, and fermentation scale-up company based in Bedford, MA. He leads technology scouting, licensing and partnership activities. Prior to his work at Conagen, he worked as a scientist with expertise in reprogramming E. coli, yeast, plant, soil bacteria, and cyanobacterial metabolism. He is the founder of two startup companies, a lecturer in biomolecular engineering, the author of over ten peer-reviewed scientific publications and listed as an inventor on four patents. He obtained his B.S. from Harvey Mudd College, his Ph.D. at UC Berkeley with Jay Keasling at the Joint BioEnergy Institute, and his postdoctoral training at Harvard with Pam Silver at the Wyss Institute for Biologically Inspired Engineering. He serves on the editorial board of several scientific journals and is a panel reviewer for the DOE and NSF.

June Medford
Colorado State University

June Medford is a Professor of Biology at Colorado State University where she has been a faculty member since 1996. She completed her Ph.D. in 1986 at Yale University and did her postdoc with Monsanto's Plant Molecular Biology Group. She is also the founder and president of the biotechnology company Phytodetectors. A recognized world leader in plant synthetic biology, Dr. Medford is at the forefront in developing methodologies and applications for synthetic biology implemented in plants. She developed the first plant sentinels, plants transformed with the computationally designed ability to detect and respond to exogenous substances, as well as programmable genetic controllers and new technology using a synthetic desalination circuit. Dr. Medford is active in promoting the potential of plant synthetic biology to a wide audience of scientists and policy makers, and is a member of several scientific advisory boards and three editorial boards.

Donald Ort
University of Illinois

Deputy Director Donald Ort is the Robert Emerson Professor in Plant Biology and Crop Sciences at the University of Illinois. His research seeks to understand and improve plant growth and photosynthetic performance in changing environmental conditions, such as increasing CO2 temperature and drought. Don's research ranges from improving photosynthetic efficiency to the molecular and biochemical basis of environmental interactions with crop plants to ecological genomics. His research spans from the molecular to crop canopies in the field. Don earned his bachelor's degree in biology from Wake Forest University and his doctorate in plant biochemistry from Michigan State University. He has served as the president of the American Society of Plant Biologists, the International Society of Photosynthesis Research, and the International Association of Plant Physiology. He also served as editor-in-chief of Plant Physiology and is an associate editor of Annual Review of Plant Biology. Don has received numerous awards and recognitions, including election to the National Academy of Science and being named one of Thomson Reuters' Most Influential Scientific Minds. He has published over 250 peer-reviewed papers in journals that include Science.

Tufan Oz
University of Florida

Dr. Tufan Oz is a researcher at University of Florida. He obtained his PhD at METU Ankara, Turkey. During his graduate studies he worked on various aspects of plant molecular biology and biotechnology including gene cloning, expression profiling and genetic transformation. Before joining UF, he worked as a postdoctoral fellow at Palacky University Olomouc, Czech Republic, and as a researcher at ACPFG Adelaide, Australia. Since 2014, he has been working on genome editing using CRISPR/Cas9 in Dr. Fredy Altpeter's research group at the UF Agronomy Department. His research focuses on improvement of crops such as sugarcane and barley using genome editing and genetic transformation approaches.

Christian Rogers
Sainsbury Laboratory, University of Cambridge

Christian is the Scientific Programme Manager for the Bill & Melinda Gates Foundation-sponsored project Engineering the Nitrogen Symbiosis for Africa (ENSA). Led by the University of Cambridge, ENSA brings together ten institutions in a focused initiative to engineer a nitrogen-fixing symbiosis in cereals to increase yields for resource-poor farmers in sub-Saharan Africa. Christian leads the engineering technology team at the Sainsbury Laboratory Cambridge University (SLCU) which centralises ENSA's engineering activities in barley and maize. He also founded the Crop Engineering Consortium (CEC) which brings together the major Gates Foundation sponsored biotechnology projects ENSA and RIPE with scientists at the cutting edge of crop engineering technology and provides essential low-cost foundational platforms in gene synthesis, DNA assembly, plant transformation and NGS sequencing. Christian has worked extensively in Africa to establish capabilities in biotechnology by training scientists in Ghana, Uganda, Ethiopia and the BecA-ILRI hub in Kenya.

John Shanklin
Brookhaven National Laboratory

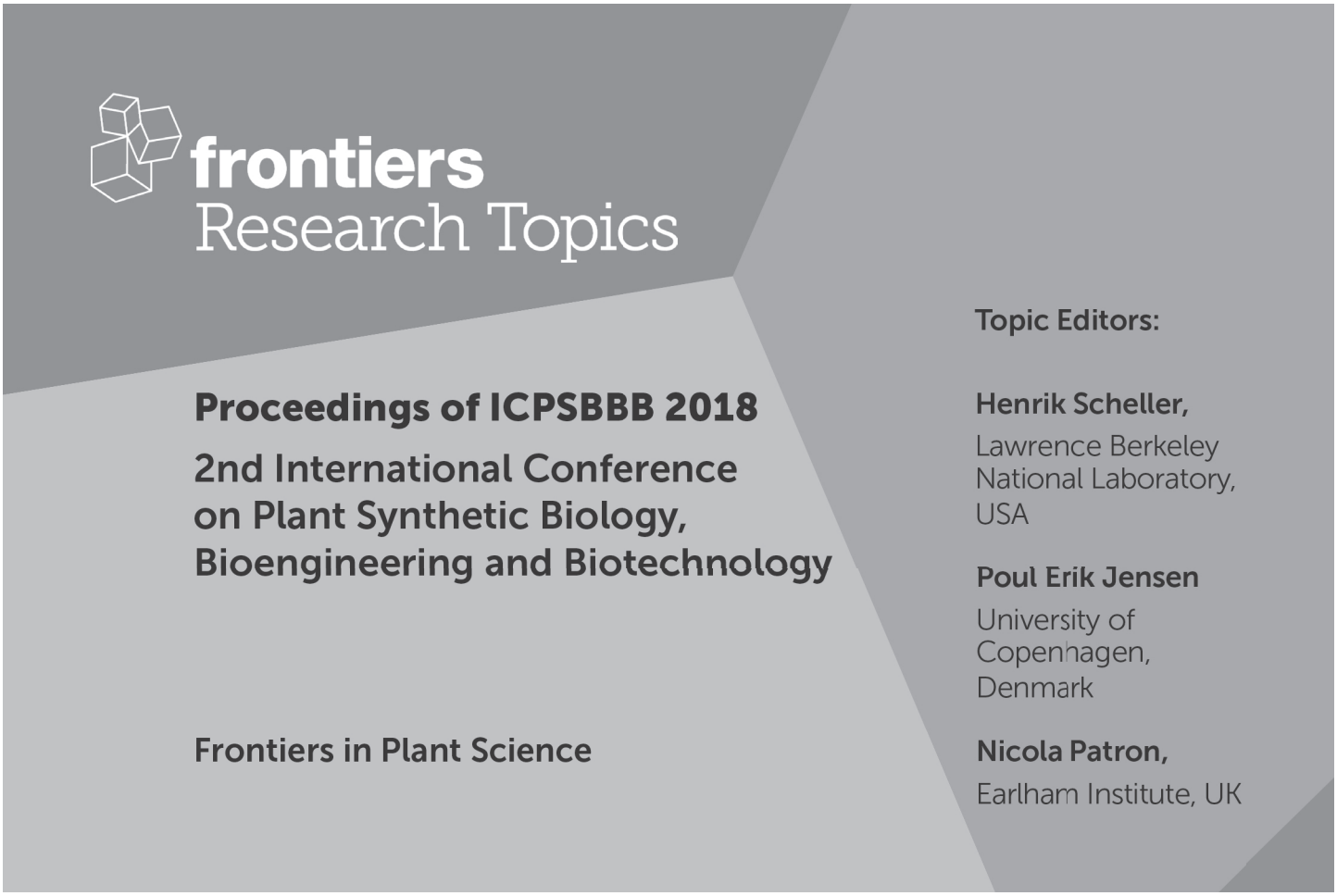
John Shanklin grew up near Manchester England. He moved to Wisconsin where he received a M.Sc. and Ph.D. working with Rick Vierstra on the biochemistry of selective protein turnover in plants. He moved to Michigan State's DOE Plant Research Laboratory for a Post Doc. with Chris Somerville where he became fascinated with plant lipid biochemistry and lipid modification enzymes. John established his own research program when he moved to BNL in 1992 as an Assistant Biochemist. John is the recipient of a Presidential Early Career Award, the Terry Galliard Medal for Plant Lipid Biochemistry and is an elected Fellow of the American Association for the Advancement of Science. John is now a Senior Biochemist and has served many roles at BNL including Group Leader for Plant Science, and is presently Chair of the Biology Department.

Patrick Shih
University of California, Davis

Patrick M. Shih, PhD, is an Assistant Professor at UC Davis, and Director of Plant Biosystems at the Joint BioEnergy Institute. He has a BS in microbiology and a BA in political science from the University of California, San Diego. He received his PhD from the Univ. of California, Berkeley, where he worked on engineering synthetic carbon fixation pathways into photosynthetic organisms. He was previously a postdoc with Dominique Loque (JBEI), Jay Keasling (UC Berkeley/LBNL), and Beth Sattely (Stanford) developing plant synthetic biology tools for complex metabolic engineering efforts. Patrick received his PhD from UC Berkeley studying the evolution of photosynthesis with Kris Niyogi and Cheryl Kerfeld.

Qiudeng Que
Syngenta

Dr. Qiudeng Que is a senior group leader in Syngenta's Seeds Research Unit. His responsibilities include management of trait research pipeline operations and development of novel platform technologies including genome editing. His areas of expertise include genome editing, crop transformation, transgene gene expression and molecular analysis. He has worked on the development of genetic engineering technologies for various crops in the last 20 years. His team successfully developed efficient genome editing technologies in maize and soybean and applied them for trait researches. His team is currently focused on development of novel breeding tools and traits using a suite of cellular and molecular biology tools including conventional breeding, genome editing and transgenic technologies.



Thursday, November 29th

KEYNOTE SPEAKER

Combining Parts: Coupling Photosynthetic Electron Transport to Metabolic Engineering

Poul Erik Jensen
Copenhagen Plant Science Centre, Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

Photosynthesis drives the production of ATP and NADPH mainly used to fix CO_2. Excess of redox power can be exploited for production of high-value compounds. Important natural products are often synthesized in low quantities by their host organism and can be difficult to produce by chemical synthesis because of their complex chemical structures. The cytochromes P450 (P450s) situated in the endoplasmic reticulum play key roles in natural product biosynthesis, and are powered by electron transfers from NADPH. We have shown that plant P450s expressed in chloroplasts and cyanobacteria are directed to the thylakoid membrane[1,2]. Here, the photosynthetic electron transport will support P450 catalytic activity independent of NADPH and dedicated reductases. In order to route reducing power more efficiently to P450s, we have fused them with ferredoxin (Fd) or flavodoxin-like FMN domains[3]. These fusions allow the P450s to obtain electrons for catalysis directly from the photosynthetic electron transport chain by interacting with photosystem I and make them competitive with the natural occurring ferredoxin requiring enzymes. Further dedicated redirection of reducing power can be obtained by scaffolding all the enzymes of a pathway on the thylakoid membrane. In a novel strategy, we have fused enzymes with transmembrane domains of TatB and TatC from the chloroplast twin arginine translocation system[4]. This reduced the accumulation of unwanted intermediates, side products and increased the accumulation of the end product fivefold. This work shows that chloroplasts and cyanobacteria are attractive for metabolic engineering, and suggests unexplored potential for engineering of photosynthetic electron transfer chains to accommodate heterologous enzymes.

[1] Gnanasekaran, T et al. (2016) J. Exp. Bot. 67(8):2495-506.

[2] Wlodarczyka, A et al. (2016) Met. Engineering 33: 1-11.

[3] Mellor, S et al. (2016) ACS Chem. Biol. 11(7):1862-9.

[4] Henriques de Jesus et al. (2017). Met. Engineering 44: 108-116.

SESSION 1: TISSUE CULTURE AND TRANSFORMATION OF RECALCITRANT SPECIES

INVITED SPEAKER

New Tools for an Old Problem

Kim Boutilier
Plant Developmental Systems, Wageningen University and Research, Wageningen, Netherlands

Somatic and gametophytic embryogenesis are two examples of induced plant cell totipotency, where embryos develop from vegetative or gametophytic tissues, rather than from the zygote after fertilisation. These two types of *in vitro* embryogenesis form the basis for a number of plant breeding and biotechnology applications, including clonal propagation, doubled-haploid production and regeneration after transformation, but also offer good model systems for understanding how plant cells are reprogrammed to follow a new developmental pathway. The classical tissue culture approach used to overcome recalcitrance for *in vitro* regeneration in crops is to identify empirically the explant and culture parameters that contribute to efficient production systems. Almost all of the major breakthroughs in plant tissue culture have been achieved in this way, and although successful, this approach is often time consuming and inefficient, as only a few parameters can be tested at one time. A molecular-genetic approach is often used to identify the molecular pathways driving plant cell totipotency in model plant systems, but can be confounded by genetic redundancy, lethality, or pleiotropy.

Our lab is using a third approach, chemical screening, to overcome recalcitrance for *in vitro* embryogenesis in crops, and to better understand the mechanistic basis for induced totipotency. In this approach, individual compounds from commercial targeted and non-targeted compound libraries are screened for their ability to enhance embryogenesis, while keeping the tissue culture conditions constant. I will highlight the general concepts behind chemical screening and demonstate how we have used this approach to identify competence factors for gametophytic and somatic embryogenesis.

INVITED SPEAKER

Maize Transformation at Pioneer - Meeting Future Demands for Genome Modification

Bill Gordon-Kamm, *Keith Lowe, Hoerster George, Ning Wang, Emily Wu, Ajith Anand, Maren Arling, and Todd Jones*
Crop Genome Engineering, Corteva Agriscience Agricultural Division of DowDuPont, Johnston, IA

Although cereal transformation has continued to improve over the years, the process has generally remained constrained to a few genotypes per crop, and methods are still slow and labor intensive. The overall impact has been that cereal transformation, genome modifications and high-throughput transformation remain beyond the reach of almost all academic labs. Recent progress in our labs is rapidly changing this situation for cereals. By focusing on the overexpression of the maize Babyboom (BBM) and Wuschel2 (WUS2) genes, we can routinely produce high transformation frequencies in numerous previously non-transformable maize inbreds. Recent optimization of the promoters driving BBM and WUS2 expression has resulted in direct germination of somatic embryos to recover transgenic plants (eliminating all callus steps), making maize inbreds such as B73 and Mo17 easily transformable using Agrobacterium strain LBA4404. Of even greater import to genome modification methods, this process is largely genotype independent and regenerated plants can be sent to the greenhouse in less than half the time of conventional methods. Another limitation for many monocots is the intensive labor and greenhouse space required to supply immature embryos for transformation. As a new alternative to immature embryos, we use BBM and WUS2 to recover transgenic events directly from either embryo slices from mature seed or leaf segments from seedlings in a variety of Pioneer inbreds, routinely recovering healthy, fertile T0 plants. We will also describe a new system for Agrobacterium-mediated co-transformation that greatly simplifies the process from vector construction through analysis.

Identifying the Genetic Basis of Transformation Efficiency in Barley

Beata Orman-Ligeza[1], *Nicole Schatlowski*[1], *Wendy Harwood*[2], *Alison Hinchliffe*[2], *Cong Tan*[3], *Malcolm Macaulay*[3], *Pete Hedley*[3], *and Kay Trafford*[1]
(1)Genetics and Breeding, NIAB, Cambridge, United Kingdom, (2)The John Innes Centre, Norwich, United Kingdom, (3)The James Hutton Institute, Invergowrie, United Kingdom

Agrobacterium-mediated transformation requires the successful regeneration of plant material that is susceptible to transformation. In spite of exhaustive attempts, only a few barley cultivars are amenable to transformation and most labs use Golden Promise. The genetic factors underlying differences between barley cultivars in transformation efficiency are largely unknown. To improve the transformation efficiency of elite barley cultivars requires identification of these genetic factors.

Transgenic barley is usually obtained from calli induced from immature embryos after co-cultivation with *Agrobacterium*. We tested whether the large-embryo phenotype of a mutant of barley called *lys3* (*high-lysine 3*) would influence the performance of its embryos in tissue culture and affect transformation efficiency. To date, four independent *lys3* mutants have been described and they all have larger-than-normal-embryos. We tested the transformation efficiency of *lys3* mutant M1460 and found it to be similar to that of Golden Promise. Further studies suggested that the immature embryos of M1460 are particularly efficient at producing embryogenic callus.

To understand the genetic basis of M1460 transformability, we crossed M1460 to the elite UK cultivar Optic that is recalcitrant to transformation. Progeny plants from this cross and a subsequent back-cross to Optic were genotyped using the 50k barley SNP array. From this analysis, we identified two conserved genomic regions that might contribute to the high transformation efficiency of M1460. Additionally, after two generations of crosses to Optic, we found that the transformability trait from M1460 segregated away from the large-embryo phenotype. We also found that the other three *lys3* mutants with large embryos (RISØ 1508, RISØ 18, RISØ 19) have low transformation efficiencies. Together these data suggest that a locus other than Lys3 is responsible for the high transformation efficiency of M1460 and that this locus is likely to lie in one of the two genomic regions that we have identified.

SESSION 2: SYNTHETIC BIOLOGY: TOOLS AND APPLICATIONS

INVITED SPEAKER

Quantitative and Predictive Parts for Plant Synthetic Biology
June Medford
Colorado State University

Robotic Plant Protoplast Manipulation
Neal Stewart
University of Tennessee

A liquid handling robot with various features has been developed and is housed in the Center for Agricultural Synthetic Biology. The robot has been designed to start with plants tissues and end with fluorescence measurements using programmable automation. It produces protoplasts from various plant tissues, can transfect the protoplasts and incubate them for subsequent fluorescence measurements all in plate formats. Current results and near-future applications will be discussed.

Improved Plant Synthetic Biology Using a Versatile and Robust Agrobacterium-Based Gene Stacking System
Roger Thilmony
Crop Improvement and Genetics Research Unit, USDA-ARS, Albany, CA

Plant synthetic biology provides a means for the rapid genetic improvement of crops and will enable future improvements of complex traits like yield and nutritional quality through the introduction and coordinated expression of multiple genes. GAANTRY (Gene Assembly in *Agrobacterium* by Nucleic acid Transfer using Recombinase technologY) is a flexible and effective system for stably stacking multiple genes within an *Agrobacterium* virulence plasmid Transfer-DNA (T-DNA) (Collier et al. The Plant Journal 2018). The system utilizes unidirectional site-specific recombinases in vivo and an alternating selection scheme to sequentially assemble multiple genes into a single transformation construct. To demonstrate GAANTRY's capabilities, 10 cargo sequences were sequentially stacked together to produce a 28.5 kilobase pair T-DNA, which was used to generate hundreds of transgenic *Arabidopsis* events. Approximately 90% of the events identified using the dual antibiotic selection screen exhibited all of the introduced traits. A total of 68% of the tested lines carried a single copy of the selection marker transgene located near the T-DNA left border and only 8% contained sequence from outside the T-DNA. Research is also underway to demonstrate that the GAANTRY system can successfully genetically engineer rice, as well as other important crop species. Thus, GAANTRY is a powerful, yet simple to use, new tool for transgene stacking and plant synthetic biology.

Reprogramming Regulation
Nicola Patron
Earlham Institute

Engineering Root Architecture with New Tools for Controlling Gene Expression in Plants
Jennifer A.N. Brophy and Jose R. Dinneny
Plant Biology, Carnegie Institution of Washington, Stanford, CA

The shape of a plant's root system influences its ability to reach essential nutrients in the soil or to acquire water during drought. Progress in engineering plant roots to optimize water and nutrient acquisition has been limited by our capacity to design and build genetic programs that alter root growth in a predictable manner. We are building a library of synthetic transcription factors and using them to construct gene circuits that reprogram root development. The genetic circuits will be used to express mutant auxin-response transcription factors (AUX/IAAs) in specific root cell-types to alter root structure. In addition to synthetic gene circuits, we are constructing a platform for site specific integration of transgenes in Arabidopsis. This site specific integration platform should accelerate the development of genetic circuits in plants by reducing the variability in gene expression produced by random transgene integration and shortening the design-build-test cycle. We are currently testing methods of preventing random T-DNA integration after Agrobacterium-mediated DNA delivery. If successful and efficient, our platform will direct T-DNAs to integrate a specific location within the plant genome. This will accelerate the pace of plant research by generating clonal plant lines that can be phenotyped in the T1 generation.

Applying Digital Signatures to DNA for Advanced Quality Control and Security
Jenna Gallegos[1], *Diptendu Kar*[2], *Indrajit Ray*[2], *and Jean Peccoud*[1]
(1)Chemical and Biological Engineering, Colorado State University, Fort Collins, CO, (2)Computer Sciences, Colorado State University, Fort Collins, CO

There is a growing body of research concerned with traceability and identification of DNA sequences. For genetically modified organisms and other proprietary strains, the ability to carefully trace and authenticate sequences can help inventors assert their intellectual property, distance themselves from liabilities associated with variations of their invention and assist in obtaining regulatory approval. In cases where genetically modified sequences are part of an elaborate supply chain, such as the global food system, authenticity and traceability are especially important. It has been proposed that unique watermarks be inserted into the genomes of genetically modified organisms and infectious agents to improve traceability, but watermarks are independent of the data they are attached to and can be counterfeited. In the digital world, the problem of securing and authenticating the source and integrity of a document or web page is solved using encryption. Asymmetric encryption schemes using DNA sequences have

previously been proposed; we are working to significantly expand on this work by using digital signatures to encrypt DNA molecules in living cells. As a proof of concept, we have developed a system for applying digital signatures to plasmid DNA and are exploring options for extending this technology to plant and microbial genomes. The application of digital signatures to plant genomes could facilitate our ability to trace foods from farm to fork, enabling consumers to source sustainably grown food, expediting the origin of food pathogen outbreaks, and facilitating litigation over seed licensing, cross-pollination, and "escape" of organisms that have not been approved for environmental release.

Reprogramming Plants Using Synthetic Transcription Factors and Systemically Maintained RNA Scaffolds
Arjun Khakhar[1] *and Daniel Voytas*[2]
(1)Biology, University of Minnesota, Saint Paul, MN, (2) Department of Genetics, Cell Biology & Development and Center for Genome Engineering, University of Minnesota, Saint Paul, MN

Transcriptional programs sculpt plant morphology and metabolism to meet environmental challenges like droughts or pests. These same programs have been manipulated through selective breeding over generations to increase agricultural productivity and robustness. Studying the genetic basis of these improved traits has elucidated the specific alterations in the expression levels of key master regulator genes that lead to phenotypic improvement. However, breeding these traits into elite crop lines can be challenging for several reasons, including long timescales, linkage drag and hybrid incompatibility. Additionally, the reliance on natural variation for traits has not fully leveraged our understanding of plant biology for forward engineering of crops.

We aim to overcome these challenges by designing a flexible system of synthetic transcription factors to rapidly reprogram development and metabolism in an agriculturally relevant manner. Our system, called ViparíNama (Sanskrit: to change), uses RNA scaffolds to assemble transcription factors at loci of interest to modulate gene expression. We are designing these RNA scaffolds to be systemically transported and maintained in the plant through the use of parts from RNA viruses that have been optimized using a combination of next generation sequencing based screens and machine learning aided in-silico evolution. We plan to utilize this system to reprogram the metabolism of maize and create lines with enhanced production of beta-carotene and the natural insecticide DIMBOA. We are also using this system to create semi-dwarfed varieties of tomato by reprogramming gibberellin-driven developmental pathways in a tissue specific manner.

Development of Novel Optogenetic Tools By Continuous Directed Evolution of Plant Blue-Light-Dependent Interactions
Huachun Liu
Molecular Biology Institute, UCLA, Los Angeles, CA

Arabidopsis thaliana Cryptochrome2 (AtCRY2) is a blue-light receptor that mainly regulates plant photomorphogenesis through blue-light-specific interactions with numerous protein partners. Such blue-light-specific interactions have been exploited in optogenetics to manipulated biological events in a timely and precisely manner. AtCRY2-AtCIB1 photodimerizer is one of the best characterized protein pairs for optical control of protein interactions. However, the application of CRY2-CIB1 was impeded by concomitant homodimerization of AtCRY2, and limited dynamic range. To overcome the limitations, this study focused on the development of a novel pair of blue-light-dependent interacting proteins: AtCRY2-AtBIC1. AtBIC1 (Blue-light Inhibitor of Cryptochromes 1) was recently identified as an endogenous inhibitor of AtCRY2. BIC1 interacts with CRY2 and inhibits homodimerization of CRY2 in the blue light. Additionally, the size of BIC1 is very small (only 15 kDa), which could allow gene fusion with minimal impact. We used a novel continuous directed evolution method developed by David Liu's lab, PACE (Phage Assisted Continuous Evolution), to evolve AtCRY2 to increase its interaction affinity with BIC1 in the blue light. The PACE method coupled random mutagenesis with selection, enabling dozens of rounds of automatic evolution in a single day with minimal human efforts. Using the method, we successfully isolated mutants of CRY2 with stronger interactions with BIC1. To increase the dynamic range and signal-to-background ratio of CRY2-BIC1 dimerizer, a counter evolution method was proposed to reduce background in darkness. Our study could provide improved tools for optical control of protein interactions.

INVITED SPEAKER

Ensa: Engineering the Nitrogen Symbiosis for Africa
Christian Rogers
Sainsbury Laboratory, University of Cambridge, Cambridge, United Kingdom

The ENSA project brings together teams from ten institutions with the aim of engineering a nitrogen-fixing symbiosis in cereals for the benefit of small-holder farmers in sub-Saharan Africa. The ability to take up mineral nutrients, particularly nitrogen and phosphorus, is generally the major limitation to crop productivity in agricultural. Global food production is heavily dependent on the application of inorganic fertilisers to augment limiting nutrients. In the natural environment many plants enter symbiotic associations with microorganisms to facilitate nutrient acquisition. Early in evolution plants acquired the ability to associate with beneficial arbuscular mycorrhizal fungi, an association particularly important for the acquisition of phosphorus. Legumes have additionally evolved beneficial interactions with nitrogen-fixing bacteria, allowing direct access to atmospheric nitrogen as a source of nutrition. The nitrogen-fixing symbiosis evolved from the pre-existing fungal symbiosis,

with many shared aspects between these two associations. This evolutionary history means that many of the molecular components utilised by legumes to establish the nitrogen-fixing symbiosis are already present in cereals, because of their ability to enter the arbuscular mycorrhizal association. The ENSA project is working to understand the evolutionary emergence of the nitrogen-fixing symbiosis in legumes and engineer the existing symbiotic mechanisms in cereals to accommodate nitrogen-fixing bacteria.

In silico Model for Mining the Cis-Regulatory Determinants of Tissue-Specific Gene Expression

Mitra Ansariola[1,2], Shawn O'Neil[3], Valerie N. Fraser[1,2], Sergei Filichkin[2], and ***Molly Megraw***[2,4]
(1)Molecular and Cellular Biology Graduate Program, Oregon State University, Corvallis, OR, (2)Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, (3)Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR, (4)Department of Computer Science, Oregon State University, Corvallis, OR

Gene expression across tissues is regulated by an unknown number of determinants, including prevalence of transcription factors (TFs) and their binding sites along with other aspects of cellular state. Recent studies have suggested the importance of both genetic and epigenetic aspects, at least two of which have substantial literature support as causal determinants of tissue specificity: TF binding sites, and chromatin accessibility at those sites. In order to investigate the extent and relative contributions of these potential determinants, we produced three datasets for both leaf and root tissues of Arabidopsis thaliana plants: TSS-seq data to identify Transcription Start Sites, OC-seq data to identify regions of Open Chromatin, and RNA-seq data to assess gene expression levels. For those genes that are differentially expressed between root and leaf, we constructed a model incorporating chromatin accessibility with TF binding information upstream of TSS locations, with the goal of predicting the tissue in which each of these genes would be upregulated. The resulting model was highly accurate when both chromatin structure and sequence were considered (over 90% auROC and auPRC), allowing one to predict the tissue in which a given gene will express. Specifically, one can use the model to (1) create "in silico knockouts" of TFs that strongly influence the predicted tissue of expression, and (2) identify collections of TFs whose absence moves a specific native gene promoter across the decision boundary to predict expression in a different tissue. The ultimate goal of this work is to develop a rational design process for the tissue-specific targeting of plant gene promoters.

Transcriptional Rewiring: Editing Plant Stess Response Networks

Oliver Windram
Life Sciences, Imperial College London, London, United Kingdom

Plant defence responses are modulated by substantial transcriptional reprogramming, up to 40% of the genome can be differentially expressed following pathogen challenge. This group of differentially expressed genes includes hundreds of transcription factors suggesting a complex transcriptional network regulates plant stress response. We have previously shown that transcriptional rewiring can be used to generate novel phenotypic diversity that can help adapt yeast to stresses associated with heterologous expression constructs. Transcriptional rewiring involves the fusion of transcription factor coding sequences with non-natural promoter sequences which in turn can have pleotropic cascade effects on downstream target cell pathways. Here I will show how we are using a combination of Synthetic and Systems Biology, inspired by our studies in yeast, to select plant regulators for targeted rewiring approaches that seek to enhance plant tolerance to pathogens.

Parts-Prospecting for the Methanol Economy: Yhbo and Formaldehyde Resilience

Jenelle A. Patterson[1], *Hai He[2], Mark A. Wilson[3], Arren Bar-Even[2], and Andrew D. Hanson[1]*
(1)University of Florida, Gainesville, FL, (2)Max Planck Institute of Molecular Plant Physiology, Golm, Germany, (3)University of Nebraska, Lincoln, NE

Formaldehyde (HCHO) is a highly reactive carbonyl compound that cross-links and formylates proteins, DNA, and small molecules, making it both cytotoxic and genotoxic. The mechanism of HCHO damage, and the enzyme systems that repair or prevent it, are critical to natural and synthetic methylotrophy – and hence to the proposed methanol economy. Methanol produced in various ways is a potential cheap and sustainable carbon source for biofuel production and other SynBio applications. As the oxidation of methanol to HCHO is the first step in methanol metabolism, HCHO production is an unavoidable facet of methylotrophy. The accumulation of HCHO can damage the metabolic machinery in platform cells and increase their mutation rate, leading to rapid loss of engineered production traits. Little is known about whether or how cells repair HCHO-damage to proteins, DNA, and small molecules. Comparative genomic analysis indicates that the enzyme YhbO is associated with HCHO-related proteins. YhbO belongs to the DJ-1 protein family, which is implicated in carbonyl damage repair. Deletion of *yhbO* in *Escherichia coli* strain BW25113 increases sensitivity to exogenous HCHO; this phenotype is specific to HCHO and is independent of carbon source. Interestingly, deleting *yhbO* in other *E. coli* K12 backgrounds (e.g., MG1655) does not lead to HCHO-sensitivity. YhbO thus has potential as a damage-repair part in engineering synthetic methylotrophy. We are now

testing possible mechanisms by which YhbO confers HCHO resilience, (e.g., by deformylating proteins) using genetic and biochemical approaches and investigating the gene(s) responsible for the difference between the BW25113 and MG1655 strains.

Friday, November 30th

KEYNOTE SPEAKER

Reverse Engineering of the Omega-3 LC-PUFA Pathway in Transgenic Plants - How to Make a More Nutritious Vegetable Oil

Johnathan Napier, *Lihua Han, Sjur Sandgrind, Richard, P. Haslam, Olga Sayanova*
Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

We have been evaluating the possibility of producing omega-3 LC-PUFAs in different transgenic hosts, to provide a sustainable source of these important nutrients, with a specific interest in producing de novo these health-beneficial fatty acids independent of oceanic sources. Attempts to metabolically engineer plants with the primary algal biosynthetic pathway for LC-PUFAs has been successfully carried out in a range of species, allowing insights into factors constraining the accumulation of these fatty acids in non-native hosts. The use of lipidomics (including MALDI-MS Imaging) has allowed us to overcome metabolic bottlenecks in the transgenic pathway, ultimately leading to the breakthrough production of a transgenic oilseed crop which contains up to 30% omega-3 LC-PUFAs in its seed oil. This omega-3 trait represents probably the most complex plant metabolic engineering to undergo field-trials to date – we will report on our most recent observations. We have also evaluated the use of glasshouse-grown GM Camelina seed oil as a replacement for fish oil in aquafeed diets, observing effective substitution in feeds for salmon and sea bream. These data further confirm the potential of these novel oils and their potential role in human nutrition, direct or indirect. More recently, we have adopted synthetic biology approaches to generate libraries of parts (genes, promoters, 3-UTRs) to identify the optimal combination of elements to direct transgenic accumulation of these important non-native fatty acids. Further enhancements can also be in envisaged via the use of CRISPR-Cas9 editing to remove negative factors constraining maximal accumulation.

SESSION 3: PLANT BIOFOUNDRY AND METABOLIC ENGINEERING

INVITED SPEAKER

Engineering the Composition and Content of Plant Oils

John Shanklin[1]*, Zhiyang Zhai[1], Jantana Keereetaweep[1], Tam Nguyen[1,2], Ed. Cahoon[2], Saroj Parajuli[3], Baskaran Kannan[3], Janice Zale[3] and Fredy Altpeter[3]*
Biosciences Department, Brookhaven National Laboratory 463, Upton, NY[1]. Center for Plant Science Innovation, Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE[2]. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida, IFAS, Gainesville, FL[3].

Plant triacylglycerols (TAGs) i.e., oils are a renewable source of highly-reduced, i.e., energy dense carbon. Because they are produced from photosynthetically fixed CO2, they also represent attractive and sustainable sources of biofuels and bioproducts with the potential to reduce our dependence on fossil resources. The uses of triacylglycerols depends on the structure of the fatty acids esterified to the glycerol backbone. Current crop plants accumulate a relatively narrow range of fatty acids, relative to the diversity found in non-crop species. In my presentation I will describe the engineering of w-7 fatty acid accumulation as a feedstock for 1-octene production. Naturally occurring desaturase enzymes had low turnovers, so we created a highly-improved version with the use of directed evolution. Additional modifications to fatty acid metabolism allowed us to create a crop plant containing a majority of its fatty acids as w-7s. For increasing oil yield per acre, I will discuss the rationale for increasing the accumulation of TAG in the vegetative tissues of high-biomass crops such as sugarcane. Initial results are encouraging in that we have increased natural levels of TAG by approximately two orders of magnitude by manipulating only a few oleogenic factors. We have recently turned our attention to the understanding details of the regulation of WRINKLED1, the major transcription factor controlling fatty acid synthesis. WRI1 is rapidly and conditionally degraded, and our new understanding of its regulation suggest novel approaches to stabilizing it that will increase fatty acid synthesis and TAG accumulation.

Synthetic Design of New Plant Oil Traits for Oilseed Crops As Sustainable and High-Quality Feedstock

Haejin Kim[1], *Edgar Cahoon*[2], *and Thomas Clemente*[3]
(1)Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, (2) Biochemistry, University of Nebraska, Lincoln, NE, (3)Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE

Using soy products as fish food ingredients is sustainable and viable for fish aquaculture. However, the current soy-based feed lacks EPA/DHA omega-3 fatty acids and oil-based feed components, like astaxanthin, needed to obtain the pink-color of some fish meat. Because of these deficiencies, soy-based aquaculture feed currently requires supplementation with fish oil and high-priced astaxanthin, particularly for farm-raised fishes like salmon and trout. In addition, oils with high very-long-chain polyunsaturated fatty acids content are easily oxidized, which limits the shelf life of fish due to the development of off-flavors and odors. For that reason, a high vitamin E antioxidant trait is needed to provide oxidative stability. Conventional plant breeding and biotechnology approaches typically target only one or a small number of traits at a time. In contrast, the emerging discipline of synthetic biology offers tools for making step changes in crop improvement by enabling integration of many trait genes into the crop genome in a single genetic transformation. The goal of our research is the stacking of multiple oil traits: EPA/DHA, astaxanthin, and vitamin E, to obtain a single soybean line with optimized aquaculture feed value. To produce EPA, five transgenes including desaturase and elongase from diverse organisms were synthesized with barley *HGGT***,** which is involved in the production of vitamin E. For astaxanthin production, *β*-ring oxygenase and astaxanthin synthase from *Adonis aestivalis* were used. An 8-transgene expression vector with seed-specific promoters was constructed and transformed into soybean. T3 seeds successfully produce EPA, vitamin E, and astaxanthin. To evaluate the upper limit of transgene numbers that can be introduced into plant from one construct, we also constructed ten modules with multi trait genes to generate improved sustainability and biofuel and industrial quality traits. These trait targets include genes for heat tolerance, seed size, oil content, oil quality, and protein quality.

INVITED SPEAKER

Production of the Platform Chemical Muconic Acid in Plant Biomass

Aymerick Eudes
Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, CA

Muconic acid (MA) is used for the production of chemicals such as adipic acid, terephthalic acid, and caprolactam. Synthesis of these polymer precursors utilizes petroleum-derived chemicals, and the development of alternative strategies for bio-based production of MA has garnered significant interest. Plants represent advantageous hosts for engineered metabolic pathways towards the manufacturing of chemicals. We demonstrate that plants can be used for the bio-manufacturing of MA. In particular, co-expression of bacterial salicylate hydroxylase (NahG), catechol 1,2-dioxygenase (CatA), salicylate synthase (Irp9), and feedback-resistant 3-deoxy-D-arabino-heptulosonate synthase (AroG) resulted in the conversion of the shikimate-derived salicylic acid pool into MA. This value-added co-product was easily recovered after biomass pretreatment. The elucidation and implementation in bioenergy crops of MA biosynthetic routes that divert phenylpropanoid pathway intermediates away from lignin biosynthesis will be presented. These engineering strategies combine in plant biomass the production of value-added chemicals with low-recalcitrance traits towards sustainable development of biorefineries.

Model-Guided Metabolic Engineering of Increased 2-Phenylethanol Production in Plants

Shaunak Ray[1], *Joseph Lynch*[2], *Clint Chapple*[2], *Natalia Dudareva*[2], *and* ***John A. Morgan***[2]
(1)School of Chemical Engineering, Purdue University, West Lafayette, IN, (2)Department of Biochemistry, Purdue University, West Lafayette, IN

2-Phenylethanol (2-PE) is a natural aromatic with properties that make it a candidate oxygenate for petroleum-derived gasoline. In plants, biosynthesis of 2-PE competes with the phenylpropanoid pathway for the common precursor phenylalanine. The phenylpropanoid pathway in plants directs significant carbon flux towards lignin biosynthesis, a major biopolymer in plant cell walls that impedes the process of biofuel production. We therefore propose a genetic engineering strategy at the phenylalanine branch point, whereby a portion of the carbon flux towards lignin biosynthesis is diverted towards the production of an economically valuable product, 2-PE. Transgenic *Arabidopsis thaliana* were generated that overexpress aromatic aldehyde synthase (AAS) in tandem with tomato phenylacetaldehyde reductase (PAR) introducing a pathway to produce 2-PE. To analyze the competition between lignin and 2-PE biosynthesis, excised stems and leaves were exogenously fed with $^{13}C_6$-ring labeled Phe, and isotopic enrichment of downstream metabolites were quantified in time-course to calculate fluxes. Combining metabolic flux analysis with measurements fr**om** *in vitro* kinetic assays of pathway enzymes revealed that

endogenous Phe limits 2-PE production. This prediction was tested by combining the overexpression of PAR/AAS with: (1) the overexpression of a feedback-insensitive 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase known to have hyper-induced phenylalanine biosynthesis, and (2) with the double mutant *pal1 pal2* known to have reduced activity of the competing enzyme, phenylalanine ammonia lyase (PAL). Furthermore, to evaluate the effect of subcellular partitioning on the extent of competition between PAL and AAS, the PAR/AAS tandem overexpression construct was fused to chloroplast transit peptides to localize 2-PE biosynthesis in plastids. The high accumulation of plastidial Phe combined with the lack of competition from cytosolic PAL resulted in significantly elevated 2-PE levels validating the predictions derived from kinetic modeling. Combining kinetic modeling with time-course *in vivo* metabolomics led to successful rational engineering of 2-PE accumulating plants.

INVITED SPEAKER

Redesigning Plant Phytoalexin Biosynthesis

Patrick Shih
Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA

Plants produce a wealth of biologically active compounds, and thus a major impetus for studying plant natural product biosynthesis has been the goal of elucidating and harnessing these metabolic pathways as a means to produce molecules of interest. The field of synthetic biology has opened the door not only to the engineering of new metabolisms into heterologous hosts, but also the designing of novel pathways that are not known to exist in nature. Here, we expand the biological repertoire of plant metabolism by taking a "plug-and-play" approach to rationally engineer synthetic pathways for the production of target non-natural compounds. We demonstrate that we can introduce enzymes from various organisms in order to produce a variety of previously undescribed phytoalexins, compounds involved in plant pathogen defense. Our engineered non-natural products display enhanced properties against known fungal pathogens, highlighting the potential in creating new biopesticides.

Molecular Farming of Spider Silk Analogues for Regenerative Medicine

Congyue A. Peng[1]*, Delphine Dean*[2]*, and William R. Marcotte Jr.*[1]
(1)Genetics & Biochemistry, Clemson University, Central, SC, (2) Bioengineering, Clemson University, Central, SC

Sustainable and cost-effective production of spider dragline silk (spidroin) is desired owing to its biocompatibility and unprecedent mechanical performance. Here, we report the construction of *Nephila clavipes* dragline silk gene analogue, coding the respective native spidroin terminal domains and the abbreviated number of central repeat domains. The mini-spidroin constructs were introduced into tobacco leaves via agrobacterium mediated transformation. Mini-spidroins were purified through affinity chromatography and the removal of affinity tag was achieved through intein activation. The plant derived mini-spidroin features high water holding capacity and remains in liquid-phase after freeze-dry, while most of its counterparts with *E. coli* origin became powders. The condensed mini-spidroins were treated with acidic acid and crosslinked with glutaraldehyde. Solutions for the formation of macromoleculer complexes was prepared by further diluting the treated mini-spidroins in phosphate buffer and mixing with chitosan-acidic acid solution. A counteract charged gellan gum solution was layered with the mini-spidroin solution. For fibers, the film formed at the interface of the two solution was pulled and air-dried; for hydrogels, gelation occurred after the layered solution were dwelled at room temperature without disturbance. Cell adherence to the fibers and hydrogels were tested using dental pulp stem cells and fibroblast cells. The *bona fide* fibers showed poor cell adherence. However, the hydrogels provided adequate attachment for both dental pulp stem cells (DPSC) and fibroblast cells to grow and proliferate. Future studies implementing cues that promote DPSC alignment and neural differentiation will potentiate the use of mini-spidroin based hydrogels in neuron regeneration.

INVITED SPEAKER

From Discovery to Market: Fermentation for Ingredients and Natural Products

Matthew Mattozzi
Conagen Inc., Bedford MA, USA

Plants and their secondary metabolites have served as fragrances, flavors, and pharmaceuticals for millennia. While the discovery of new secondary metabolites is extremely important, cost barriers such as limited availability and high molecule complexity can prevent their adoption by consumers. The emerging field of synthetic biology is beginning to democratize this process. By determining the metabolic pathways by which plants make small molecules and re-constructing them an organism amenable to fermentation, we can greatly increase the availability of natural products. Here we present case studies in the natural flavorings (peach lactones) and sweeteners (steviol glycosides) space and how Conagen has produced natural product molecules heterologously via fermentation. Current and future

applications of bioproduction will be discussed, and several case studies will be presented that illustrate the impact already being felt in the food and beverage space. We are interested in working with academics to sponsor projects for natural product and metabolic pathway discovery.

SESSION 4: GENE-EDITING: FROM REFINED TOOLS TO CROP IMPROVEMENT

INVITED SPEAKER

Multi-Allelic Precision Editing of the Complex Sugarcane Genome By Homology Directed Repair of CRISPR/Cas9 Induced DNA Breaks

Tufan Oz[1,2]*, Ratna Karan*[1]*, Aldo Merotto*[1]*, and Fredy Altpeter*[1,2]
(1)Agronomy Department, University of Florida - IFAS, Gainesville, FL, (2)DOE Center for Advanced Bioenergy and Bioproducts Innovation, Gainesville, FL

Genome editing tools such as CRISPR/Cas9 have been employed in several crop genomes. They enable precise targeting and introduction of double stranded DNA breaks in vivo. Subsequent cellular repair mechanisms, predominantly non-homologous end joining (NHEJ), act as critical steps to endogenous gene editing or correction. However, there is very limited control over these mechanisms, which generate an abundance of random insertions and deletions (indels). Frameshift mutations associated with these indels of unspecified size and sequence might result in loss of function phenotypes of agronomic importance. Gain of function mutations, on the other hand, generally require precise nucleotide substitutions in the target locus. This can be accomplished with the aid of a homologous repair template and involves the cellular homology directed repair (HDR) mechanism. We will present data that supported efficient HDR mediated precision editing of multiple alleles of the acetolactate synthase (ALS) gene in the highly polyploid sugarcane and conferred herbicide resistance.

INVITED SPEAKER

Gene Editing Across Wide Genetic Background via Haploid Induction

Qiudeng Que, *Guozhu Tang, Dakota Starr, Jenny Su, Yuejin Sun, Zhongying Chen, Shujie Dong, Jamie McCuiston, Weining Gu and Tim Kelliher*
Syngenta Crop Protection, LLC, Research Triangle Park, NC

Deployment of genome edited variants across broad genetic background is limited by our ability to edit elite germplasm directly. Here we report that various *in vivo* haploid induction (HI) systems can be used to deliver editing machinery transiently and induce edits in nascent zygotes and/or embryos of both maternal and paternal haploids in monocot and dicot species. The concept was discovered in maize using a native haploid inducer line, extended to dicots using an engineered CENH3 HI system, and then explored in wheat via wide cross with maize pollen expressing CRISPR-Cas. Sequencing data indicated that the edited haploids lacked inducer DNA and editing machinery. The newly developed technology enables direct editing of diverse commercial crop varieties by delivering editing components in gamete nuclei destined for post-fertilization genome loss. Edited haploid lines can be directly incorporated into doubled haploid (DH) testing and breeding programs.

Enhancing Photosynthesis, Vitamin E Production and Tolerance to Herbicides By Targeting Homogentisate Catabolism Using CRISPR/Cas9 Genome Editing System in Soybean

Cuong Nguyen[1]*, Rebecca Cahoon*[2]*, Hanh Nguyen*[3]*, Phat Do*[1]*, Shirley Saito*[3]*, Gary Stacey*[1,4]*, Thomas Clemente*[3]*, Edgar Cahoon*[2]*, and Minviluz Stacey*[1]
(1)Plant Sciences, University of Missouri, Columbia, MO, (2) Biochemistry, University of Nebraska, Lincoln, NE, (3)Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE, (4)Biochemistry, University of Missouri, Columbia, MO

Soybean (*Glycine max*) is a major plant source of protein and oil and produces important secondary metabolites beneficial for human health. In a previous study, we identified and characterized the function of homogentisate dioxygenase (HGO), which catalyzes the committed enzymatic step in homogentisate catabolism, through a forward genetic screen screen of a soybean fast neutron (FN) mutant population. The soybean fast neutron mutant line, MO12, produced brown seeds with higher concentrations of homogentisate and Vitamin E in seeds. In this study, we used CRISPR/Cas9 genome editing system to further study the effect of *HGO* genes on the homogentisate metabolism pathway in soybean. The dual gRNA CRISPR/Cas9 system was utilized to generate single and double *hgo1 hgo2* mutants of soybean. The *homozygous hgo1* mutant plants are phenocopies of the MO12 mutant line, whereas the *hgo2* mutant plants showed a similar phenotype to the wildtype, *Cv*. Throne. The homogentisate content in the seed of the double *hgo1 hgo2* mutant line was comparable to that of the *hgo1* mutant, indicating that GmHGO2 has no significant contribution to homogentisate catabolism in soybean seeds. However, we observed improved growth performance of the double mutant in the field. The double mutant line showed increased tolerance to HPPD-inhibiting herbicide, which interferes with homogentisate biosynthesis, and an increase in photosynthetic rate compared to wild type and single mutants. Overall, our results showed that GmHGO1 is the major isoform responsible for homogentisate catabolism in seeds, but both GmHGO1 and GmHGO2 contribute to homogentisate turn-over in vegetative tissues. Moreover, our results show that targeting homogentisate catabolism is an effective approach to improving soybean agronomic traits and that CRISPR/Cas9 system is a powerful tool for plant metabolic engineering in soybean.

SESSION 5: ENGINERING OF THE CHLOROPLAST GENOME

INVITED SPEAKER

Advances in Synthetic Biology Approaches for Production of High Value Products in Chloroplasts

Henry Daniell
School of Dental Medicine, University of Pennsylvania, Philadelphia, PA

Chloroplasts are ideal bioreactors for production of proteins but require synthetic biology approaches to express eukaryotic genes utilizing prokaryotic protein synthetic machinery. A codon optimization algorithm has been developed utilizing highly expressed chloroplast genes from 133 sequenced chloroplast genomes using Java (Plant Physiology 172:62). High level expression of the largest human protein (891 kDa) – pentameric form of human Blood Clotting Factor VIII was demonstrated using this synthetic approach (Plant Biotechnology Journal 16: 1148). In addition, selectable marker genes have been removed to facilitate regulatory approval after expression of therapeutic, food or feed enzymes or proteins in lettuce chloroplasts. Recent advances in this field including commercial scale production of human therapeutic proteins in FDA-approved cGMP facilities (Biomaterials 70: 84-93), development of tags to deliver protein drugs to targeted human cells or tissues (Biomaterials 80: 68-79), methods to quantify in planta drug dose using proteomic quantitation by parallel reaction monitoring analysis (Plant Physiology 172:62-77), long-term stability of proteins/enzymes at ambient temperature (Molecular Therapy, 24: 1342-1350), testing human drug doses in large animals (Molecular Therapy 25: 512-522), toxicology, pharmacokinetic and pharmacodynamics studies to obtain regulatory approval will be presented (Annual Review of Genetics, 50: 595-618; Genome Biology 17:134).

Five newly launched leaf-products will be compared here with 23 commercial microbial-enzyme products for textile, detergent or juice industries. Crude leaf-extract enzymes are functional at low concentrations without protease inhibitors. Contact-angle water droplet absorption by the FAMAS bioscouring videos exceeds 3-second industry requirements. Leaf-lipase/mannanase crude-extracts remove chocolate/mustard oil stains more efficiently at 70°C than commercial enzymes (<10% activity). Endo/exoglucanase crude leaf-extracts remove dye efficiently from denim surface and de-pilled knitted fabric. Leaf-pectinase powder efficiently clarified orange juice pulp. Thus, leaf-production platform offers a novel low-cost approach by elimination of fermentation, purification, concentration, formulation and cold-chain to revolutionize healthcare and enzyme industries.

INVITED SPEAKER

Engineered RNA-Binding Protein for Gene Regulation in Non-Green Plastids

Qiguo Yu[1], Alice Barkan[2], and ***Pal Maliga***[1]
(1)Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, (2)Institute of Molecular Biology, University of Oregon, Eugene, OR

Non-green plastids are desirable for the expression of recombinant proteins in edible plant parts to enhance the nutritional value of tubers or fruits. However, plastid transgenes are expressed at extremely low levels in the amyloplasts of storage organs such tubers. Here we report a two-component regulatory system consisting of an engineered PPR10^{GG} RNA binding protein and a cognate BS^{GG} binding site upstream of a GFP reporter gene in the plastid genome. The BS^{GG} binding site is not recognized by the resident potato PPR10 protein, restricting GFP protein accumulation to low levels in leaves. When the engineered PPR10^{GG} protein is expressed from the tuber-specific patatin promoter, GFP accumulation is enhanced 20-fold, from 0.06% to about 1.3% of total soluble protein in tubers while having little impact on GFP accumulation in leaves. The two-component regulatory system enables high-level transgene expression in non-photosynthetic plastids without interfering with chloroplast gene expression in leaves.

Engineering the Chloroplast Genome with Synthetic Biology Tools for Microcompartment Construction.

Vishalsingh Chaudhari *and Maureen Hanson*
Cornell University, Ithaca, NY

Photosynthetic efficiency of C3 plants suffers from the slow catalytic rate and the reaction of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) with O_2 instead of CO_2, leading to the costly process of photorespiration. Cyanobacteria are able to utilize a form of Rubisco exhibiting a higher catalytic rate by encapsulating the enzyme within a microcompartment known as a carboxysome. Engineering the cyanobacterial CO_2 concentrating mechanism (CCM) into chloroplasts is an approach to enhance photosynthesis in important C3 plants such as soybean, poplar, and Brassica species. Microcompartments may also be used to compartmentalize other biochemical reactions to confer new capabilities on transgenic plants. Achieving such a goal requires improving the technology for expressing multiple proteins from chloroplast operons. We have been developing strategies and vectors for expressing as many as 12 cyanobacterial genes from the chloroplast genome. Our strategy uses parts flanked by two restriction sites on each side where the distal site is a Type-II rare cutter (8bp recognition) and proximal site is TypeIIS (S for shifted cleavage). Usage of Type-IIS restriction enzyme, which cuts outside the recognition site and produces non-palindromic overhang, brings several unique advantages to traditional BioBrick strategy while maintaining its features. First, more than two parts can be inserted in a vector backbone in a single round of cloning. Second, usage of LguI allows to make a 'scar-less' 5'UTR-CDS junction or an in-frame joining of protein domains with 1-substitution (as opposed

to two in the latest Biobrick standards). Third, the flexibility in the overhang sequence creates advantages similar to Golden Gate cloning. The strategy allows designing of case-specific approaches which can assemble even more number of parts simultaneously. And lastly, it reduces the domestication requirement for parts as it utilizes longer recognition sites for 3 out of 4 of the enzymes.

Saturday, December 1st

KEYNOTE SPEAKER

Sensing and Computing in Plant-Associated Microbes
Christopher Voigt
Massachusetts Institute of Technology

SESSION 6: TRANSGENIC APPROACHES FOR IMPROVING TRAITS AND PERFORMANCE OF CROPS

INVITED SPEAKER

Optimizing Photorespiration for Improved Crop Productivity
Paul F South[1], Amanda P Cavanagh[2], and ***Donald Ort[2]***
(1)Global change and photosynthesis research unit, USDA-ARS, Urbana, IL, (2)Institute for Genomic Biology, University of Illinois, Urbana, IL

Population growth, increasing global affluence and an expanding bioeconomy are conspiring to increase mid-century agricultural demand by 60–120%, a challenge that current methods of increasing crop productivity cannot meet. Traits that have increased productivity during the Green Revolution are near maximum efficiency leaving photosynthesis as the only target to double yield potential. Photorespiration is required in C3 plants to metabolize toxic glycolate formed when Rubisco oxygenates rather than carboxylates ribulose-1,5-bisphosphate. Depending on growing temperatures photorespiration can reduce yields by 20-50% in C3 crops. Optimization of the photorespiratory process by only 5% could be worth millions annually in increased productivity. Synthetic biology has provided new opportunities in altering photorespiratory metabolism to improve photosynthetic efficiency. Using a synthetic biology approach, we tested hundreds of prototype plants from a range of multigene construct designs aimed at reducing photorespiration stress. Flux through the synthetic pathways was maximized by inhibiting glycolate export from the chloroplast. The synthetic pathways tested improved quantum yield by 20%. and increased productivity by >40% in replicated field trials. Engineering alternative photorespiration pathways while inhibiting native photorespiration can drive significant increases in C3 crop yield and resistance to temperature stress under agricultural conditions.

INVITED SPEAKER

Uniform Transgene Expression and Relatively Minor Position Effects in Maize and Soybean
Scott Betts
Corteva

The GRAIN Platform: Identification of Gene Targets for Improving Crop Yield
Frank A. Skraly[1]*, Kristi D. Snell[1], Madana M.R. Ambavaram[1], Meghna R. Malik[2], and Oliver P. Peoples[1]*
(1)Yield10 Bioscience, Woburn, MA, (2)Metabolix Oilseeds, Saskatoon, SK, Canada

Achieving step changes in the seed yield of major crops is not likely to come about with single genetic manipulations, but rather with combinations of changes that are conceived through modeling, experimental iteration, and logical reasoning. Drawing upon its many years of metabolic engineering and modeling expertise, Yield10 is developing the GRAIN (Gene Ranking Artificial Intelligence Network) platform, which encompasses metabolic and regulatory modeling, along with a robust transformation and field trial system. Combinations of genetic changes for increased yield may encompass both traditional plant breeding and molecular breeding tools such as editing, transgenics, and rearrangements of native genetic elements. The GRAIN platform seeks to optimize combinations of these changes by utilizing stoichiometric, kinetic, thermodynamic, and co-expression analyses to narrow the list of actionable targets from many thousands to relatively few, providing plant scientists with manageably small libraries of manipulations to test. The iterative nature of GRAIN is enabled by using camelina as a model crop, because of its ease of transformation and regeneration, its rapid and compact growth, and the relatively few regulatory hurdles associated with field trials. Using this approach, Yield10 is identifying and de-risking yield traits for major agricultural crops.

Genetic Engineering for Enhancing Abiotic Stress Tolerance in Canola (*Brassica napus*)

Aqsa Tabasum, Mohan Singh, and ***Prem Bhalla***
Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Australia

Canola is the second largest oilseed crop worldwide used for human and animal feed. Canola cultivation has grown rapidly over the past five years owing to a high demand for canola oil and meal and is now Australia's third largest broad-acre crop. Australia is the world's second largest exporter of canola seed. With rising global demand for canola for food and non-food applications, its production is expected to increase by 40% by the year 2025. However, impending global climatic changes are predicted to hamper crop productivity. Salinity, drought and high temperatures are significant environmental factors that limit agricultural yields. In this context, genetic improvement of crops for abiotic stress tolerance is vital to maintaining our food supply.

Helicases, an important class of DEAD-box protein family, are primarily known to unwind duplex nucleic acids to perform many housekeeping activities. These highly conserved enzymes play an essential role in several cellular processes including RNA metabolism and regulation of gene expressions. The development of abiotic stress tolerant canola lines by heterologous overexpression of a DNA/RNA helicase gene will be presented.

Functional Analyses of Ovate-Interacting Trms in Tomato

Biyao Zhang[1], *Qiang Li*[2], *Neda Keyhaninejad*[2], *and Esther van der Knaap*[2]
(1)Horticulure, University of Georgia, Athens, GA, (2)University of Georgia, Athens, GA

OVATE is the representative member of OVATE Family Proteins (OFPs) and plays an important role in tomato fruit shape. The single or double natural NILs of ovate and another OFP mutant *sov1* contribute to the elongated and pear-shaped fruits in many cultivated tomatoes. By using OVATE as a bait, 11 TRM (TONNEAU1 Recruiting Motif) proteins were found to interact with OVATE in the yeast 2 hybrid system1. Conserved motif prediction with all OVATE-interacting TRMs revealed a 10-amino-acid domain, M8, is located in an overlapping region of all clones captured in Y2H system. Both α-galactosidase activity assays in yeast and BiFC in tobacco demonstrated that M8 motif is crucial for interaction between OFPs and TRMs proteins1. To further study the function of TRMs in tomato, especially in the fruit, we generated mutations in several OVATE-interacting TRM genes, *TRM3/4*, *TRM5*, *TRM17/20a*, *TRM25*, *TRM26a*, *TRM30/34a* and *TRM19* in the wild species tomato LA1589 (*Solanum pimpinellifolium*) by CRISPR/Cas9 technology. More than three mutant alleles for each TRM gene have been created, including null alleles and weak alleles with amino acids changes in the M8 motif. Null alleles should reveal their potential roles in organ shape or other developmental processes, while M8-modified alleles can help to verify the specific functions of this motif in protein interactions in whole plants. Recently, progress has been made with *TRM5* and *TRM3/4*. The ovate and sov1 double NIL feature fruit with an increase in elongation, proximal end angle and degree of obovoid. The elongated shape is largely recovered to wild type phenotype of round fruit by a null in *TRM5*. Similar trends are also detected in ovary shapes at anthesis. Therefore, *TRM5* plays a profound role in tomato fruit shape. The poster will present progress on tomato phenotypes with altered TRM function.

Increasing Gene Editing Efficiency in Citrus through Transient Expression of CRISPR-Cas9 Proteins and Heat Treatments

Saroj Parajuli[1], *Huo Heqiang*[2], *and Zhanao Deng*[1]
(1)IFAS-GCREC, University of Florida, Wimauma, FL, (2)IFAS-MFREC, University of Florida, Apopka, FL

The CRISPR-Cas9 system has been used to edit genes in several citrus genotypes. Early demonstrations of successful genome editing in citrus were based on targeting the phytoene desaturase (PDS) gene. Despite of these early successes, the gene editing efficiency of this system in citrus needed to be increased substantially for routine use. In this study, we demonstrated enhanced gene editing efficiencies in two citrus genotypes (Carrizo citrange and Duncan grapefruit) by transient expression of two Cas9 proteins [SpCas9 and high fidelity spCas9 (HF-spCas9)] and heat treatments. Mutations were detected in citrus leaf and callus tissues 96 and 144 hours after *Agrobacterium* inoculation. Heat treatment at 37 ˚C for 48 hours increased mutation efficiency by several folds. Transient expression of Cas9 proteins and gRNAs in citrus tissues will be beneficial in selecting gRNAs with higher editing efficiencies for producing stable genome edited citrus plants.

Editing SWEET Genes for Understanding Their Roles in Citrus Susceptibility to Huanglongbing Disease

Saroj Parajuli[1], *Huo Heqiang*[2], *Yi Li*[3], *Fred G. Gmitter*[4], *and Zhanao Deng*[1]
(1)IFAS-GCREC, University of Florida, Wimauma, FL, (2)IFAS-MFREC, University of Florida, Apopka, FL, (3)Department of Plant Science, University of Connecticut, Storrs, CT, (4)IFAS-CREC, University of Florida, Lake Alfred, FL

Citrus greening, also known as Huanglongbing (HLB), is a devastating bacterial disease threatening citrus industries worldwide. There is an urgent need to develop a long-term, effective genetic solution to this threat. Various gene expression and gene editing approaches are being explored to achieve HLB resistance in citrus. Several disease susceptibility genes have been identified in model and crop plants. Among them are the sugars will eventually be exported transporters (SWEETs) genes. In rice, SWEET11, SWEET13 and SWEET14 genes are involved in rice susceptibility to Xanthomonas oryzae oryzae (Xoo). Knocking-out of these genes increased rice resistance to Xoo. Different gene expression studies in citrus suggest that SWEET genes may be involved in citrus susceptibility to HLB. To understand the roles of citrus SWEET genes in HLB susceptibility, we targeted SWEET1 and SWEET10 genes in two citrus genotypes ('Duncan' grapefruit and 'Carrizo' citrange) and edited them using the CRISPR/Cas9 system. Six different types of insertion and deletion of nucleotides were observed in different transgenic lines, ranging from addition of a single nucleotide to the deletion of up to 23 nucleotides. Most of the mutations were either addition or deletion of a single nucleotide at the targeted sites. Deep sequencing of the targeted region revealed that the mutation ranged from 2% to 98% in SWEET1-edited lines and up to 100% in SWEET10 edited lines. These gene-edited plants will be evaluated for their susceptibility/resistance to HLB and other bacterial diseases.

Metabolic Engineering for Production of Lipids in Vegetative Sugarcane Biomass

Saroj Parajuli[1], *Baskaran Kannan*[2,3], *Ratna Karan*[3], *Hui Liu*[4,5], *Eva Garcia-Ruiz*[6], *Deepak Kumar*[7], *Vijay Singh*[7,8], *Huimin Zhao*[6,8], *Steve Long*[8,9], *John Shanklin*[4,5], *and Fredy Altpeter*[2,3]
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Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like sugarcane has been proposed as a strategy to boost lipid yields per acre for biodiesel production. The energy content of plant oils in the form of triacylglycerols (TAGs) is two-fold greater compared to carbohydrates. However, vegetative plant tissues do not accumulate oil to a significant amount since fatty acid synthesis in these tissues serves primarily membrane construction, in addition TAGs undergo rapid turnover. Therefore, our objectives include:

1.) increasing fatty acid synthesis by expressing a transcription activator of fatty acid biosynthetic genes,

2.) increasing fatty acid synthesis by suppressing lipid re-import into the plastids from the cytoplasm,

3.) increasing TAG synthesis from diacyl-glycerol and acyl-CoA by over-expression of rate limiting enzymes,

4.) optimizing TAG storage by limiting the access of lipases to TAG storage compartments.

Constitutive single or multiple gene expression/suppression cassettes were generated and co-delivered with the selectable nptII expression cassette by biolistic gene transfer into sugarcane. Plants were regenerated on geneticin containing culture medium and analyzed for presence and expression of target constructs by PCR and RT-PCR, respectively. Plants were analyzed for TAG content by Gas-Chromatography and Mass Spectrometry (GC-MS).

The presented research outcome will add value to sugarcane for production of advanced biofuels.

Engineering Plant-Microbe Communication for Environmental Sensors.

Tyler Toth
Biological Engineering, MIT, Cambridge, MA

Whole-cell biosensors have certain desirable traits for in field applications because they are self-regeneratable, feasible, and scalable. Moving beyond single species-based biosensors, the plant-microbe community is a completely engineerable system where sensing, signal processing, and response via chemical or protein production can be distributed across different plant and bacterium cells. In order to relay information between plant and bacterium, a two-way chemical language which is orthogonal and specific is required. Here we take motivation from legume-rhizobium symbiosis to develop microbe to plant communication using lipo-chitooligosaccharides (LCOs). The 20+ gene clusters for LCO synthesis from *Rhizobium* **s**p. IRBG74 and *Sinorhizobium meliloti* 1021 have been placed under synthetic transcriptional regulation in order to control expression with desired inputs. As a proof-of-concept, reporter assays in the plants Lotus japonicus and Medicago truncatula validate perception of these bacteria derived LCO signals. Additionally, we take motivation from Agrobacterium tumefaciens to develop plant to microbe communication using opines. Opine-specific transcriptional regulators function in a chassis bacterium *Azorhizobium caulinodans*ORS571. Expression of opine-specific transporters reduces the threshold for opine detection by ~1,000 fold into the micromolar range. Validation of microbial detection of plant-derived opines is ongoing. In summary, engineerable two-way chemical communication between plants and bacteria using LCOs and opines is a step towards plant-microbe community-based biosensors.

Identifying the Genetic Basis of Transformation Efficiency in Barley

Beata Orman-Ligeza[1], *Nicole Schatlowski*[1], *Wendy Harwood*[2], *Alison Hinchliffe*[2], *Cong Tan*[3], *Malcolm Macaulay*[3], *Pete Hedley*[3], *and Kay Trafford*[1]
(1)Genetics and Breeding, NIAB, Cambridge, United Kingdom, (2)The John Innes Centre, Norwich, United Kingdom, (3)The James Hutton Institute, Invergowrie, United Kingdom

Agrobacterium-mediated transformation requires the successful regeneration of plant material that is susceptible to transformation. In spite of exhaustive attempts, only a few barley cultivars are amenable to transformation and most labs use Golden Promise. The genetic factors underlying differences between barley cultivars in transformation efficiency are largely unknown. To improve the transformation efficiency of elite barley cultivars requires identification of these genetic factors.

Transgenic barley is usually obtained from calli induced from immature embryos after co-cultivation with *Agrobacterium*. We tested whether the large-embryo phenotype of a mutant of barley called *lys3* **(***high-lysine 3***)** would influence the performance of its embryos in tissue culture and affect transformation efficiency. To date, four independent *lys3* mutants

have been described and they all have larger-than-normal-embryos. We tested the transformation efficiency of *lys3* mutant M1460 and found it to be similar to that of Golden Promise. Further studies suggested that the immature embryos of M1460 are particularly efficient at producing embryogenic callus.

To understand the genetic basis of M1460 transformability, we crossed M1460 to the elite UK cultivar Optic that is recalcitrant to transformation. Progeny plants from this cross and a subsequent back-cross to Optic were genotyped using the 50k barley SNP array. From this analysis, we identified two conserved genomic regions that might contribute to the high transformation efficiency of M1460. Additionally, after two generations of crosses to Optic, we found that the transformability trait from M1460 segregated away from the large-embryo phenotype. We also found that the other three *lys3* mutants with large embryos (RISØ 1508, RISØ 18, RISØ 19) have low transformation efficiencies. Together these data suggest that a locus other than *Lys3* is responsible for the high transformation efficiency of M1460 and that this locus is likely to lie in one of the two genomic regions that we have identified.

Overexpression of Rice *Cullin* Gene Confers Salt Tolerance in the Salt-Sensitive Arabidopsis Mutant Lacking *cul3a*

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By genome-wide association study (GWAS), candidate loci associated with salt tolerance were identified in Thai rice population. The putative causative genes yielded from GWAS that have Arabidopsis homologs were selected for functional characterization. We found that Arabidopsis mutant of *Cullin3a* **(***cul3a***)** was more sensitive to salt stress than wild-type (WT). CUL3a is a member of ubiquitin E3 ligases, which regulate protein stability. However, the function of CUL3a in salt stress responses is not known. In this study, we overexpressed a rice *cullin3* gene in the Arabidopsis WT and the Arabidopsis mutant of *cul3a***.** All overexpressing lines exhibited higher germination rates than the *cul3a* mutant under salt stress. When 7-day old seedlings were treated with 120 mM NaCl for 5 days, all overexpressing lines exhibited higher growth measured by fresh weights and dry weights than the mutant. In addition, when compared with the *cul3a* mutant, they showed higher carotenoid contents under salt stress. Taken together, the rice cullin3 gene can revert the salt-sensitive phenotype of the Arabiopsis *cul3a* mutant under salt stress suggesting the function of rice cullin3 in the mechanism of salt stress response in rice.

Rapid Prototyping of Constructs in a Chloroplast Cell-Free System for Plastid Engineering

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Chloroplasts are attractive targets of plant engineering with high foreign protein production capacity and genetic control that enables the expression of several genes stacked into operons. Transferring multi-genic functions requires the characterization of genetic parts and fine-tuning of protein expression, but the time-consuming transformation of chloroplasts prevents engineers from carrying out this task in plants. To overcome this problem, a cell-free system – based on purified and subsequently lysed tobacco chloroplasts – was used which enables the expression of proteins from a template.

In order to tune protein expression, different transcription initiation rates were designed with the RBS Calculator to produce luciferase under T7 promoter. Luciferase expression correlated with the predicted RBS strengths, suggesting that RBS Calculator can be used to fine-tune protein expression not just in bacteria, but also in chloroplasts.

To demonstrate the prototyping capacity of the cell-free system, two enzymes of the capsaicin biosynthetic pathway were selected. The sequences were also codon-optimized and their RBS was tuned to cover a high range of protein expression levels. The design and testing of 55 constructs for this study only took us 4 weeks, which would have never been possible by transforming plants.

In order to test a multi-gene system, the Klebsiella 'refactored' v2.1 cluster codifying for 16 genes of nitrogenase was expressed in chloroplast and *E. coli* cell-free systems. RT-PCR analysis confirmed the presence of all open reading frames in both systems but revealed differences in mRNA levels.

The chloroplast cell-free system prevents mistakes like using wrong codon-optimization or RBS, and could allow for up to 1000 constructs to be prototyped weekly. Those producing the desired expression pattern could be selected for plastid transformation in the plant. In addition, the cell-free system could also serve as a test-bed to finely balance expression of multi-gene clusters.

Synthetic Promoter Development for Drought Stress Response in Hybrid Poplar

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Abiotic stress, such as that caused by drought, is problematic to crop production, especially those for which irrigation is not economically feasible. Bioenergy crops, such as hybrid poplar, must be endowed with robust coping mechanisms for stress. To that end, we are partnering with collaborators to develop stress-inducible synthetic promoters to drive stress-coping genes only when needed by hybrid poplar plants. Our strategy is to mine poplar –omics data for signature promoter motifs that are drought-responsive, then combine motifs in multimers upstream of the core cauliflower mosaic virus (CaMV) -46 35S promoter region. Herein we found 30 candidate DNA motifs using *p*-value threshold of 0.01 from promoters of drought-responsive co-expressed genes. These domains each were comprised of 3 to 9 conserved motifs that were detected among the 30 candidate regions of approximately 100 bases each. Our mined domains, which included part of the native promoters were fused to a green fluorescent protein gene and screened under proxy drought stress in poplar mesophyll protoplasts. The inducible motifs have been synthesized into a purely synthetic promoter architecture and will be screened in a robotic protoplast system to determine temporal and spatial inducibility of drought stress response in poplar.

Quantitative and Genome-Wide Analysis of a Transcription Factor BZR1 Interactions with Its Partner Transcription Factors and Target Genes

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Our previous research demonstrates that Brassinosteroid and auxin signaling pathways are integrated with both light and temperature. This integration occurs through the interaction of three transcription factors (TFs): BZR1, ARF6, and PIF4. Using ChIP-Seq, we showed that these TFs bind to overlapping sets of target genes to control cell elongation and photomorphogenesis, which explains conceptually how diverse signals are integrated into coherent cell growth decisions. While ChIP-seq binding profiles show direct binding of TFs and multiprotein complexes under complex cellular conditions, how cis-regulatory element combinations and interacting TFs define the overlapping target gene sets and fine-tune TF activities remain to be answered. Here, we analyze TFs-genome binding affinity *in vitro* on a massively parallel array. For this approach, optics are built into a high-throughput sequencer, which are then used to visualize *in vitro* binding of a fluorescently-labeled TF to plant genome DNA fragments immobilized as clusters on a flow cell. In principle, this method enables quantitative measurement of binding affinities of BZR1, ARF4 and PIF4 individually to the genomic cis-regulatory elements under defined conditions without interference of chromatin and unknown factors. Furthermore, both equilibrium binding constants and dissociation kinetics can be determined for a TF at each of the millions of genomic DNA fragments. More importantly, by labeling TFs with different fluorescent substrates, the binding of pairwise or multiple TFs can be measured simultaneously to detect cooperation or competition between TFs at each DNA fragment. Such analysis can reveal how combinatorial TF-TF and TF-DNA interactions refine target selectivity and allow transcriptional crosstalk.

Processing Genome Edited, Lignin-Modified Sugarcane Biomass with a Xylose Fermenting Yeast Strain Drastically Elevates Biofuel Production

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Sugarcane is a prime feedstock for commercial production of bioethanol. Utilizing both sucrose and cell wall bound sugars for fermentation will enhance the biofuel yield per unit area. The recalcitrant structure of lignocellulosic biomass is a major barrier for efficient biomass-to-ethanol conversion. Co-editing of more than 100 copies of the caffeic acid O-methyltransferase (COMT) gene resulted in modified lignin biosynthesis and elevated the saccharification efficiency more than 40% compared to un-modified sugarcane biomass. The combination of feedstock engineering via genome editing of the lignin synthesis pathway of sugarcane and co-fermentation of xylose and glucose with a recombinant xylose utilizing yeast strain allowed to produce 148% more ethanol compared to that of the un-modified biomass and control strain. The lignin reduced biomass led to a substantially increased release of fermentable sugars (glucose and xylose). The engineered yeast strain efficiently co-utilized glucose and xylose for fermentation, elevating ethanol yields. This strategy will significantly improve the economic feasibility of producing biofuels from lignocellulosic biomass.

Altering Chloroplast Size in Sugarcane By RNAi Suppression of FtsZ, Is Bigger Better?

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Sugarcane is one of the most productive biofuel crops due to its superior photosynthetic efficiency. Sugarcane has a C4 type metabolism for fixation of carbon, allowing it to be very well adapted to biomass production in tropical and subtropical regions. Genetic improvement of photosynthetic efficiency could potentially be achieved by developing a photosynthetically more effective canopy. To evaluate the effect of chloroplast size on light penetration into the canopy and biomass production, we modified the expression of Filamenting temperature sensitive mutant Z (FtsZ). FtsZ is a key player in the division of both bacteria and chloroplasts. FtsZ is a cytoskeletal GTPase that self-assembles in vitro into filaments and other conformations similar to those formed by its eukaryotic structural homologue, tubulin. RNAi –suppression of the nuclear encoded FtsZ in sugarcane resulted in five-fold increase in chloroplast size. Field performance of transgenic plants will be reported.

Overexpression of OsTF1L, a Rice HD-Zip Transcription Factor, Promotes Lignin Biosynthesis and Stomatal Closure That Improves Drought Tolerance

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Drought stress seriously impacts on plant development and productivity. Improvement of drought tolerance without yield penalty is a great challenge in crop biotechnology. Here, we report that the rice (*Oryza sativa*) homeodomain-leucine zipper transcription factor gene, *OsTF1L* (*Oryza sativa transcription factor 1-like*), is a key regulator of drought tolerance mechanisms. Overexpression of the *OsTF1L* in rice significantly increased drought tolerance at the vegetative stages of growth and promoted both effective photosynthesis and a reduction in the water loss rate under drought conditions. Importantly, the *OsTF1L* overexpressing plants showed a higher drought tolerance at the reproductive stage of growth with a higher grain yield than non-transgenic controls under field-drought conditions. Genome-wide analysis of *OsTF1L* overexpression plants revealed up-regulation of drought-inducible, stomatal movement and lignin biosynthetic genes. Overexpression of *OsTF1L* promoted accumulation of lignin in shoots, whereas the RNAi lines showed opposite patterns of lignin accumulation. In addition, *OsTF1L* overexpression enhances stomatal closure under drought conditions resulted in drought tolerance. More importantly, OsTF1L directly bound to the promoters of lignin biosynthesis and drought-related genes involving *poxN/PRX38, Nodulin protein, DHHC4, CASPL5B1* and *AAA-type ATPase*. Collectively, our results provide a new insight into the role of *OsTF1L* in enhancing drought tolerance through lignin biosynthesis and stomatal closure in rice.

Ethephon Induced Alkaloid Production in Two Varieties of the Medicinal Plant *Catharanthus Roseus*

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Many plant-derived secondary metabolites have chemical properties that give them therapeutic value for the treatment of cancers, hypertension, and other common illnesses. In the medicinal plant *Catharanthus roseus*, the secondary metabolites of interest are the monoterpene idole alkaloids (MIAs) vinblastine and vincristine, which are naturally produced at relatively low levels in the most commonly used varieties. As a result, pharmaceutical scientists instead extract intermediate compounds from the MIA biosynthetic pathway and chemically synthesize the final products *in vitro*—a difficult and costly process. Medicinal chemists have historically used methyl jasmonate, a plant signaling hormone, to induce higher levels of vinblastine and vincristine *in planta* for their research, but this particular treatment is too expensive for practical use in a large-scale agricultural or biopharmaceutical production setting. In the course of our research, we examined alternatives to chemical synthesis and methyl jasmonate induction by assaying alkaloid concentrations in two of the most commonly used varieties of *C. roseus*. We present our findings here, as well as the effect of an ethylene derivative as an induction agent. The ultimate goal of this research is to understand how to upregulate biosynthesis of medically-relevant indole alkaloids in the native host plant.

Particle Bombardment for Transient Gene Expression in Citrus Plant Cells Using the Helios® Gene Gun

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Transient gene expression can provide valuable data about various aspects of protein characteristics, such as its subcellular localization and intra/inter cellular trafficking, its expression levels, stability and degradation, its interactions with other proteins, and its activity. In citrus, transient gene expression is typically carried out using Agrobacterium inoculation, but the efficiency of this method varies considerably depending on the citrus variety and the experimental conditions. Particle bombardment is a very convenient method to deliver nucleic acids into the cells directly. Because it is a physical method, it does not depend on biochemical features of the structural components that are typically present on cell surfaces and can overcome physical barriers such as the cell wall. The Helios® Gene Gun (Bio-Rad, Hercules, CA) is a hand-held device that uses a low-pressure helium pulse to accelerate DNA/RNA-coated sub-cellular sized particles into a wide variety of target cells either for *in vitro* or *in vivo* applications. Using this system, we optimized parameters for robust particle bombardment into citrus plant cells and transient gene expression. In addition, we have also achieved stable protein expression in bombarded citrus cell suspensions and in cultured tissues, which could lead to the production of transgenic plants. The capability of the gene gun to deliver different type of nucleic acids (plasmids, mRNA and viral RNA/DNA) allows us to develop strategies for DNA-free gene editing to produce disease resistant citrus plants.

***LOC_Os01g68450* gene Increases Salt Tolerance By Photosynthesis Process Enhancement**

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Salt stress is one of the limiting factors for rice growth and productivity. Salt tolerant mechanisms and genes involving in this process have been investigated. Previously, *LOC_Os01g68450* was proposed to be one of the key genes involving in salt stress tolerance in rice, based on co-expression network analysis. The transgenic Arabidopsis with LOC_Os01g68450 expression was evaluated for salt tolerant phenotype according to the ability in pigment maintenance and photosynthesis parameters under salt stress condition, in comparison with wild type (WT) and the Atduf mutant, Arabidopsis mutant with T-DNA insertion at AtDUF2358 gene, Arabidopsis ortholog of LOC_Os01g68450 gene. It was shown that the transgenic lines in both backgrounds had the higher pigment contents and photosynthesis parameters under salt stress condition, when compared to WT and mutant line. These suggested that LOC_Os01g68450 gene could increase salt tolerance by photosynthesis enhancement process.

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