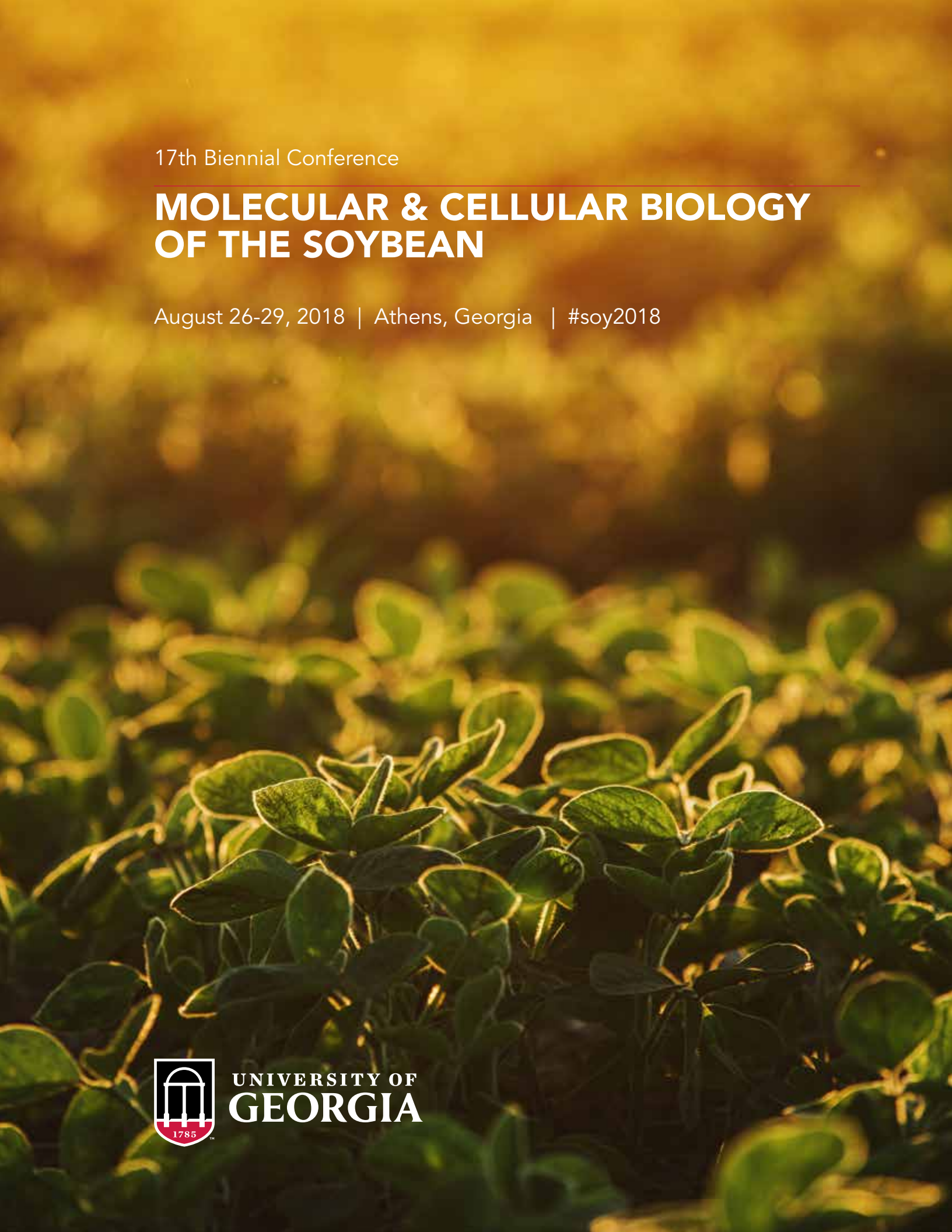

17th Biennial Conference

MOLECULAR & CELLULAR BIOLOGY OF THE SOYBEAN

August 26-29, 2018 | Athens, Georgia | #soy2018

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Iron horse in soybean field. Photo by Evan McCoy. See next page for information about the sculpture.

Dear SOY2018 colleagues,

Thanks for coming to the 17th Biennial Conference on the Molecular and Cellular Biology of the Soybean! We look forward to a few days of scientific exchange and fellowship. Welcome to Athens, Georgia, a city rich in history and culture. We hope that you experience the best of what Athens has to offer.

This year will go down in history as the year that land planted with soybeans overtakes land planted with corn in the U.S. Soybeans are now the country's No. 1 crop. It is an exciting time for soybean researchers. This meeting will showcase some new research in gene characterization, genome editing, soybean engineering, and soybean biology and environmental interaction.

The registration list is full of names new to this group. A hearty welcome goes out to all who are attending the biennial conference for the first time. May this conference be the first of many!

We appreciate the help from all of the session chairs and their time and effort spent convening sessions. Their participation ensures the topics covered are timely and relevant. Furthermore, they all have strived to include a graduate student or post-doc in their sessions.

Finally, we are fortunate to have many wonderful sponsors and want to be sure to thank them for their support. Please take time to look at the sponsors listed in the program and thank their representatives at the meeting.

Sincerely,

The SOY2018 local organizers
Scott Jackson
Zenglu Li
Wayne Parrott
Jennifer Leverett
Maddilyn Johnson

Layout and design: Cindy Allen
Registration: Regina Fitzpatrick
Webmaster: David Allen

SOY 2018

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THE IRON HORSE

The following is taken from an article by Ellie Holt that appeared in the *Atlanta Journal Constitution* on Nov 20, 2017. It has been abridged and edited for the purpose.

The Iron Horse was sculpted by Abbott Pattison, an acclaimed artist from the Chicago Institute of Art and the University of Georgia's sculptor in residence at the time, the 12-foot-tall piece was created in UGA's old Fine Arts Building and originally placed in front of a residence hall on May 25, 1954.

Controversy surrounded the two-ton abstract sculpture within hours of its installation. Public display of art was not the norm at UGA in the early 1950s. According to reports, students and faculty voiced differing opinions about the sculpture, and students began to vandalize the artwork before violently beating it and setting a fire beneath it. UGA authorities and the Athens Fire Department were called to the scene to extinguish the fire. The statue was moved into hiding early the next morning.

In 1959, L.C. Curtis, a horticulture professor at UGA, moved it to his farm in Greene County, about 20 miles from Athens. In 2011, UGA purchased the 660-acre Curtis farm for agricultural research and renamed it the Iron Horse Plant Sciences Farm (commonly known as the Iron Horse Farm).

The Iron Horse has become a significant attraction over the years, with visitors stopping at all hours of the day.

MEETINGS OF THE BIENNIAL CONFERENCE ON MOLECULAR & CELLULAR BIOLOGY OF THE SOYBEAN

17th: Athens, GA (2018)
16th: Columbus, OH (2016)
15th: Minneapolis, MN (2014)
14th: Des Moines, IA (2012)
13th: Durham, NC (2010)
12th: Indianapolis, IN (2008)
11th: Lincoln, NE (2006)
10th: Columbia, MO (2004)
9th: Urbana-Champaign, IL (2002)
8th: Lexington, KY (2000)
7th: Knoxville, TN (1998)
6th: Columbia, MO (1996)
5th: Athens, GA (1994)
4th: Ames, IA (1992)
3rd: Ames, IA (1990)
2nd: Ames, IA (1988)
1st: Ames, IA (1986)

TWEETING AND PHOTOGRAPHY

- For those who tweet, please use #soy2018
- Some speakers and poster presenters have indicated their preference for no photographs and or tweeting of their slides/posters. Please respect their wishes!

UGA Soybean Advanced Yield Trial at Iron Horse Farm
Photo by Ethan Menke

SOY2018 AGENDA

SUNDAY, AUGUST 26, 2018

3 p.m. Registration opens (Grand Hall 1-6 Foyer)

3 p.m. Poster posting (Grand Hall 7)

3:30 p.m. SOYBASE WORKSHOP (Grand Hall 1-6)
Everyone welcome, no preregistration required

5:30 p.m. WELCOMING & OPENING REMARKS (Grand Hall 1-6)

5:45 p.m. KEYNOTE ADDRESS (Grand Hall 1-6)
Gary Stacey, University of Missouri
S00: *Soybean should be more than just a crop*

6:15 p.m. LIFETIME ACHIEVEMENT AWARD PRESENTATIONS (Grand Hall 1-6)
- Perry Cregan, retired, USDA-ARS, Beltsville
- Junyi Gai, Nanjing Agricultural University

6:45 p.m. OPENING RECEPTION (Atrium)
Two courtesy drink tickets are provided. Cash bar available

8:30 p.m. Dinner on your own. ***A Flagpole Guide to Athens*** is included in your registration packet and it contains a map of down town and a listing of the various venues available.

MONDAY, AUGUST 27, 2018

7:45 a.m. Registration opens (Grand Hall 1-6 Foyer)

PESTS & DISEASES (Session 1, Grand Hall 1-6)
Chairs: Melissa Mitchum, University of Missouri
Dechun Wang, Michigan State University

8 a.m. **Rick Masonbrink, Iowa State University**
S01: *Effector duplication and diversification in the soybean cyst nematode genome*

8:20 a.m. **Melissa Mitchum, University of Missouri**
S02: *The complex interplay between soybean resistance and nematode virulence*

8:40 a.m. **Hao-Xun Chang, Michigan State University**
S03: *Foliar chlorosis of Sudden Death Syndrome is a dominant and qualitative symptom depending on STAY-GREEN genes of soybean*

9 a.m. **Dechun Wang, Michigan State University**
S04: *The genetics of aphid resistance in soybean*

9:20 a.m. **Gustavo McIntosh, Iowa State University**
S05: *Changes in membrane lipids in soybean leaves in response to soybean aphid infestation*

9:40 a.m. Coffee Break (Grand Hall 7 Foyer)

MONDAY, AUGUST 27, 2018 *continued*

PESTS & DISEASES (Session 2, Grand Hall 1-6)
Chairs: Kerry Pedley, USDA-ARS, Maryland
Anne Dorrance, The Ohio State University

10:20 a.m. **Aarda Kacharoo, University of Kentucky**
S06: *Potyviral effector targets and viral co-opting of the untranslated protein response in soybean*

10:40 a.m. **Chantal McCabe, USDA-ARS, Iowa**
S07: *Leveraging RNA-Seq to characterize resistance to brown stem rot and the Rbs3 locus in soybean*

11 a.m. **Mehdi Kabbage, University of Wisconsin**
S08: *Soybean defense mechanisms against* ***Sclerotinia sclerotiorum***

11:20 a.m. **Kerry Pedley, USDA-ARS**
S09: *Rpp1 encodes a novel protein that controls immunity to soybean rust*

11:40 a.m. **Anne Dorrance, The Ohio State University**
S10: *How can they be so different? QDRL for watermolds and true fungi*

12 p.m. Lunch (Atrium)

1 p.m. **Anne Sylvester, National Science Foundation**
What is new with the Plant Genome Program at NSF? (Grand Hall 1-6)

ABIOTIC STRESS (Session 3, Grand Hall 1-6)
Chairs: David Hyten, University of Nebraska
Lisa Ainsworth, University of Illinois

1:20 p.m. **David Schachtman, University of Nebraska**
S11: *Changes in the soil, root and rhizosphere bacterial microbiome of soybean lines that differ in their tolerance to alkaline soils*

1:40 p.m. **Lorenzo Rossi, University of Florida**
S12: *The mutual impact of cerium oxide nanoparticles and cadmium on soybean physiology and root anatomy*

2 p.m. **Jamie O'Rourke, USDA-ARS, Iowa**
S13: *Exploring early responses to nutrient deficiency stress in soybean*

2:20 p.m. **Pauline Lemonnier, University of Illinois**
S14: *Transgenic strategies to maximize soybean response to rising carbon dioxide concentrations*

2:40 a.m. **Aaron Lorenz, University of Minnesota**
S15: *Enhancing resistance to iron deficiency chlorosis in soybean through high-throughput phenotyping and QTL fine mapping*

3 p.m. Coffee Break (Grand Hall 7 Foyer)

SYMBIOSES & PHYTOBIOME (Session 4, Grand Hall 1-6)
Chairs: Gary Stacey, University of Missouri
Leslie Dormier, USDA-ARS, Illinois

3:20 p.m. **Katalin Toth, University of Missouri**
S16: *Soybean RIN4, a crucial player in symbiosis development*

3:40 p.m. **Chris Anderton, DOE, Environmental Molecular Sciences Lab**
S17: *A metabolic view inside the soybean root nodule*

Continued on next page

MONDAY, AUGUST 27, 2018 *continued*

4 p.m. **Amy Welty-Bernard, Iowa State University**
S18: *Towards an understanding of root architecture-microbe interactions: spatial analysis of the soybean root microbiome*

4:20 p.m. **Shin-Yi Lee Marzano, South Dakota State University**
S19: *Exploring the microbiome from soybean grown in different rotations to increase soybean productivity*

4:40 p.m. **Leslie Dormier, USDA-ARS, Illinois**
S20: *Detection of diverse virus-like sequences in metatranscriptomes of soybean phyllosphere microbiomes*

5 p.m. POSTER COMPETITION & SOCIAL (Grand Hall 7)
Chairs: Pengyin Chen, University of Missouri
Jamie O'Rourke, USDA-ARS, Iowa
Two courtesy drink tickets are provided. Cash bar available
Please stand by your **even**-numbered posters from 5-6 p.m.
Please stand by your **odd**-numbered posters from 6-7 p.m.

7 p.m. Dinner, on your own

TUESDAY, AUGUST 28, 2018

8 a.m. Registration opens (Grand Hall 1-6 Foyer)

COMPOSITION & NUTRITION (Session 5, Grand Hall 1-6)
Chairs: Kristin Bilyeu, USDA-ARS, Missouri
Ed Cahoon, Michigan State University

8 a.m. **Hae Jin Kim, University of Nebraska**
S21: *New oil traits for soybean as a sustainable and high quality feedstock for aquaculture*

8:20 a.m. **Kyujung Van, The Ohio State University**
S22: *Approaches for identifying QTL associated with protein and oil contents and compositions in soybean seed*

8:40 a.m. **Brian Diers, University of Illinois**
S23: *Mapping seed protein QTL and progress in cloning them*

9 a.m. **Thomas E. Clemente, University of Nebraska**
S24: *Development of an improved baking oil soybean by combining high saturated fatty acid and high oleic acid traits*

9:20 a.m. **Kristin Bilyeu, USDA-ARS, Missouri**
S25: *The next commodity soybean*

9:40 a.m. Coffee Break (Grand Hall 7 Foyer)

FUNCTIONAL GENOMICS (Session 6, Grand Hall 1-6)
Chairs: Aaron Lorenz, University of Minnesota
Bob Stupar, University of Minnesota

10:20 a.m. **François Belzie, Université Laval**
S26: *SoyaGen: A translational genomics project for short-season soybean in Canada*

TUESDAY, AUGUST 28, 2018 *continued*

10:40 a.m. **Jianxin Ma, Purdue University**
S27: *Molecular links underlying pleiotropic traits in soybean*

11 a.m. **Andrew Bent, University of Wisconsi**n
S28: *Converging mechanisms confer soybean cyst nematode resistance*

11:20 a.m. **Lila Vodkin, University of Illinois**
S29: *A molecular catalog of independent mutations that inactivate Argonaute5 with effects on the gene silencing pathway in soybean*

11:40 a.m. **Jeonghwa Kim, University of Missouri**
S30: *The classical dt1-t allele responsible for tall determinate stem architecture in soybean is caused by two of the identified missense alleles of dt1*

12 p.m. Lunch (Atrium)

12 p.m. Lunch (Olympia 2) **All early career professionals** are invited to take their lunch to Olympia 2 for a session with Anne Sylvester & Diane Okamuro from NSF

BREEDING & GENOMICS (Session 7, Grand Hall 1-6)
Chairs: Oswald Crasta, Corteva
Brian Diers, University of Illinois

1:20 p.m. **Bill Beavis, Iowa State University**
S31: *Genomic applications in a multi-trait soybean variety development program*

1:40 p.m. **John Woodward, Corteva**
S32: *Using genomics for high-resolution haplotype characterization and identifying novel trait-associated variation*

2 p.m. **Warren Kruger, Monsanto**
S33: *Integration of precision phenotyping and genomics will drive future improvements in breeding and product placement*

2:20 p.m. **Marcos Quiroga, GDM Seeds**
S34: *The role of germplasm as a key factor in the changes of crop production systems and yield increase in South a.m.erica; A genomic approach*

2:40 a.m. **Leah McHale, The Ohio State University**
S35: *Increasing the rate of genetic gain for yield in soybean breeding programs*

3 p.m. Coffee Break (Grand Hall 7 Foyer)

BIOTECHNOLOGY & GENOME EDITING (Session 8, Grand Hall 1-6)
Chairs: John Finer, The Ohio State University
Thomas Clemente, University of Nebraska

3:20 p.m. **Heidi Kaeppler, University of Wisconsin**
S36: *Genotype independent, meristem-based soybean transformation*

3:40 p.m. **Hyeon-Je Cho, Corteva**
S37: *A novel DuPont Pioneer proprietary organism discovered for plant transformation*

4 p.m. **Andika Gunadi, The Ohio State University**
S38: *Harnessing soybean promoters to optimize homology independent targeted integration*

4:20 p.m. **Don Ort, South Dakota State University**
S39: *Lowering the cost of photorespiration*

Continued on next page

TUESDAY, AUGUST 28, 2018 *continued*

4:40 p.m. **Raquel Lía Chan, Instituto de Agrobiotecnología del Litoral**
S40: *The long way from the lab to the field: Soybean plants expressing the sunflower HaHB4 gene outyield their controls in different environments*

5 p.m. **Zhan-Bin Liu, Corteva Agriscience**
S:41 *Soybean genome editing at Corteva Agriscience*

6 p.m. Buses depart from the Classic Center for **dinner at Smithonia Farm**. Conference name tags are required to board the bus. The first bus will return at 9 p.m. Buses run every 15 minutes. The last bus returns at 10:30 p.m.

WEDNESDAY, AUG. 29, 2018

8 a.m. Registration opens (Grand Hall 1-6 Foyer)

GENOME BIOLOGY (Session 9, Grand Hall 1-6)
Chairs: Paul Chomet, NRGene
Michelle Graham, USDA-ARS, Iowa

8 a.m. **Janine Sherrier, University of Georgia**
S42: *Development of transport pathways in root nodules*

8:20 a.m. **Chuming Xu, University of Georgia**
S43: *Gene identification and phenotypic prediction of complex traits using transcriptome data*

8:40 a.m. **John Harada, University of California - Davis**
S44: *Functional genomics of soybean seed development*

9 a.m. **Blake Meyers, Donald Danforth Plant Science Center**
S45: *Conservation, novelty and loss of plant small RNA pathways in soybean and other legumes*

9:20 a.m. **Bob Stupar, University of Minnesota**
S46: *Cloudy with a chance of mutations: Gene editing and functional analyses in soybean*

9:40 a.m. Short Coffee Break (Grand Hall 7 Foyer)

INTEGRATIVE GENOMICS (Session 10, Grand Hall 1-6)
Chairs: Leah McHale, The Ohio State University
Jianxin Ma, Purdue University

10 a.m. **Bao-Hua Song, University of North Carolina - Charlotte**
S47: *Insight from the wild---dissection of broad-spectrum resistance to Soybean Cyst Nematode (SCN) using an integrative approach*

10:20 a.m. **Marc Libault, University of Oklahoma**
S48: *Identification of conserved root hair-specific transcriptional regulatory elements by comparative -omic analysis*

10:40 a.m. **Jer-Young Lin, University of California - Los Angeles**
S49: *Dissecting the methylomes of seed development*

11 a.m. **Zhixi Tian, Chinese Academy of Sciences**
S50: *Dissecting agronomic traits using genomic and genetic approaches in soybean*

11:20 a.m. **Dahlia Nielsen, North Carolina State University**
S51: *Networks underpinning symbiosis revealed through cross-species eQTL mapping*

11:40 a.m. Conference ends

Photo by Scott Jackson

SPEAKER ABSTRACTS

S-00
Soybean should be more than just a crop

Gary Stacey | University of Missouri | staceyg@missouri.edu

Prior to the widespread adoption of molecular biology and genomics, soybean served the plant community as a leading model system for studies of physiology and biochemistry. For example, many of the biochemical pathways used to populate the popular *Arabidopsis* Information Resource database came from studies first performed in soybean. However, the soybean research community was slow to adopt the new technology, which allowed the rise of the so-called model legumes, *Medicago truncatula* and *Lotus japonicus*, largely focused on studies of the nitrogen fixation symbiosis. Subsequently, while the maize community aggressively pursued federal funding, contributing significantly to the establishment of the NSF Plant Genome Program, soybean again was not a major player. While soybean commodity funding eventually did make a significant contribution to the development of genomic resources, research on soybean, as opposed to maize and rice, for example, maintained a largely parochial, narrow research focus on traits of agronomic or commercial interest. The net effect of this history, regardless of intent, was that the soybean research community and, more importantly, soybean producers have been ill served with, at a minimum, many lost opportunities for advancements, both basic and applied. My intention in this talk is to show how past history does not need to dictate the future. What is needed is a change in mind set among the community to pursue a more open, broader, and aggressive path with the goal to again establish soybean as a leading model for plant research. The major beneficiary of such an approach will be soybean producers and the industry that they support.

S-01
Effector duplication and diversification in the soybean cyst nematode genome

Rick Masonbrink | Iowa State University | Remkv6@iastate.edu
Tom R. Maier, Iowa State University
Usha Muppirala, Iowa State University
Etienne Lord, Agriculture and Agri-Food Canada
Parijat S. Juvale, Iowa State University
Jeremy Schmutz, Joint Genome Institute
Nathan T. Johnson, Worcester Polytechnic Institute
Dmitry Korkin, Worcester Polytechnic Institute
Melissa G. Mitchum, University of Missouri
Benjamin Mimee, Agriculture and Agri-Food Canada
Sebastian Eves-van den Akker, University of Cambridge
Matthew Hudson, University of Illinois
Andrew J. Severin, Iowa State University
Thomas J. Baum Iowa State University

Heterodera glycines, commonly referred to as the soybean cyst nematode (SCN), is an obligatory and sedentary plant parasite that causes over a billion-dollar yield loss to soybean production annually. Although there are genetic determinants that render soybean plants resistant to certain nematode genotypes, resistant soybean cultivars are increasingly ineffective because their multi-year usage has selected for virulent *H. glycines* populations. The parasitic success of *H. glycines* relies on the comprehensive re-engineering of an infection site into a syncytium, as well as the long-term suppression of host defense to ensure syncytial viability. At the forefront of these complex molecular interactions are effectors, the proteins secreted by *H. glycines* into host root tissues. The mechanisms of effector acquisition, diversification, and selection need to be understood before effective control strategies can be developed, but the lack of an annotated genome has been a major roadblock. Here, we use PacBio long-read technology to assemble a *H. glycines* genome of 738 contigs into 123Mb with annotations for 29,769 genes. The genome contains significant numbers of repeats (34%), tandem duplicates (18.7Mb), and horizontal gene transfer events (151 genes). Using previously published effector sequences, the newly generated *H. glycines* genome, and comparisons to other nematode genomes, we investigate the evolutionary mechanisms responsible for the emergence and diversification of effector genes.

S-02
The complex interplay between soybean resistance and nematode virulence

Melissa G. Mitchum | Division of Plant Sciences and Bond Life Sciences Center, University of Missouri | goellnerm@missouri.edu

Though soybean cyst nematode-resistant soybean varieties are available to minimize yield loss, producers are faced with limited options for rotation once virulent SCN populations develop in their fields. The widespread lack of genetic diversity for SCN resistance genes in commercial soybean varieties has significantly increased the prevalence of virulent SCN populations and reduced the effectiveness of current sources of resistance. I will discuss recent results of studies aimed at evaluating how SCN adapts to new sources of resistance and various resistance gene combinations in rotation to determine how best to deploy resistance for strategic management and improve resistance durability. Furthermore, I will discuss how these efforts are being used to identify the genetic basis of nematode adaptation to soybean resistance in an effort to develop methods to more rapidly assess the virulence of field populations.

S-03
Foliar chlorosis of sudden death syndrome is a dominant and qualitative symptom depending on STAY-GREEN genes of soybean

Hao-Xun Chang | Michigan State University | changh21@msu.edu
Ruijuan Tan, Michigan State University
Glen L. Hartman, USDA-ARS, University of Illinois
Hyunkyu Sang, Michigan State University
Zixiang Wen, Michigan State University
Leslie L. Domier, USDA-ARS, University of Illinois
Steven A. Whitham, Iowa State University
Dechun Wang, Michigan State University
Martin I. Chilvers, Michigan State University

Fusarium virguliforme is a fungal pathogen that causes soybean sudden death syndrome (SDS). The pathogen inhabits soil and produces multiple phytotoxins translocated from infected roots to leaves causing foliar chlorosis and necrosis. While overall soybean resistance to *F. virguliforme* is partial and inherited quantitatively, this study hypothesized the soybean-phytotoxins interaction in leaves follows the inverse gene-for-gene model demonstrated in the wheat-phytotoxins system. A soybean phenotypic category was developed to breakdown complex SDS foliar symptoms into four groups that each exhibited partial resistance (mild to no symptoms), susceptibility (both chlorosis and necrosis), chlorosis, or necrosis. Focusing on chlorosis, linkage and genome-wide association mapping identified two loci harboring the paralogous STAY-GREEN genes of soybean (GmSGR1 and GmSGR2), which were dominantly and qualitatively inherited. The expressions of STAY-GREEN genes were up-regulated in response to phytotoxin treatment, and either a functional GmSGR1 or GmSGR2 enabled the development of SDS foliar chlorosis. The recessive resistance to SDS foliar chlorosis only occurred when both STAY-GREEN genes were mutated. However, soybean varieties harboring this recessive resistance have limited breeding merit because of compromised photosynthesis efficiency. Our results demonstrate the development of SDS foliar chlorosis depends on the chlorophyll degradation and leaf senescence pathway, which shares the same mechanism as the stay-green phenotype that was first described in pea as one of Mendel's seven genetic traits. Therefore, the development of SDS foliar chlorosis is a mirror image of Mendel's stay-green trait, but not a case following the inverse Flor's gene-for-gene model.

S-04

The genetics of aphid resistance in soybean

Dechun Wang | Michigan State University | wangdech@msu.edu

The soybean aphid is an important insect of soybean. Use of aphid resistance cultivars is the most economical and environmentally friendly method to control the insect. Since the first discovery of soybean aphids in the US in 2000, significant efforts have been put in the genetic study of soybean resistance to the insect. To date, resistant loci have been mapped to seven genomic regions on five chromosomes. A review of the mapping results, the effects of the resistant loci, and the effects of combining multiple resistant loci will be presented.

S-05

Changes in membrane lipids in soybean leaves in response to soybean aphid infestation

Gustavo C. MacIntosh | Iowa State University | gustavo@iastate.edu

Khoi Nguyen, Iowa State University

Jessica Hohenstein, Iowa State University

Aphids are specialized insects that feed on phloem sap. They alter plant metabolism, affect plant growth and development, and are also vectors for plant viruses. Their feeding habits result in little mechanical damage and aphids avoid triggering many plant defenses commonly elicited by other herbivores. It has been proposed that aphids can suppress effective defenses through the induction of decoy responses. Several lines of evidence suggest that lipid-derived signals and phytoalexins have an effective role as defenses against these insects. Previously, we showed that soybean aphids, Aphis glycines Matsumura, affect fatty acid accumulation in soybean plants. Here, we performed lipidomics and transcriptome analyses of soybean leaves that were infested with soybean aphids for 7 days to identify further changes in lipid composition triggered by aphid feeding. We found significant changes in membrane lipid species including digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), phosphatidylglycerol (PG), lysophosphatidylethanolamine (lysoPE), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA). Transcriptome data identified a large number of differentially expressed genes associated with lipid metabolism, including changes in different fatty acid desaturases, and a strong induction of phospholipase A, phospholipase C, and phospholipase D. We also observed a strong induction of the oxylipins biosynthesis pathway, and negative regulation of sphingolipids biosynthesis. The production and transport of cuticular waxes, including the synthesis of long-chain fatty acids is strongly repressed after aphid feeding. Our results suggest that membrane phospholipids play an important role in the soybean response to aphid feeding, and that fatty acid-, PA-, and PI-derived signals likely mediate this interaction.

S-06

Potyviral effector targets and viral co-opting of the untranslated protein response in soybean

Aardra Kachroo | University of Kentuck | apkach2@uky.edu
Hexiang Luan, Nanjing Agriculture University and University of Kentucky
MB Shine, University of Kentucky
Haijan Zhi, Nanjing Agriculture University
Pradeep Kachroo, University of Kentucky

The seed-borne and aphid-transmitted potyvirus, soybean mosaic virus (SMV) causes mosaic and severe necrosis in soybean, affecting both seed yield and quality. Analysis of SMV-infected soybean plants shows ultrastructural changes associated with ER membrane reorganization ultimately resulting in the untranslated protein response (UPR). UPR, which involves the accumulation of unfolded proteins at the ER, often occurs during the ER stress response, and is an important coping mechanism for plants undergoing biotic or abiotic stresses. Depending on the severity and length of stress conditions, UPR can either induce autophagy as a mechanism of cell survival or result in cell death. The mechanisms underlying ER stress activation or viral perception by ER stress sensors in the plant are not known. Our work suggests that physical interactions between soybean eukaryotic elongation factor alpha (eEF1A) and the ER-localized SMV P3 protein might mediate viral perception leading to UPR. Chemical induction of UPR promotes SMV infection in soybean. Conversely, knockdown of eEF1A expression inhibits the ability of soybean plants to induce UPR, and enhances soybean resistance to SMV. Thus, plants lacking eEF1A or the associated eEF1B show reduced UPR in response to SMV infection and better resist viral accumulation. P3-responsive changes in the subcellular localization of eEF1A and the involvement of eEF1A in the soybean cell death response are reminiscent of the effects of the Human immunodeficiency virus 1 Nef protein on mammalian eEF1A and programmed cell death. This raises the possibility that P3 affects eEF1A function by altering its subcellular localization in infected cells. The comparable virulence-related roles of SMV P3 and HIV-1 Nef in promoting viral virulence further suggest possible parallel mechanistic functions for these viral proteins.

S-07

Leveraging RNA-seq to characterize resistance to brown stem rot and the Rbs3 locus in soybean

Chantal McCabe | USDA-ARS | Chantal.McCabe@ars.usda.gov
Silvia Cianzio, Iowa State University
Jamie O'Rourke, USDA-ARS
Michelle Graham, USDA-ARS

Brown stem rot (BSR), caused by the fungus *Phialophora gregata*, reduces soybean yield by up to 38%. While three dominant resistance loci have been identified (Rbs1-Rbs3), the gene networks responsible for pathogen recognition and defense remain unknown. Further, identification and characterization of resistant and susceptible germplasm remains difficult. We conducted RNA-Seq of infected and mock-infected leaf, stem, and root tissues of a resistant (PI 437970, Rbs3) and susceptible (Corsoy 79) genotype. Combining historical mapping data with genotype expression differences allowed us to identify a cluster of receptor like proteins (RLPs) that are candidates for the Rbs3 resistance gene. Reads mapping to the Rbs3 locus were used to identify potential novel single nucleotide polymorphisms (SNPs) within candidate genes that could improve phenotyping and breeding efficiency. Comparing responses to infection revealed little overlap in differential gene expression between genotypes or tissues. Gene networks associated with defense, DNA replication, and iron homeostasis are hallmarks of resistance to P. gregata. This novel research demonstrates the utility of combining contrasting genotypes, gene expression, and classical genetic studies to characterize complex disease resistance loci.

S-08
Soybean resistance mechanisms against Sclerotinia sclerotiorum

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Sclerotinia sclerotiorum, a predominately necrotrophic fungal pathogen with a broad host range, causes a significant yield limiting disease of soybean called *Sclerotinia* stem rot (SSR). Resistance mechanisms against SSR are poorly understood, thus hindering the commercial deployment of SSR resistant varieties. We used a multiomic approach utilizing RNA-sequencing, Gas chromatography-mass spectrometry-based metabolomics and chemical genomics in yeast to decipher the molecular mechanisms governing resistance to *S. sclerotiorum* in soybean. Transcripts and metabolites of two soybean recombinant inbred lines, one resistant, and one susceptible to *S. sclerotiorum* were analyzed in a time course experiment. The combined results show that resistance to *S. sclerotiorum* in soybean is associated in part with an early accumulation of JA-Ile ((+)-7-iso-Jasmonoyl-L-isoleucine), a bioactive jasmonate, increased ability to scavenge reactive oxygen species (ROS), and importantly, a reprogramming of the phenylpropanoid pathway leading to increased antifungal activities. Indeed, we noted that phenylpropanoid pathway intermediates such as, 4-hydroxybenzoate, ferulic acid and caffeic acid were highly accumulated in the resistant line. In vitro assays show that these metabolites and total stem extracts from the resistant line clearly affect *S. sclerotiorum* growth and development. Using chemical genomics in yeast, we further show that this antifungal activity targets ergosterol biosynthesis in the fungus, by disrupting enzymes involved in lipid and sterol biosynthesis. Overall, our results are consistent with a model where resistance to *S. sclerotiorum* in soybean coincides with an early recognition of the pathogen, leading to the modulation of the redox capacity of the host and the production of antifungal metabolites.

S-09
Rpp1 encodes a novel protein that controls immunity to soybean rust

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Susceptible soybean plants infected by virulent isolates of *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust, are characterized by tan-colored lesions and erumpent uredinia on the leaf surface. Seven loci, Rpp1 – Rpp7, that provide varying degrees of resistance to *P. pachyrhizi* (Rpp) have been identified through germplasm screening and mapped within the soybean genome. Two genes, Rpp1 and Rpp1b, map to the same region on soybean chromosome 18. Rpp1 conditions an immune response (IR), characterized by a lack of visible symptoms, to avirulent P. pachyrhizi isolates. In contrast, resistance provided by Rpp1b – Rpp7 results in red-brown foliar lesions. Rpp1 was previously mapped to a region spanning approximately 150 Kb between markers Sct_187 and Sat_064 in L85-2378 (Rpp1), an isoline developed from the susceptible Williams 82 cultivar and the resistant soybean accession PI 200492 (Rpp1). To identify Rpp1, we developed a bacterial artificial chromosome contig spanning the locus from PI 200492. Sequencing of this region identified three homologous nucleotide binding site-leucine rich repeat (NBS-LRR) candidate resistance genes, with each containing an additional N-terminal ubiquitin-like protease 1 (ULP1) domain. Silencing of the Rpp1 candidates compromised the IR in the soybean accession PI 200492. Our results indicate that Rpp1 is a novel ULP1-NBS-LRR protein that plays a key role in the IR.

S-10

How can they be so different? QDRL for watermolds and true fungi

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Quantitative disease resistance loci (QDRL) towards *Phytophthora sojae*, *Pythium irregulare*, *Py. lutarium*, *Py. oopapilum*, *Py. sylvaticum*, *Py. ultimum var. sporangiiferum*, *Py. ultimum var. ultimum*, and *Fusarium graminearum* were mapped in several advanced recombinant inbred line (RIL) populations. RIL populations from the SoyNAM population were derived from Plant Introduction PI 427136 and adapted genotypes 4J105-3-4, HS6-3976, LD02-9050, S06-13640, LG05-4835 and LG00-3372 all crossed with susceptible cultivar IA3023; an additional RIL population was derived from PI 438489B × Magellan. Unexpectedly, the vast majority of the QDRL towards each pathogen mapped to different loci, even within individual RIL populations segregating for resistance to more than one pathogen. In the IA3023 × PI 427136 population, the correlation coefficients were not significant for seed rot severity and percent germination for *Py. lutarium* compared to *Py. oopapilum*, but were significant for the same traits between *Py. lutarium* and *Py. oopapilum* and between *Py. oopapilum* and *Py. sylvaticum*. Overall, two to six QDRL were identified in each population to each pathogen and most were minor, contributing less than 10% of the phenotypic variance (PV) which is expected for necrotrophic pathogens with broad host ranges. Interestingly, there were several major QDRL (PV>20%) for *P. sojae*, *Py. ultimum var. ultimum*, and *Py. ultimum var. sporangiiferum* identified in these NAM populations. Based on fine mapping in two additional RIL populations derived from crosses with PI567301B and PI567516C, numerous candidate genes were identified that are associated with resistance to *F. graminearum*. Using a systems genetics approach, putative causal genes for resistance to *P. sojae* were identified in an advanced RIL population derived from Conrad × Sloan. Efforts are ongoing towards assessing the effectiveness of these genes and QDRL, identifying additional candidate alleles for these pathogens, and more importantly combining resistance alleles and developing germplasm with durable multiple resistance.

S-11

Changes in the soil, root and rhizosphere bacterial microbiome of soybean lines that differ in their tolerance to alkaline soils

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The microbial communities present within the endosphere, rhizosphere, and soil near the plant roots play roles in root development, nutrient acquisition, and in some cases promote growth. Due to limited iron availability in alkaline soils, iron deficiency causes decreased plant growth and declines in yield. The aim of this research project is to characterize the bacterial microbiomes in and around soybean roots in a range of soybean lines that differ in tolerance to alkaline soils. Recombinant inbred lines that showed more extreme tolerance or sensitivity to alkaline soils from the progeny of a cross between a sensitive and tolerant parent were selected for this study. The field site used had naturally occurring regions of alkaline pH with lower available iron and areas with more neutral pH. The endosphere, rhizosphere, and bulk soil of soybean plants were sampled at three growth stages. The V4 region of the 16s rRNA gene was sequenced using Illumina MiSeq and multiple bioinformatics analyses were conducted to provide data on the presence, absence, and abundance of microbial taxa. Data will be presented on how microbial communities change across a season, in alkaline soils compared to more neutral pH soils and in sensitive and tolerant lines. In the future testing of culturable microorganisms will be pursued based on hypotheses derived from theses culture independent studies to determine if any microbes have protective activity against the adverse effects of alkaline soil.

S-12

The mutual impact of cerium oxide nanoparticles and cadmium on soybean physiology and root anatomy

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The pollution of cultivated soils with both conventional and emerging contaminants is affecting plants, animals and humans. In this specific scenario, elevated levels of cadmium (Cd) in soil due to various anthropogenic activities have led to higher Cd concentrations in various crop tissues, making it a food safety concern. Meanwhile, engineered nanoparticles (ENPs) are increasingly detected in irrigation water and agricultural soils due to the rapid advancement of nanotechnology. As a result, understanding the interactions of coexisting ENPs and trace elements is critical to gaining insights into the accumulation of these two groups of chemicals in cultivated plants. Importantly, soybean (*Glycine max* (L.) Merr.) is a main protein source of the human diet that can accumulate metals (including Cd) in the grains. The investigations focused on measurements of the plant physiological parameters, with specific emphasis on the net photosynthesis rate, stomatal conductance, Fv/Fm, CeO_2NPs and Cd contents. Root anatomical parameters were also taken into account. Root apoplastic barriers were measured using a fluorescent dye under a fluorescent microscope and the association of Cd and CeO_2NPs with plant root exudates was examined. The results indicated that CeO_2NPs led to higher variable fluorescence to maximum fluorescence ratio, suggesting that CeO_2NPs enhanced the plant light energy use efficiency by photosystem II. In addition, the presence of CeO_2NPs did not affect Cd accumulation in soybean, but Cd significantly increased the accumulation of Ce in plant tissues, especially in roots and older leaves. The altered Ce in planta distribution was partially associated with the formation of root apoplastic barriers in the co-presence of Cd and CeO_2NPs.

S-13

Exploring early responses to nutrient deficiency stress in soybean

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Iron (Fe) and phosphate (Pi) are micro and macro-nutrient required by all plants. While previous studies have demonstrated Pi deficiency stresses can affect Fe stress pathways, this study provided the fist opportunity to directly compare expression profiles of plants subjected to –Fe and –Pi simultaneously. These analyses allowed us to confirm that soybean utilizes the same genes and biological pathways in response to both nutrient deficiencies, but at different times. Our data clearly indicates that soybean responds to -Fe deficiency, but Pi resupply. Genes that were shared by both –Pi and –Fe responses are involved in broad defense processes while genes unique to either –Fe or –Pi are involved in specific stress responses and hormone biosyntheses. Leveraging the gene expression profiles generated by our lab with previously identified QTL for iron and phosphate deficiency tolerance, we have identified high priority candidate genes underlying each of the QTL not obvious from gene annotations alone. Combined, these studies provide an improved understanding of genes and gene networks contributing to soybean nutrient acquisition, utilization, and homeostasis that can be exploited to improve abiotic stress tolerance through traditional breeding and cutting edge molecular processes.

S-14

Understanding and Improving Soybean Response to Elevated Atmospheric [CO_2]

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During the past two decades, soybean responses to the rising atmospheric [CO_2] have been characterized at the SoyFACE (Free Air Concentration Enrichment) facility in Illinois. As a C3 crop, soybean typically shows increased photosynthesis, aboveground biomass production and seed yield at elevated [CO_2], while partitioning efficiency (or harvest index) is decreased. However, a meta-analysis comparing 18 genotypes showed there is a significant genotypic variation in the physiological and yield responses of soybean to elevated [CO_2]. Cultivars with greater partitioning efficiency in ambient [CO_2] tend to maintain a higher partitioning efficiency in elevated [CO_2]. In this project, a set of 9 historical cultivars released in the U.S. from 1930 to 2014 were grown at SoyFACE to determine if conventional breeding has altered soybean responses to elevated [CO_2]. We hypothesize the selection for greater yields and partitioning efficiency have also selected for soybean genotypes that are more responsive to elevated [CO_2]. Additionally, we are testing a transgenic approach to improve the yield response to elevated [CO_2] in a modern soybean cultivar. We hypothesize that soybean could have insufficient sugar export capacity from the photosynthetic source to sink tissues at elevated [CO_2] as suggested by sugar-mediated inhibition of photosynthetic capacity. To overcome this possible limitation, soybean was transformed to overexpress a proton/sucrose symporter in the phloem companion cells. We believe that combining conventional breeding and genetic transformation could help adapt soybean crop production to future atmospheric [CO_2].

S-15

Enhancing resistance to iron deficiency chlorosis in soybean through high-throughput phenotyping and QTL fine mapping

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Iron deficiency chlorosis (IDC) is an important abiotic stress of soybeans in the Upper Midwest, robbing millions of bushels per year. Varietal tolerance to IDC is the preferred management tool of farmers, making the development of tolerant varieties an important goal for soybean breeders. Unfortunately, variation in IDC tolerance is under polygenic control in most breeding populations, and little is known about the physiological and molecular mechanisms underlying tolerance. The University of Minnesota Soybean Breeding Program routinely screens all breeding lines for IDC tolerance using a nursery with consistent and strong IDC pressure. However, human rating of tolerance is laborious and subjective. This presentation will present current activities and results using imagery from unmanned aerial systems for rating IDC tolerance in the context of a public soybean breeding program. Beyond routine screening and breeding activities, progress in fine mapping an important IDC tolerance QTL has been made. Progress on this fine mapping effort will be presented. Identification of the underlying causal polymorphism will contribute to our understanding of the mechanisms underlying varietal tolerance to IDC in soybean.

S-16

A protein involved in plant immunity and symbiosis

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A paradigm shift is occurring in the field of symbiotic nitrogen fixation with a growing realization of the central importance of the plant immune response in the earliest steps of rhizobial infection and establishment of the symbiosis. In order to better understand the biology of this mutualistic interaction, we conducted a phosphoproteomic study on soybean root and root hairs in response to the compatible symbiont *Bradyrhizobium japonicum*. A protein well characterized in plant immune responses was found to be phosphorylated within one-hour post-inoculation in soybean root hairs in response to *B. japonicum*. RNAi-targeted gene silencing and CRISPR-Cas targeted knock-out of one of the homologs resulted in a significant reduction in nodule formation. We introduced phosphomimetic (a mutation to D) and phospho-minus (a mutation to A) point mutations in the gene and constitutively expressed the mutated protein in soybean transgenic roots. The expression of the phosphomimic version of one phosphor-site resulted in a significant reduction in nodule formation, while expression of the dephosphorylated version of the same residue did not significantly impact nodulation. On this same protein, a second phosphorylation site was identified located within a 15 amino acid region, which appears to be present only in proteins derived from leguminous plants. When a phospho-minus version of this phosphorylation site was introduced into transgenic soybean roots, significantly fewer nodules were formed, suggesting that the site might be required for the symbiotic signaling. Interestingly, one of the receptor-like kinases involved in the early symbiotic signaling, phosphorylates this above mentioned residue located within a legume-specific motif in vitro. Currently, efforts are made to confirm the phosphorylation *in planta*.

S-17

A metabolic view inside the soybean root nodule

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Understanding the metabolic processes within plant systems known to obtain nitrogen through biological nitrogen fixation (BNF) is of enormous interest. Attaining such knowledge could be transformative in developing more sustainable agricultural practices and in efforts to genetically enable vital crops to fix their own nitrogen (e.g., maize). The symbiotic association between soybean (*Glycine max* Williams 82) and nitrogen-fixing Rhizobiaceae (*Bradyrhizobium japonicum*), for example, generates specialized organs, so-called root nodules, where BNF occurs. However, little is known about the array of metabolites involved, and their spatial distribution, which can influence this symbiosis. Recently, our group described a method for rapidly profiling the metabolome of intact soybean root nodules using laser ablation electrospray ionization mass spectrometry (LAESI-MS). This method also provided spatially resolved metabolic information on the anatomical compartments of the root nodule. For example, soyasaponins were primarily detected in the epidermis, whereas heme B and jasmonoyl aminocyclopropane carboxylate were mainly observed in the infection zone, and monosaccharides and flavonones were both in the outer and inner regions. Further exploration of the soybean root nodules with high (spatial and mass) resolution molecular tomography, via matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), revealed unexpected distribution of the metabolic activities within the soybean nodule. While many metabolites exhibited distinct spatial compartmentalization, several metabolites were asymmetrically distributed throughout the nodule (e.g., S-adenosylmethionine). These results establish a more complex metabolic view of plant-bacteria symbiosis (and BNF) within soybean nodules than previously hypothesized. Further, these findings suggest that spatial perspectives in metabolic regulation should be considered to unravel the overall complexity of interacting organisms, like those relating to associations of nitrogen-fixing bacteria with host plants.

S-18

Towards an understanding of root architecture-microbe interactions: spatial analysis of the soybean root microbiome

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Plant health is intrinsically tied to belowground root systems and the activity of associated microbes in and around roots. Although interactions between plant and beneficial microbiota have been implicated in increased plant fitness and stress tolerance, crop breeding strategies have yet to identify and leverage the traits of root and associated microbiomes that contribute to crop plant performance. One of our long-term objectives is to enhance soybean plant performance, including tolerance to stressors such as drought, by 1) identifying community profile markers that could be used to screen plant germplasm that select for beneficial root communities and 2) isolating candidate biologicals for soybean management systems. We are currently generating high-resolution spatial maps, and low-resolution temporal maps, of root microbiomes for two soybean genotypes that represent extremes in root system architecture – a high yielding elite genotype with high water-use efficiency characterized by deep roots and a cultivar from Jilin, China characterized by shallow, lateral roots. Plant root tissue and associated rhizosphere soil were harvested from the field and the lab experiments at time intervals encompassing two distinct developmental phases of soybean root growth: 1) vegetative stages in which root growth is slow and linear and 2) reproductive stages in which root growth is rapid and N-fixation strong. We designed the spatial mapping in the laboratory to capture plant recruitment and microbial colonization activities, namely by sampling up to six discrete root regions. Previous studies with the growth system used in the laboratory demonstrated repeatable rhizosphere and endosphere microbiome profiles associated with soil water content, but did not address spatial differences across the root system. Our results should provide insights into the interactions between root traits and root-associated communities, thus potentially highlighting key transitions belowground.

S-19

Exploring the microbiome from soybean fields to increase soybean health and productivity

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While yields of most major crops grown in the U.S. have been rising steadily for decades, this is not the case for all crops in all regions of the world. Reasons for yield stagnation include climate change, limitations on water supply for irrigation, input availability, or changes in soils that might include soil loss, degradation, or cumulative effects on soil microbial communities, including both pathogens and beneficial microbes, after decades of continuous production of the same crops. We believe that proactive research can identify changes in soybean microbiome that may allow us to forestall negative effects associated with short-duration crop rotations. In an ongoing study, soil and soybean samples were collected in Illinois where "yield gaps" were observed between different long-term rotation regimes. In this presentation, I will first summarize the effect of rotation on yield responses, soil health indicators, and soybean-associated microbiomes. A second part of the presentation I will update on a follow-up characterization of a virus originally discovered from soybean phyllosphere. We were able to "rescue" the virus from contigs assembled from a metatranscriptome survey. To build a cause-and-effect relationship between the virus and the potential host, we transfected the viral constructs to white mold fungus, *Sclerotinia sclerotiorum*. The virus replicates in *S. sclerotiorum* and causes reduced virulence of the white mold. Thirdly, we use viruses discovered in the microbiomes to determine the *S. sclerotiorum* RNA silencing genes responsible for processing the foreign nucleic acids. These genes can be potential candidates for downstream development of an RNA silencing-based approach to control the white mold fungus that causes Sclerotinia stem rot in soybean.

S-20

Detection of diverse virus-like sequences in metatranscriptomes of soybean phyllosphere microbiomes

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Analysis of soybean phyllosphere metatranscriptomes from large numbers of field-grown leaf samples over multiple years allows the unbiased detection and relative quantification of viruses of soybean and associated organisms without prior knowledge of their occurrence. Metatranscriptome analysis also obviates the need to culture host organisms prior to virus characterization, which can be problematic with biotrophic pathogens. In addition to previously described soybean-infecting viruses, sequences derived from novel viruses of soybean, soybean aphids and soybean associated fungi were detected in multiple years. The analysis assembled the complete nucleotide sequence of a new soybean-infecting member of the genus Nepovirus that was named soybean latent spherical virus. A novel picorna-like virus with distinct molecular features was discovered from soybean leaf samples and independently detected in soybean aphids (*Aphis glycines*) and named *Aphis glycines* virus 1. The most diverse group of viruses detected were of fungal origin. The analyses identified 25 partial genome sequences that represented at least 22 mycovirus genomes, only one of which had been described previously. The novel mycovirus genomes showed similarity to nine distinct lineages of viruses with RNA genomes including the genera Alphapartitivirus, Botybirnavirus, Endornavirus, Mitovirus, Mycoflexivirus, Ourmiavirus, Totivirus, Victorivirus, family Tombusviridae, and order Mononegavirales. One sequence was detected that was similar to members of the family Gemycircularvirus, which is a group of viruses with single-stranded DNA genomes. Viruses infecting pathogens and pest of soybean can be beneficial to soybean production in that they have the potential to debilitate their hosts thereby reducing the severity of damage caused to crops.

S-21

New oil traits for soybean as a sustainable and high-quality feedstock for aquaculture

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Global seafood demands are increasing, and aquaculture is a way to satisfy the demands. Soy products as fish food ingredients are sustainable and viable for fish aquaculture. However, the current soy-based feed lacks sufficient EPA/DHA omega-3 fatty acids and other oil-based feed components, like astaxanthin pigments, 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 salmon and trout. In addition, oils with high omega-3 fatty acid content are prone to oxidation, 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 to extracted soybean oil and increase shelf-life to farmed fish. 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 event. The goal of this 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 (*Ostreococcus tauri* Δ6-desaturase, *Physcomitrella patens* Δ6-elongase, *Thraustochytrium* sp. Δ5-desaturase, *Phytophthora sojae* 12-desaturase, *Phytophthora infestans* 3-desaturase) were synthesized with barley HGGT, which is involved in the production of vitamin E. *Adonis aestivalis* βring oxygenase (keto2) and astaxanthin synthase (HBFD) were used for astaxanthin production. An 8-transgene expression vector under seed-specific promoters was constructed and transformed into soybean. T3 seeds with up to 8% omega-3 EPA, 1,000 µg/g vitamin E, and 150 µg/g astaxanthin have been generated. In addition, elongase 5 (OtElo5) and desaturase 4 (O809D4) transgenes from *Ostreococcus tauri* are being combined with the five EPA transgenes for DHA production for transformation into soybean.

S-22

Approaches for identifying QTL associated with protein and oil contents and compositions in soybean [*Glycine max* (L.) Merr.] seed

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Soybean [*Glycine max* (L.) Merr.] processors desire cultivars with both elevated seed protein and oil contents as well as specific amino acid and fatty acid profiles. Thus, significant efforts have previously been made towards the identification and validation of QTL associated with seed composition traits. Protein content is generally negatively correlated with oil content and seed yield, making the development of cultivars with both high protein and high oil difficult. Our first approach in the task of identifying and validating QTL was a meta-analysis of QTL mined from literature for seed contents of protein and oil, as well as seed amino acid and fatty acid compositions. A total of 55 meta-QTL for these seed traits were detected on 6 of 20 chromosomes. Meta-QTL identified narrower confidence intervals than original QTL and candidate genes were identified within each meta-QTL. For our second approach, we conducted a genome-wide association study (GWAS) using phenotypic data collected from five environments for 621 soybean accessions and 34,014 SNP markers. Among the seed composition traits analyzed, three and five genomic regions significantly associated with seed protein and oil contents, respectively, were identified. QTL on chromosomes 15 and 20 associated with seed protein and oil contents were reconfirmed by our GWAS approach, both exhibiting a negative relation between the two traits. A multi-trait mixed model allowed identification of "common effect" loci on chromosome 5 that increased oil with no effect on protein and chromosome 10 that increased protein with little effect on oil, demonstrating the possibility of reducing the negative relationship between protein and oil. Frequencies of positive effect haplotypes from chromosomes 5, 10, 15 and 20 for protein and oil in germplasm across maturity groups and geographic regions will be helpful for finding genetic sources for improvement of these seed traits for specific geographic regions.

S-23

Mapping seed protein QTL and progress in cloning

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Soybean [*Glycine max* (L.) Merrill] is grown primarily as a source of protein and oil and the value of the crop is dependent on the concentration of these components in the meal. Over the last two decades, many quantitative trail loci (QTL) controlling seed protein and oil concentration have been mapped as shown by 240 associations between seed protein and genetic markers listed on Soybase. The two protein and oil QTL that have been mapped in a large number of studies and have generally been shown to have the greatest effect on these traits are cqSeed protein-003, which maps onto chr 20, and cqSeed protein-001, which maps onto chr 15. The high protein allele for cqSeed protein-003 was shown to negatively impact yield for two different allelic sources tested in the northern US, but the impact on yield in the southern US has been inconsistent. The high protein allele for cqSeed protein-001, which has a smaller effect on protein than cqSeed protein-003, was not significantly associated with lower yield, but there was a trend of lower yield for lines that carry this high protein allele. There have been efforts to fine map both QTL with the eventual goal of cloning them. Through selecting and testing progeny of recombinant plants, we have mapped cqSeed protein-003 to an interval less than 80 kb. Within the interval, candidate genes were identified based on the Williams 82 genome assembly. We are now knocking out the most likely candidate gene in the interval to verify whether it is cqSeed protein-003.

S-24

Development of an improved baking oil soybean by combining high saturated fatty acid and high oleic acid traits

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Soybean (*Glyicine max* (L.) Merr) is a commodity crop highly valued for its protein and oil content. The high percentage of polyunsaturated fatty acids in soybean oil results in low oxidative stability, which is a key parameter for usage in baking, high temperature frying applications, and extended shelf life of packaged products containing soybean oil inclusion. Introduction of a seed-specific expression cassette carrying the Arabidosis transcription factor WRINKLED1 (AtWri1) into soybean, led to seed oil with levels of palmitate up to approximately 20%. Stacking of the AtWri1 transgenic allele with a transgenic allele harboring the mangosteen steroyl-ACP thioesterase (GmFatA) resulted in an oil with total saturates up to 30%. The creation of a triple stack in soybean, wherein the AtWri1 and GmFatA alleles were combined with a FAD2-1silencing allele led to the synthesis of an oil with 25% saturates and up to 60% oleate. Constructs were then assembled that carry a dual FAD2-1 silencing element/GmFatA expression cassette, alone or combined with an AtWri1 cassette. These plasmids are designated pPTN1289 and pPTN1301, respectively. Soybean events carrying the T-DNAs of the respective binary plasmids have been characterized under both greenhouse and field conditions. Transgenic events carrying the T-DNA of pPTN1289 displayed an oil with stearate levels between 18% to 25%, and oleate in the mid to upper 60%, with reduced palmitate (<5%). While events combined with the AtWri1 (pPTN1301) had similar levels of stearic and oleate levels, but containing wild type levels of palmitate. The modified fatty acid composition results in an oil quality with higher oxidative stability, and functionality attributes for end use in baking applications.

S-25

The next commodity soybean

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Soybean value depends on the position on the value chain. Soybean is processed into two main products, the vegetable oil and a protein-rich meal. While it was originally grown in the US as a forage crop, soybean became the major domestic oilseed crop up to 2005 when nutrition facts labels were required to include trans fat content. Changes in the food industry reduced demand for commodity soybean oil. Soybean presently derives over two thirds of its value from the meal. Competition from other protein sources put pressure on soybean meal to provide increased value for livestock feed formulations. Out of this scenario is an opportunity for a new soybean that delivers increased value through functional traits for both oil and meal. Identifying the optimum set of seed composition characteristics that improve the value of soybean while maintaining high yield potential is critical to the success of the crop. The high oleic/low linolenic acid oil trait plus the low raffinose family of oligosaccharides meal trait is a combination that has potential to be the next commodity soybean. Understanding the biological effects of this combination will be important to ensure more functional soybeans can be successfully deployed and utilized throughout the value chain.

S-26

SoyaGen: A translational genomics project for short-season soybean in Canada

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SoyaGen is a translational genomics project that aims to exploit genomics to consolidate and further expand the soybean crop, already the third most important in Canada at close to 7.3M acres in 2017. An extensive characterization of genetic diversity, both at the nucleotide and structural levels, was achieved through whole-genome sequencing of a representative set of lines, yielding a catalogue of close to 5M SNPs/indels and 100K structural variants. Having captured haplotype diversity in Canadian short-season soybean in this fashion, these variants can be rapidly and accurately imputed following inexpensive genome scans (using GBS or arrays) on any line. To render this information more useful to breeders, we have developed a tool to facilitate the translation of SNP haplotype information in genic regions into a set of alleles. Thanks to a detailed characterization of the allelic combinations at four known genes controlling maturity (E1 to E4), we are studying how these maturity "packages" perform under a broad range of agro-climatic conditions in Canada. These powerful genotyping tools and extensive datasets are also being used to identify new genes/QTLs underlying additional traits of interest to breeders, such as resistance to pests and diseases. One such example will be provided in the form of a genome-wide association study for partial resistance to *Sclerotinia sclerotiorum*, the causal agent of *Sclerotinia* stem rot, or white mold. This collaborative project represents a highly concerted research effort and enjoys strong financial support from a wide array of stakeholders (grower organizations and seed companies) as well as both Genome Canada and Génome Québec.

S-27

Molecular links underlying pleiotropic traits in soybean

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Soybean stem growth habit is a key adaptation and agronomic trait that directly affects plant height, flowering time and duration, node production, leaf morphology, root architecture, maturity, water use efficiency, abiotic stress tolerance, and, ultimately, soybean yield. Based on the timing of the termination of apical stem growth, most elite soybean cultivars are classified into three categories of stem architecture, commonly known as determinate, semi-determinate, and indeterminate types. Classical genetic analyses demonstrated that soybean stem growth habit is regulated by an epistatic interaction between two major genes: Dt1 and Dt2. We have recently cloned Dt1, a floral repressor that prevents terminal flowering to form indeterminate stems, and Dt2, a MADS-domain factor gene that directly represses Dt1 expression in shoot apical meristems to form semi-determinate stems. More recently, we have identified several Dt2 targets associated with responses to water stress and stomatal development by a combination of RNA-seq and ChIP-seq analyses with a Dt2-specifc antibody, linking stem growth habit and flowering time to abiotic stress responses at the molecular level. Potential downstream implications of molecular links underlying these traits are under investigation. We anticipate our study will provide novel insights into the mechanisms modulating semi-determinacy and pave new strategies for optimizing plant architecture of soybean, and potentially other legume crops for enhanced environmental resilience and yield potential.

S-28

Converging mechanisms confer soybean cyst nematode resistance

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Soybean cyst nematode is perennially the most damaging disease of soybean in North America. We have been studying soybean Rhg1, the most widely used locus for SCN disease resistance in cultivated soybeans. We also have been studying other soybean loci associated with effective Rhg1 resistance, and QTLs other than Rhg1 that provide protection against SCN populations that overcome the PI88788 source of Rhg1. A number of intriguing functional and genome evolution features have emerged. We previously reported that Rhg1 encodes a toxic version of α-SNAP. α-SNAP and NSF proteins are core eukaryotic housekeeping proteins that sustain cellular vesicle trafficking by recycling SNARE protein bundles. We have now found co-evolved α-SNAP and NSF modifications in different SCN-resistant soybean genotypes. This includes a special NSF allele on a separate chromosome from Rhg1, encoding an NSF protein not typical to other plants or animals. This NSFRAN07 is present in 11% of the >19,000 G. max lines in the USDA collection, but is 100% associated with resistance-conferring Rhg1. The NSFRAN07 protein is apparently required for the viability of soybeans that carry resistance-conferring Rhg1 alleles, a finding with practical implications for resistance breeding. In other work, we will report the striking finding that a separate type of SCN resistance involves altered regulation of another SNAP proteins. Lastly, we will present new findings about Rhg1-mediated resistance. We previously reported that Rhg1 is a complex locus carrying up to ten repeats of a four-gene block, with three unrelated genes at Rhg1 that each make contributions to SCN resistance. We will present evidence that Rhg1 represents a resistance stack in which distinct resistance mechanisms are encoded by at least two of the Rhg1 genes, which have converged into tight linkage and undergone copy number expansion.

S-29

A molecular catalog of independent mutations that inactivate Argonaute 5 with effects on the gene silencing pathway in soybean

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The "saddle" pattern of mixed black and yellow colors on the same soybean seed results from interactions of silencing alleles at the I (inhibitor) locus with recessive alleles of the K1 locus, recently identified to encode an Argonaute5 (AGO5) protein of the RNAi pathway (Cho et al., Plant Cell 2017). Recessive k1 alleles overcome the effect of the dominant I and ii alleles that normally inhibit seed color by producing short-interfering RNAs (siRNAs) targeting chalcone synthase (CHS) mRNAs. In a Clark mutant (PI547439) with homozygous ii k1 alleles, we found a 129 bp deletion in the AGO5 gene that would lead to an inactive protein. We then used NGS amplicon sequencing of the 6.2 kb AGO5 gene (Glyma.11G190900) from a number of additional spontaneous saddle pattern mutant lines from the USDA germplasm collection and determined that five more of them had different lesions in the AGO5 gene relative to each parent variety. Generally, these were small deletions or insertions in one of the 20 exons that would cause altered protein structure or premature termination. The letter K of this locus appears to have been chosen as the gene symbol from Kurakake, the Japanese variety that was used as the source of the saddle trait in the first reported inheritance studies in 1929. We examined two varieties named Kurakake and they both contained the same 4 bp deletion within Exon 6 of the AGO5 gene that would cause premature termination. However, there are other genes that produce a saddle phenotype as does the ii k1 combination, including the ik allele of the I locus and the k3 allele. We are currently attempting to determine other genetic lesions in addition to the K1 (AGO5) gene that affect the silencing pathway in a visible manner producing the saddle phenotype.

S-30

The classical dt1-t allele responsible for tall determinate stem architecture in soybean is caused by two of the identified missense alleles of dt1

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Soybeans [*Glycine max* (L.) Merr.] have diverse morphological variations in stem architecture, and the variations are generally classified into two major categories based on the architecture of stem termination, termed indeterminate and determinate. Indeterminate lines (Dt1) continue stem growth after flowering, resulting in relatively tall plants with many nodes; determinate lines (dt1) abruptly halt stem growth just after flowering, which results in thick stem tips and comparably shorter plants than indeterminate ones. Two additional classifications in soybean stem growth architecture, semi-determinate (Dt2) and tall determinate (dt1-t), were proposed to explain soybean lines which have intermediate stem length between indeterminate and determinate soybeans. Two of the genes affecting stem architecture have been cloned in soybean in soybean: Dt1 and Dt2. Dt1 (GmTfl1, Glyma.19g194300) is a homolog of *Arabidopsis* Terminal Flower 1 (TFL1) where the functional gene participates in forming indeterminate stems in soybean and four missense mutations have been described as responsible for determinate types. Overexpression alleles of Dt2 (Glyma.18G273600) result in semi-determinate stem types in the presence of Dt1. The four missense alleles of dt1 are: R62S, P113L, R130K, and R166W. A previous study determined there was a third allele at the dt1 locus termed dt1-t that was responsible for the tall determinate stem architecture. The aim of this work was to clarify the molecular basis of the dt1-t allele. Whole genome re-sequence data of the classical donors of the tall determinate trait 'Soysota' and 'Peking' revealed that they possess either the R62S or R130K alleles of Glyma.19g194300. Further research is directed at molecular breeding for the dt1-t alleles to determine the effect on stem architecture and lodging in MG V targeted soybean lines with the R62S or R130K alleles compared with dt1 alleles typically utilized in the US south to control lodging.

S-31

Application of genomics to multi-trait soybean variety development

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The objective of this study was to investigate the impact of integrating Marker Assisted Selection (MAS) for single gene and oligo-genic traits and Genomic Selection (GS) for yield in a soybean variety development system through use of simulations, decision classifier metrics, and cost analysis. The breeding goals of the system are to identify soybean varieties adapted to maturity zones II, III and IV, with the greatest genotypic values for yield, while assuring that the varieties will not lodge and are resistant to *Phytophthora* Root Rot and race 1 of Soybean Cyst Nematode. Impacts were evaluated in terms of cost and ability to meet the breeding goals. Results indicate that MAS for lodging, PRR, SCN and maturity can be implemented with greater efficiency and efficacy than phenotypic selection and that GS for yield is more efficient than phenotypic selection when used to identify lines to cross. However, GS is not efficient or effective for selecting lines to advance through the various stages of variety development.

S-32

Using genomics for high-resolution haplotype characterization and identifying novel trait-associated variation

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Maintaining genetic diversity within commercial breeding programs is essential for sustaining genetic gain over time. However, continuous selection of loci conditioning favorable phenotypes for yield reduces available diversity. Recent advances in genomic technologies have enabled cost-effective ways to monitor and track haplotype diversity at high resolution. Furthermore, low-cost, highly informative sequence information has been leveraged to enable rapid identification of genomic variation associated with relevant breeding traits. Corteva's product development teams have utilized such genomic information to evaluate diversity within their breeding germplasm, improve marker-assisted breeding efficiency, and accelerate genetic gain.

S-33

Integration of precision phenotyping and genomics will drive future improvements in breeding and product placement.

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2018 is a significant year for soybean in US as it is the largest planted crop. At the same time, average yields in 2016 and 2017 for soy are at historic highs (~50+ Bu/ac), and the yield record for soy was set in 2016 at 171 Bu/ac. These are impressive outcomes although momentum in soy has been building for over a decade. Over the last 15 years, average yield of soybean has continued to increase at the same time as ~15M acres of production were added. The development and adoption of new technologies in breeding have been vital in driving the development of better adapted varieties with higher yields. Investments in genomics and phenotyping are improving the precision of our breeding pipelines. Rapid adoption of these technologies into commercial breeding programs presents its own challenge and requires reimagination of the breeding process to execute at scale. Integration of technologies impact precision throughout the pipeline from population development to product characterization and require reconfiguration of trait deployment, marker, and testing strategies to be effective.

S-34

The germplasm as a key factor in the changes in the soybean production systems and yield increase in South American

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The increase in soybean yields in the last 30 years has been achieved through genetic gain, management gain and the interaction between them. In the decades of the 70´s and 80´s the soybeans in South America were introduced with the "full-season" crop concept used mainly in the USA Mid-West area, under the assumption that maximum frost-free period would maximize biomass which in time would determine maximum yields and better tolerance to stress. However, in the mid 90´s in Argentina, the presence of biotic and abiotic problems related to this model leaded the industry to release early maturity group varieties capable of exploring better environments during the critical period of soybean (R3 to R5) and avoiding biotic stress factors. Early maturity groups and indeterminate growth habit, combined with new available technologies (no-till system and GMO Events with Glyphosate resistant soybeans among others), provided not only higher yield potential but also yield stability. In a similar scenario but from 2006 and on, Southern Brazil suffered a big change in the use of genetic resources in soybeans, moving rapidly from determinate cultivars of longer Maturity Groups to Indeterminate Earlier cultivars, leading to a big increase in the yields and a wider window of opportunity for planting. In several areas, indeterminate and early maturity genotypes were key to the double crop or "safrinha" system (soybean-corn double crop) which rapidly grew in area. At present, the use of early maturities with indeterminate growth habit cultivars is also growing in the Cerrados area and it is yet to be seen how spread out this practice will be in the future. The aim of this work is to demonstrate how changes and diversity in genetic offer throughout the last decades influenced deep changes in the production systems in different areas of South America.

S-35

Increasing the rate of genetic gain for yield in soybean breeding programs

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Increases in soybean yield through breeding have been slower than growers expect, with a rate of genetic gain estimated at 0.43 bu/ac/yr. The rate of genetic gain from a single cycle of selection can be predicted by the breeders' equation ($\Delta G = S[\sigma A/\sigma P]$), where the change in yield from the parental generation to the progeny of selected parents in a single breeding cycle (ΔG) is directly proportional to the selection differential between parents and progeny (S) and the additive genetic variance (σA), and inversely proportional to the total phenotypic variance (σP), including variance due to the environment and error. Accordingly, there are several possible targets for improving the rate of genetic gain in soybean yield, including increased selection intensity, increased measurement accuracy, increased genetic diversity and additive genetic variance, and decreased length of time required for each breeding cycle. Our coordinated team effort spans eleven breeding programs across the North Central region to address several of these targets, including: (1) improving selection accuracy by incorporating pedigree, spatial, and additional phenotypic data to progeny row selections, (2) increasing useful additive genetic variance by using genomic prediction to mine germplasm from the USDA Soybean Germplasm Collection and identifying high-yield alleles from *Glycine soja*, and (3) decreasing the time per cycle of breeding selection through the application of genomic selection models derived from Northern Uniform Soybean Regional Test data. In addition, we are developing a metric to assess realized genetic gain for yield without bias from annual environmental fluctuations. These efforts are ongoing and the work in progress will be presented.

S-36
Genotype independent, meristem-based soybean transformation

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Genetic engineering/editing systems are critical tools in functional genomics research and crop improvement applications in the globally important legume crops, including soybean. Current public soybean transformation systems are limited, however, by genotype specificity, high complexity and low efficiency of the processes, and/or variable response and availability of explant target tissues. Research was conducted to develop an efficient, genotype-independent, meristem-based transformation system for soybean targeting storable, value-added, meristem explants. Embryonic axis explants isolated from imbibed, primed seed of several genotypes of soybean were targeted for DNA delivery via biolistics and Agrobacterium tumefaciens. Targeted explants were either freshly isolated, or had been dehydrated, stored for several weeks, and rehydrated prior to DNA delivery. Following DNA delivery treatments and 4 weeks of selection on spectinomycin containing culture medium, greening leaf tissue was observed. Shoots with fully developed, green trifoliate leaves were isolated and moved to rooting medium approximately 6-8 weeks after DNA delivery treatments. Expression of screenable marker genes (RFP and GUS) was monitored in putative transgenic shoots, rooted plantlets, and T1 seed. Molecular assay of primary regenerants and seedling progeny indicated stable transgene integration in T0 plants and inheritance of the transgenes in T1 progeny. Mean transformation efficiencies obtained in preliminary experiments (based on no. of events obtained per no. of explants treated) ranged from 2.1%-13.6%. Several parameters were tested during protocol optimization and results from those studies will be discussed. Protocol efficiency and flexibility were significantly enhanced via development of viable, storable, primable, "value added" explants (VAE's) competent to the transformation process.

S-37
A novel DuPont Pioneer proprietary organism discovered for plant transformation

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A novel organism capable of transforming plant cells with a recombinant screening plasmid in plant cell based high-throughput assay was identified. DuPont Pioneer's proprietary bacterial collection was used for bacterial screening. 16S rDNA sequence, fatty acid methyl esters (FAME) and MALDI-TOF resulted in a species level match to Ochrobactrum. Additional characterization utilizing the genomic sequence, coupled with multi loci sequence analysis, resulted in identifying our isolate as a new species of Ochrobactrum. This new isolate was deposited to Agricultural Research Service Culture Collection (NRRL, Accession Number NRRL B-67078) and is named "*Ochrobactrum haywardense* H1". The *O. haywardense* H1 strain was classified as not being a plant pathogen. The *O. haywardense* H1 strain was engineered with a plasmid (s) capable to deliver DNA to various monocot and dicot plants including soybean and to produce phenotypically normal transgenic soybean events. Transgenes were stably inherited in subsequent generations and showed classical Mendelian segregation. Through tissue culture optimization and subsequent advances of the vector system, we have developed a highly efficient *Ochrobactrum*-mediated soybean transformation system capable of delivering highly efficient transformation in various DuPont Pioneer elite soybean cultivars. This system offers genotype flexibility while producing high-quality events, the advantage of speed to market, and captures significant cost savings.

S-38
Harnessing soybean promoters to optimize homology independent targeted integration

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Recent advances in CRISPR genome-editing technology has created new opportunities for targeted integration of foreign DNA sequence into the soybean genome (targeted knock-in). Targeted knock-ins using CRISPR can expand the options for DNA insertion beyond what is possible through breeding, and can introduce more precise genetic modifications than conventional transgenic methods. However, targeted knock-in in plants using the commonly used homology dependent CRISPR strategies generally have < 10% efficiency. In animal tissues, a homology independent targeted integration (HITI) knock-in strategy using CRISPR resulted in significantly higher targeted knock-in efficiencies compared to homology dependent approaches. Here, we present our approaches for optimization of HITI in lima bean and soybean tissues. A rapid particle-bombardment-based screening assay was developed for measuring targeted knock-in efficiency. Several previously characterized promoters derived from soybean were used to drive the expression of Cas9 nuclease and guide RNA (gRNA), as well as the expression of a reconstituted green fluorescent protein (GFP) reporter gene (donor DNA). Knock-in efficiencies were measured by dividing the mean number of cells with reconstituted GFP in the HITI treatments by the mean number of GFP-expressing cells obtained following introduction of reconstituted GFP. In lima bean cotyledonary tissues, extrachromosomal HITI was optimized to greater than 20% relative efficiency following modifications to the gRNA construct, and peak number of GFP expressing cells was consistently observed after 48 hours post-bombardment. Fine-tuning the regulation of CRISPR components and donor DNA through titration of co-bombarded materials as well as promoter modifications was useful for improving targeted knock-in efficiencies. Assessments of HITI in soybean tissues are in progress. As a first report of knock-in through HITI in plants, these results provide pertinent information towards developing robust CRISPR-based targeted DNA insertion strategies for crop improvement.

S-39
Lowering the costs of photorespiration

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Photorespiration is essential for C3 plants in the earth's oxygen containing atmosphere but operates at a massive expense of fixed carbon dioxide and energy. Photorespiration is initiated when the initial enzyme of photosynthesis, ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco), reacts with oxygen instead of carbon dioxide and produces the toxic compound glycolate that is then recycled by photorespiration. Photorespiration can be modeled at the canopy and regional scales to determine its cost under current and future atmospheres. A regional-scale model reveals that photorespiration currently decreases US soybean yields by 36% while consuming 40% of plant's ATP and 30% of the plant's NADPH production during peak canopy photosynthesis. Furthermore, photorespiration will continue to impact yield under future climates despite increases in carbon dioxide. Although photorespiration is tied to other important metabolic functions, the benefit of improving its efficiency appears to outweigh any potential secondary disadvantages. Synthetic biology has provided new opportunities in altering photorespiratory metabolism to improve photosynthetic efficiency. Indeed metabolic bypasses to photorespiration have been generated and have demonstrated improvements in growth. Using a synthetic biology approach we have assembled a series of multigene constructs that contain alternate metabolic pathways to bypass the native photorespiratory pathway while preventing glycolate flux into the native pathway. Greenhouse studies of the best performing events of T1 homozygous single insert plants of these photorespiratory bypasses in tobacco showed >30% biomass increases, which were confirmed and extended by replicated field trails during the 2016 & 2017 growing season. We are implementing the best performing pathways into soybean.

S-40

The long way from the lab to the field: Soybean plants expressing the sunflower HaHB4 gene outyield their controls in different environments

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Transgenic soybean plants bearing the sunflower gene HaHB4 were born in a fundamental research project aimed at understanding how sunflower plants are so adaptable to different stressing environments. HaHB4 is a transcription factor belonging to the HD-Zip family, unique to plants. This characteristic led researchers to propose such proteins as master switches to trigger responses to deal with abiotic stresses, including drought. Initially, *Arabidopsis* model plants transformed with constructs able to express this transgene showed a marked tolerance to water deficit. Later, soybean cv. Williams was transformed with PromoterHaHB4:HaHB4 and evaluated in controlled (culture chamber, greenhouse) and field trials during several years. One independent event was selected exhibiting the best performance in different environments of Argentina and US. HaHB4 transgenic soybean (B10H) outyielded or was equal to controls in all tested sites and yield increase was larger in water stressing situations. Data from the last summer in Argentina (2017/2018), where a severe drought occurred, indicated variations in biomass and seed yield across experimental plots for all tested genotypes (B10H, Williams 82 control and commercial cv. NS3228) in three environments. Under heat stress or drought, seed yield of B10H was always the largest (26%-95% yield increase), but was outyielded by the commercial line in one well-watered environment. B10H grown in the greenhouse also had a better performance than Williams. Results suggest that introduction of HaHB4 in the best adapted cultivars of each region may improve their performance when heat shock and/or water deficit takes place. This study improves our understanding of the possibilities, difficulties and significant time requirement of upscaling a technology from model plants such as Arabidopsis to evolutionary distant species like soybean. This is a rare successful case because B10H is expected to be released to the market in 2018/2019 thanks to cooperative efforts of molecular biologists and agronomists.

S-41

Soybean genome editing at Corteva Agriscience

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CRISPR/Cas technology enables precise improvement of commercially relevant crops by transgenic and non-transgenic methodologies. With its superior specificity, flexibility and multiplexing capability of the CRISPR/Cas technology, we have been introducing targeted edits within the soybean genome to improve oil and protein composition, and herbicide tolerance. To improve soybean oil composition, multiplexing knockout of four fatty acid desaturase genes (2 FAD2 and 2 FAD3) in soybean had created soybean variants with high oleic, low linolenic acid oil composition in seeds. Sulfonylurea herbicide prevent branched amino acid biosynthesis in plants by inhibiting acetolactate synthase (ALS). Resistance to sulfonylurea herbicide chlorsulfuron can be achieved by mutating a single amino acid in the ALS gene from proline to serine. There are four ALS genes in soybean. Targeted P178S editing of one of soybean ALS gene (ALS1), with a double strand DNA donor template, had been achieved that confers resistance to sulfonylurea herbicide in soybean transformation process. Looking forward, the combination of highly efficient and specific CRISPR/Cas9 technology, crop reference genomes, and robust germplasm-independent plant transformation system are coming together to enable precise genome editing in soybean.

S-42
Development of transport pathways in root nodules

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During the development of nitrogen-fixing root nodules, novel transport pathways support and sustain fluxes of material between the host plant and microbial symbionts. In mature nodules, plants fuel nitrogen fixation via the provision of photosynthetically-derived fixed carbon to the microbes; the microbes, in turn, provide fixed nitrogen to the host plant. Transport requirements vary within the different stages of root-nodule development, as do the transport structures and subcellular structures which facilitate the movement of molecules between host and microbe. In this work, we show the cellular and subcellular structures underpinning the transports of these molecules during different stages of symbiotic development within indeterminate nodules.

S-43
Gene identification and phenotypic prediction of complex traits using transcriptome data

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Changes in gene expression can play an important role in phenotypic variation and evolution. Thus, transcriptomic data for multiple traits, may be considered a bridge between the genotype and phenotype. Complex traits typically involve many genes, with small effects on phenotypic variation. Recent studies have shown that noncoding variants affect phenotypic variation, presumably via gene regulation. In this study, we used transcriptome data from early developing stage tissues (3 leaf stage or germinating shoots) and three methods, ridge regression, SVM-liner and SVM-rbf to create transcriptome-based models to predict adult traits in two species, common bean and maize. The predictive accuracies, estimated from cross-validations in different prediction methods, were as high as 0.93 for seed weight in common bean and 0.83 for flowering time in maize. Transcriptome-based prediction showed closely predictive accuracies to genomic best linear unbiased prediction (GBLUP) and outperformed the latter approach in some traits. The importance of genes were ranked based on the order of their elimination in the recursive feature elimination. We found the 500 most important genes in the predictive model were significantly enriched in some GO categories known to be involved in the regulation of a specific trait further supporting the observation that variation in the gene plays a role in the phenotypic variation. We further identified expression quantitative trait loci (eQTLs) for genes and performed association analysis between the eQTLs and traits. We found significant correlations between the importance of genes in transcriptome-based prediction and the significance of their eQTLs in the association analysis. Our study 1) showed that adult phenotypes can be predicted with early stage transcriptome data and 2) provides evidence that noncoding variants may affect phenotypic variation by compacting gene expressions through complex cis- and/or trans-regulation networks.

S-44

Functional genomics of soybean seed development

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The seed is an elegant model system for studies of plant development. It consists of three major regions - embryo, endosperm, and seed coat - and each region is further compartmentalized into subregions, tissues and cell types. In order for the seed to grow and mature, the development of each seed subregion must be coordinated. Seed development is also divided into two phases temporally. During the morphogenesis phase early in seed development, the embryo and endosperm are established and partitioned into specific subregions, each with its own unique biological function. Late in seed development, the embryo and endosperm undergo the maturation phase during which storage macromolecules accumulate to massive amounts, and the embryo becomes tolerant of desiccation. Although seed development has been characterized extensively, the gene regulatory networks that operate temporally and spatially within the seed remains to be determined. We are employing a functional genomics approach to define the gene regulatory networks that govern seed development. We used laser-capture microdissection coupled with RNA sequencing experiments to profile mRNA and micro RNA populations genome-wide in subregions, tissues, and cell types of soybean seeds throughout development. We also characterized the DNA methylome of seeds at several stages of development. To complement this information, we identified the binding sites of specific transcription factors and modified histones that control the transcriptional activities of target genes. Our studies to dissect the gene regulatory networks that operate during seed development will be discussed.

S-45

Conservation, novelty and loss of plant small RNA pathways in soybean and other legumes

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Small RNAs (sRNAs) are key regulators in plant growth and development. One subclass, phased siRNAs (phasiRNAs) require a trigger microRNA (miRNA) for their biogenesis; they are generated via distinct biogenesis pathways, predominantly dependent on the activity of 22 nt miRNAs. Most 22 nt miRNAs are processed by DCL1 from miRNA precursors containing an asymmetric bulge, yielding a 22/21 nt miRNA/miRNA* duplex. miR1510 emerged in the Phaseoleae ~41 to 42 MYA with a conserved precursor structure yielding a 22 nt monouridylated form. In soybean, this miRNA triggers phasiRNA production from numerous NB-LRRs. I will describe how this analysis of miR1510 demonstrates that (1) plants can utilize post-processing modification to generate abundant 22 nt miRNA isoforms to more efficiently regulate target mRNA abundances; (2) comparative analysis demonstrates an example of selective optimization of precursor processing of a young plant miRNA. In a parallel investigation, we have found that many plants produce abundant phasiRNAs during anther development; miR2275 triggers one class, 24-nt phasiRNAs, coincident with meiosis. We recently found that this pathway is widely present in flowering plants, indicating that 24-nt reproductive phasiRNAs likely originated with the emergence of anthers. Using miR2275 as a marker, deep comparative genomic analyses demonstrated that this miR2275/24-nt phasiRNA pathway is widely present in eudicots plants, however, it is absent in legumes. Taken together, our work demonstrates that sRNA pathways in legumes demonstrate both conserved and non-conserved attributes relative to other plant lineages.

S-46

Cloudy with a chance of mutations: Gene editing and functional analyses in soybean

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Genome editing with CRISPR/Cas9 is an emerging technology for crop improvement. In soybean, applications of this technology typically require inserting stable CRISPR/Cas9 transgenes into the genome to initiate the gene editing process. In recent years, we have focused efforts towards understanding the interactions that occur between the gene editing reagents, the transformation process, the targeted genes, the host genome, and the genetic transmission of modified loci. Interesting events have been observed, including CRISPR transgene insertions into CRISPR target sites, and on-target gene editing without evidence of stable transgene integration. This talk will focus on recent developments in gene editing for a subset of genes involved in seed composition traits. This includes gene editing events focused on a single locus, and multiplex editing of up to three loci simultaneously. This talk will also address some of the logistical challenges faced in assessing phenotypes and using the targeted mutations in trait and cultivar development, due to both biological and regulatory factors.

S-47

Insight from the wild – Dissection of broad-spectrum resistance to soybean cyst nematode (SCN) using an integrative approach

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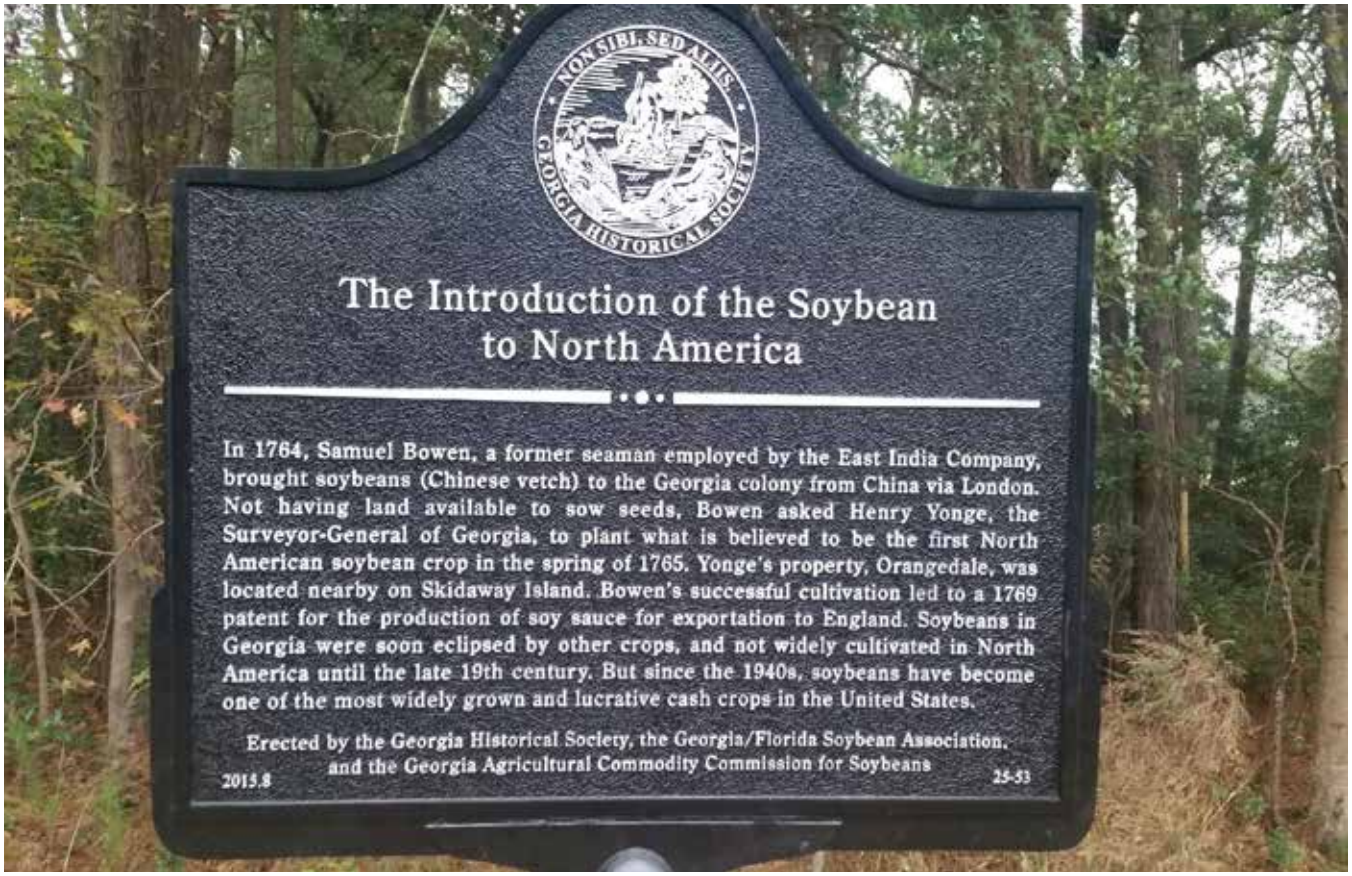
Soybean cyst nematode (*Heterodera glycine*, SCN) is the most devastating soybean pest that causes high losses in soybean production worldwide. SCN race shift uncertainties and lack of diverse resistant varieties represent two of the biggest challenges for SCN management. To meet these challenges, we identified a novel wild soybean (*Glycine soja*) genotype, S54, showing broad-spectrum resistance to two SCN types (HG2.5.7 and HG1.2.5.7) and elucidated the underlying common resistance mechanisms by integrating transcriptomic and metabolomic approaches. We identified a core set of differentially expressed genes and metabolites, including Ca2+- and salicylic acid (SA)-related signaling genes and phenolic compounds, that commonly responded to the two races. This study shows that positive regulation of Ca2+-SA signaling pathways and enhanced phenolic biosynthesis might play important roles in the broad-spectrum SCN resistance in S54. These results not only shed light on the molecular mechanisms underlying SCN resistance in wild soybean, but also facilitate developing novel and diverse soybean cultivars with broad-spectrum resistance to SCN.



This marker is erected just outside Savannah, Georgia, in commemoration of the 250th anniversary of the first soybean planted in North America.

S-48

Identification of conserved root hair-specific transcriptional regulatory elements by comparative -omic analysis

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Our understanding of the conservation and divergence of the expression patterns of genes between plant species is limited by the quality of the genomic and transcriptomic resources available. Specifically, the transcriptomes generated from plant organs are the reflection of the contribution of the different cell types composing the samples weighted by their relative abundances in the sample. These contributions can vary between plant species leading to the generation of datasets which are difficult to compare. To gain a deeper understanding of the evolution of gene transcription in and between plant species, we performed a comparative transcriptomic and genomic analysis at the level of one single plant cell type, the root hair cell, and between two model plants: *Arabidopsis thaliana* and *Glycine max*. These two species, which diverged 90 million years ago, were selected as models based on the large amount of genomic and root hair transcriptomic information currently available. Our analysis revealed in detail the transcriptional divergence and conservation between soybean paralogs (i.e., the soybean genome is the product of two successive whole genome duplications) and between *Arabidopsis* and soybean orthologs in this single plant cell type. Taking advantage of this evolutionary study, we combined bioinformatics, molecular, cellular and microscopic tools to characterize plant promoter sequences and the discovery of two root hair regulatory elements (RHE1 and RHE2) consistently and specifically active in plant root hair cells. In addition, our transcriptomic analysis also reveal a dynamic change of the soybean root hair transcriptome during its development. Specifically, 587 and 604 soybean genes are preferentially expressed during the early and the later stages of the root hair development, respectively. The role of these genes will be discussed.

S-49

Dissecting the methylomes of seed development

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We profiled soybean and *Arabidopsis* methylomes from the globular stage through dormancy and germination to understand the role of methylation in seed formation. CHH methylation increases significantly during development throughout the entire seed, targets primarily transposable elements (TEs), is maintained during endoreduplication, and drops precipitously within the germinating seedling. By contrast, no significant global changes in CG- and CHG-context methylation occur during the same developmental period. An *Arabidopsis* ddcc mutant lacking CHH and CHG methylation does not affect seed development, germination, or major patterns of gene expression, implying that CHH and CHG methylation does not play a significant role in seed development, or regulating seed gene activity. By contrast, over 100 TEs are transcriptionally de-repressed in ddcc seeds suggesting that the increase in CHH-context methylation may be a failsafe mechanism to reinforce transposon silencing. Many genes encoding important classes of seed proteins, such as storage proteins, oil biosynthesis enzymes, and transcription factors, reside in genomic regions devoid of methylation at any stage of seed development. Furthermore, we scanned soybean and *Arabidopsis* seed genomes for hypomethylated regions, or DNA Methylation Valleys (DMVs), present in mammalian cells. Seed DMVs are enriched in transcription factor genes, and are decorated with histone marks that fluctuate developmentally, resembling in significant ways their animal counterparts. We conclude that many genes playing important roles in seed formation are regulated without detectable DNA methylation events, and suggest that selective action of transcription factors as well as chromatin epigenetic events play important roles in making a seed.

S-50
Survey for the key domestication genes in soybean

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Domestication has been considered as one of the most important technological innovations in the human history. Domesticated species often exhibit convergent phenotypic evolution, termed the domestication syndrome, of which loss of seed dormancy is a component. To date, the dormancy genes that contribute to parallel domestication have not been reported. Soybean is a crop with substantial economic value, accounting for more than half of global oilseed production. It has been suggested that cultivated soybean was domesticated from wild soybean in China 5,000 years ago. Preciously, we performed a large scale assessment of soybean domestication and improvement by resequencing of 302 wild, landrace and cultivated soybean lines. Bioinformatics analysis identified a total of 121 and 109 selective sweeps during soybean domestication and improvement, respectively. We further investigate the gene responsible for specific traits via genome-wide association study, we cloned the classical stay-green G gene from soybean and found that it controls seed dormancy and showed evidence of selection during soybean domestication. Moreover, orthologs in rice and tomato also showed evidence of selection during domestication. Analysis of transgenic plants confirmed that orthologs of G had conserved functions in controlling seed dormancy in soybean, rice and *Arabidopsis*. Functional investigation demonstrated that G affected seed dormancy through interactions with NCED3 and PSY and in turn modulated abscisic acid synthesis. Therefore, we identified a gene responsible for seed dormancy, representing the first domestication gene that has been subject to parallel selection in multiple crop families. This may help facilitate the domestication of new crops.

S-51
Networks underpinning symbiosis revealed through cross-species eQTL mapping

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Interactions between species are pervasive among plants, animals, and microbes, yet the underlying molecular actors are known for only a few interactions. Many techniques have been designed to uncover genes involved in signaling between organisms. Typically, these focus on only one of the partners involved. We developed an expression quantitative trait locus (eQTL) mapping-based approach to identify cause-and-effect relationships between genes from two partners engaged in an interspecific interaction. We leverage a model plant-parasite system to demonstrate the efficacy of this approach. Gene expression measurements for ninety-eight isogenic plants (*Medicago truncatula*), each inoculated with a genetically distinct line of the diploid parasitic nematode *Meloidogyne hapla* were assayed. By sampling infected tissue from each host plant, systematic differences in gene expression across plants could be mapped to genetic polymorphisms of their infecting parasites. The effects of parasite genotypes on plant gene expression were often substantial, with up to 90-fold (p=3.2x10-52) changes in expression levels caused by individual parasite loci. Mapped loci included a number of pleiotropic sites, including one 87 kb parasite locus that modulated expression of more than sixty host genes. The 213 host genes identified were substantially enriched for transcription factors. We distilled higher-order connections between polymorphisms and genes from both species via network inference. To replicate our results and test whether effects were conserved across a broader host range, we performed confirmatory experiments using *M. hapla*-infected tomato and soybean plants. These experiments revealed that host responses are conserved across broad evolutionary distances. Our study demonstrates the efficacy of cross-species eQTL mapping in connecting genetic variation in one organism to gene expression responses in an interacting organism. The power of this approach is its ability to concurrently identify sets of hosts and pathogen genes, rather than focusing on one side of the interspecific dialogue.

Soybean diversity panel. Photo by Scott Jackson

POSTER ABSTRACTS

P-001
SoyBase: The USDA-ARS soybean genetics and genomics database

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SoyBase, the USDA-ARS soybean genetics and genomics database, provides a comprehensive collection of data, analysis tools and links to external resources of interest to soybean researchers. SoyBase is an actively curated database, with new data regularly being ncorporated, including additions to the controlled vocabularies (ontologies) for soybean growth, development and phenotypic traits, soybean genes, biparental and GWAS QTL, and genome sequences and annotations. The data in SoyBase are provided through intuitive interfaces, and are linked together wherever possible to allow easy identification and browsing of related subjects. The SoyBase home page (https://soybase.org) contains the SoyBase Toolbox, which provides quick access to a search of SoyBase, the SoyCyc metabolic pathways, the data download page, a genome sequence BLAST tool, and direct links to the genetic and sequence maps. An extensive navigation menu and site description provides facile access to all sections of SoyBase. Searching at SoyBase uses an underlying trait-based approach to return all information that is related to the search term. Numerous data types are available including genetic and QTL maps, the reference genome sequence with annotation tracks covering genetic markers, genome organization, gene annotation and expression. Pedigrees for entries in the Soybean Uniform Trials and PVP certificates have recently been updated. SoyBase includes an extensive RNA-Seq gene atlas and innovative tools for identifying fast neutron-induced mutants affecting genes or traits of interest. Several 'omics tools, for example a GO Term Enrichment tool, enable sophisticated queries on lists of genes.Please join us on Sunday at 3:30P for the SoyBase workshop.

P-002
SoySNP Registry: a database of soybean variant data

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There are over 230,000 soybean accessions in germplasm repositories worldwide, making the identification of truly unique accessions difficult. High throughput genotyping costs have dropped sufficiently to enable dense genotyping of large germplasm collections. Nevertheless, large challenges remain due to the sheer volume of such genotype data. Comparisons between genotyping projects are additionally complicated by lack of common markers among data sets, differences in accession names, SNPs called from different reference genomes, and by inconsistent data formats. Here we describe a new database for soybean genotyping data. All SNPs are assigned a new SNP ID and SNPs common between multiple datasets will have the same identifier. Old names (ex. rs and ss IDs) are kept and will be searchable. This database will be the foundation for developing new interactive tools for soybean breeders at SoyBase.

P-003

Functional characterization of a rare silicon transport allele in soybean using a CRISPR/Cas9 multiplexing approach

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Silicon (Si), the second most abundant elements in the Earth's crust, is widely recognized as beneficial for plant growth. Si plays a prophylactic role in abiotic and biotic stresses. In the present study, a diverse set of 150 soybean genotypes were screened for Si uptake and subsequently used for genome-wide association (GWA) analysis. Because of the limited variation for Si uptake, GWA failed to identify genomic loci regulating the trait. However, a Korean cultivar 'Hikmok sorip' was found to accumulate up to 3% Si (dry weight), doubling the level found in the other genotypes (1.5%). Using a bi-parental mapping population derived from 'Majesta' x 'Hikmok sorip', a highly significant QTL (Hisil) on chromosome 16 contributing over 67% of phenotypic variation was identified. Employing functional annotation of predicted gene models in the Hisil region and whole genome sequence (WGS) of parental lines, three transporter gene homologs (Hisil genes) with sequence variations were identified as the most promising candidates. The haplotype analysis performed using WGS of over 1000 genotypes revealed five more lines having alleles and high Si uptake similar to 'Hikmok sorip'. Solute transport kinetics analysed in a Xenopus oocyte assay suggested Si-efflux transport activity for all three Hisil genes. In addition, heterologous expression of Hisil gene (Lsi2c) in transgenic Arabidopsis significantly improved Si transport in leaves. For functional validation of the three Si transporter genes and to pinpoint the causal variation within the gene(s), we initiated a CRISPR/Cas9 genome editing approach. We designed two CRISPR/Cas9 constructs that expresses three gRNAs, targeting Hisil genes and 6 other genes associated with Si transport. To rapidly assess the CRISPR/Cas9 mutation, hairy root transformation was performed. Molecular assays and sequencing of individual hairy roots identified both small and relatively large deletions at the target sites.

P-004

Evolutionary differentiation among maturity and geographic groups of world soybeans based on population QTL-allele constitution of growth date traits

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Soybean is a photoperiod-sensitive crop and extremely diverse in flowering and maturity dates, which have a direct impact on its adaptability. About 10 genes conferring flowering and maturity dates have been reported and cloned. Whether these genes composes the whole genetic system of the traits is to be exhausted. To dissect the genome-wide QTL (quantitative trait loci)-allele constitution of flowering and maturity dates, a sample with 371 varieties covering 13 maturity groups (MGs) and 13 geographic regions was organized and phenotyped in two years. The restricted two-stage multi-locus model genome-wide association study (RTM-GWAS) was conducted with 20,701 SNP linkage disequilibrium blocks (SNPLDBs) as markers. A total of 52 and 59 QTL for flowering and maturity dates were detected, which explained 84.8% and 74.4% of their phenotypic variation, respectively. A total of 241 alleles of flowering date and 246 alleles of maturity date with their respective allele effects were organized into a QTL-allele matrix, respectively, which showed the genetic structure of flowering and maturity dates of the world soybeans. The detected QTL for flowering and maturity dates covered the most of those reported in SoyBase with about one-third of them have not been reported yet. There appeared specific-present alleles only in primary MGs (MGI-MGVII) but not in newly developed MG000–MG0 and MGVIII-MGX. Thus the emergence of these new MGs are due to the QTL-allele recombination rather than new mutants. Among the geographic regions, specific-present alleles were found in both the centers and non-centers in old continents as well as some in new continents. Thus these new mutant alleles might occur before new MGs came out. From the detected QTL, 110 and 99 candidate genes for flowering and maturity dates relate to 9 biological processes, respectively. The optimal crosses for shortening and extending growth periods were predicted according to the QTL-allele matrices.

P-005
Characterization of a soybean promoter responsive to drought

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The WRKY proteins form a large family of transcription factors that are involved in important physiological and biochemical processes in plants, including the response to water deficit. In a previous study, the expression pattern determined by qPCR showed that GmWRKY106 gene is differentially expressed between a drought tolerant (EMBRAPA 48) and a susceptible (BR 16) soybean genotype in water stress conditions. The putative promoter region upstream of the transcription start site (TSS) of GmWRKY106 gene was used to search for putative cis-elements. Here we present the isolation and initial characterization of three different fragments (~ 0.5, 1 and 2 Kb) of soybean GmWRKY106 promoter from both genotypes. The promoters fragments were subcloned into the pENTR/D-TOPO vector to generate entry clones, recombined into pHGWFS7 using the Gateway cloning system and transformed into K599 *Agrobacterium* cells by electroporation. As a positive control, the CaMV 35S promoter was subjected to the same cloning process. Preliminary dehydration assay has demonstrated increased GUS and GFP expression under the control of BR16 1 Kb promoter when compared to CaMV 35S promoter in hairy roots. For measuring promoter inducibility, the expression pattern determined by the promoter sequences are being characterized using stably-transformed soybean hairy roots subjected to ABA, cold, salt and dehydration treatments. A native stress inducible promoter could be a useful candidate for driving transgenes in soybean.

P-006
Discovery of the *Agrobacterium* growth inhibition sequence in virus and its application to recombinant clone screening

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While constructing soybean mosaic virus (SMV) clone vectors, we found that growth status of transformed *Agrobacterium* significantly depends on the viral strains. In particular, the clone vectors constructed with SMV SC15 can significantly suppress the growth of *Agrobacterium*. Recombinant and truncated virus vector experiments showed that the polymorphism of a P1 protein coding sequence of SC15 leads to the growth inhibition of *Agrobacterium*. But the lack of other protein encoding sequences, except for the sequence encoding coat protein, should reduce the ability of SC15 to suppress *Agrobacterium* growth. A vector (pCB301-attL-SC15P) compatible with the Gateway cloning system was constructed using this *Agrobacterium* inhibitory sequence. The results from the LR recombination reaction with pCB301-attL-SC15P and *Agrobacterium* transformation showed the valuable application potential of the *Agrobacterium* inhibitory sequence is to serve as a negative screening factor for effective recombinant clone screening in *Agrobacterium*.

P-007
Dual-targeting by CRISPR/Cas9 for precise excision of transgenes from soybean genome

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The clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 nuclease (Cas9) system has emerged as the robust gene editing tool that functions through the double-stranded break repair process leading to targeted mutagenesis in higher genomes. CRISPR/Cas9 has been simplified to a two-component system consisting of a single guide RNA (gRNA) that binds Cas9 to target genomic sites in sequence-dependent manner. In this system, target design should be the third important factor which would affect gene editing efficiency apart from cas9 species and gRNA expression properties. The present study addressed the utility of this system for excising interested genes from hairy genomes with two gRNA sequences. Soybean hairy roots was transformed by *Agrobacterium* with two constructs expressing Cas9 and two gRNAs to target an APC20 gene, using GFP as a selectable marker. Two targets selected based on Geneious 8.1 in every construct shared a spacing distance with "265bp" and "556bp" respectively. Molecular analysis of the transformed hairy roots events detected excision in both different constructs. For the construct with a "265bp" space between targets, 8 of 11 events showed a whole 265bp DNA fragment removed, other 3 events had a bi-allelic excision even though no large deletions happened. But for another "556bp" one, no large deletion happened based on up to 10 transformed events, which only observed SNPs or few bases insertion/deletion on each target site. Research above demonstrated that double targets with smaller space size tends to induce whole piece DNA deletion in CRSISPR/Cas9 system which would more likely to knock out candidate gene adequately in transgenic plants.

P-008
Advances in multiplex CRISPR/Cas9 mutagenesis in soybean

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CRISPR/Cas9 system has emerged as a powerful tool in targeted gene editing and has innovative applications in many plant species, including soybean. One of the biggest advantages of CRISPR/Cas9 is its ability to simultaneously edit multiple targets in the genome. This approach also enables unique applications, such as multigene knockouts and targeted deletions. Different strategies and toolboxes are available for designing and cloning of multiple targets in a single destination vector. In this study, we used Csy4-based excision of gRNAs to target three genes related to soybean seed composition. The efficiency of the multiplex construct was first assessed using hairy-root transformation, demonstrating that single transgenes could mutate the three targets simultaneously. Whole plant transformations (WPT) of the cv 'Bert' recovered six independent transgenic events (T0). The mutagenesis analysis revealed deletions of various sizes in all six T0 lines for all three gene targets. The majority of the detected mutations ranged from 6 bp to 104 bp in size. The target mutations in the T0 lines were stably transmitted to the T1 generation, without new modifications or reversion to wild type. T1 plants representing the progeny from three of the T0 lines (a total of 228 plants) have been analyzed for the presence of the transgene using PCR. Analysis of mutation inheritance was performed in the progeny of line WPT689-12, revealing that mutations for all three gene targets were transmitted and segregating in the T1/M1 generation. Analyses of the other T1/M1 families are ongoing. Our results demonstrated that CRISPR/Cas9 is an effective tool for multiplexing in soybean.

P-009

Increased soybean seed size and seed protein content due to a fast neutron-induced mutation

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Seed size (or single seed weight, SSW) and chemical composition are key determinants of soybean yield and seed quality. Phenotypic screening of our fast neutron (FN) mutant population identified a line, K83, that produced bigger seeds and vegetative aerial tissues compared to unmodified Williams 82. Microscopic examination of cotyledon epidermal cells showed more cells per unit area and higher stomatal index in K83 compared to Williams 82, indicating that the increased seed size is due to increased number of cells rather than due to increased cell size. Seed composition analysis indicated that K83 seeds showed consistently higher protein content compared to Williams 82 over three growing seasons. Comparative Genome Hybridization (CGH) analysis identified four deletions, encoding a total of 318 genes, in the K83 genome. Segregation analysis of BC1F2 plants indicated that the big seed phenotype is due to a single dominant locus. Moreover, the big seed phenotype co-segregated with the 361 Kb deletion in Chr. 17, indicating that a knock-out mutation in a gene(s) encoded within the deleted region is responsible for the seed phenotype. Interestingly, the deletion in Chr.17 overlaps qSw17-1, a major and stable QTL associated with seed weight in soybean. Similar to the FN K83 mutant, the increased seed weight phenotype associated with qSw17-1 is due to a dominant allele. Therefore, it is very likely that the big seed phenotype is due to the same gene in K83 and qSw17-1. A large number of seed trait QTLs have been defined in soybean (https://soybase.org/), but so far very few genes controlling seed size and quality traits have been identified to date. A main goal of our research is to complement traditional breeding approaches with induced mutagenesis technologies, including targeted gene editing, to facilitate gene discovery and seed trait improvement in soybean.

P-010

Enhancing legume transformation by altering host immune receptor–*Agrobacterium* interactions

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While *Agrobacterium tumefaciens* is renowned for its ability to deliver DNA to a wide range of plants, most soybean genotypes are recalcitrant to transformation because of a host-pathogen response that elicits a hypersensitive response and death of host tissue. Evading this host defense response in soybean would facilitate a wider range of soybean genotypes suitable for use in transgenic breeding programs. A search for soybean homologs of known plant-immunity-associated pattern recognition receptors (PRRs) identified several candidates in the soybean reference genome. Because the reference genome is from an *Agrobacterium*-resistant cultivar, re-sequencing data from an *Agrobacterium*-susceptible variety was mapped to the reference genome and analyzed for differences among the candidate genes. A PRR, Glyma.09g216400, is absent in the *Agrobacterium*-susceptible variety. Furthermore, this gene is the most similar homolog in soybean to the *Arabidopsis* Elongation Factor – Thermo unstable receptor (AtEFR), known to restrict transformation in *Arabidopsis* by recognizing the bacterial Elongation Factor – Thermo unstable (EF-Tu) protein of *Agrobacterium*. In addition, while most soybean is incompatible with *Agrobacterium*, many varieties are nodulated by *Bradyrhizobium*, which is closely related to *Agrobacterium* but possesses numerous changes in its EF-Tu protein. *Bradyrhizobium* may have evolved specific EF-Tu changes that allow evasion of the immune system or go undetected by Glyma.09g216400 or GmEFR. Replacing the recognized *Agrobacterium* protein with its ortholog from *Bradyrhizobium* through homologous recombination may allow evasion of host immune recognition, and provide an effective tool to transform resistant varieties of soybean and pulses. 10X genomic sequencing data from the susceptible soybean line and mapping of a susceptible by resistant RIL population will elucidate the other factors inhibiting *Agrobacterium*-mediated transformation of soybean.

P-011

Nested association mapping of yield, plant maturity, plant height and lodging in three *Glycine max* x *Glycine soja* RIL populations

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Given the narrow genetic base of modern soybean, discovering novel genes and alleles that are associated with beneficial traits within exotic species has the potential to substantially increase genetic gain. Traditionally, much of the genetic architecture of quantitative traits in soybean has been identified using quantitative trait loci (QTL) mapping, and more recently, genome-wide association studies (GWAS). GWAS relies on historical linkage disequilibrium (LD) and allele frequency, yet it has relatively low power to detect rare alleles. Nested association mapping (NAM) is a population design that combines these two approaches with better resolution and more power to detect associations. To dissect the genetic architecture of agronomically important traits in selected *G. soja* lines, a NAM panel compromising 464 RILs was developed using three *Glycine max* x *Glycine soja* populations. Williams 82 was used as a hub parent, crossed with plant introductions PI464890B, PI458536, and PI522226. All 464 RIL's were genotyped with the Illumina Infinium SoySNP6K BeadChip and phenotyped in eight environments across Missouri during 2016 and 2017. A GWAS was conducted using the package NAM in R. The results showed three QTLs associated with plant maturity on chromosomes 6, 11 and 12 with a positive effect on days to maturity. Another QTL was identified on chromosome 6 and was 0.3 Mb from the coding region of known flowering suppression gene, E1. A significant QTL for grain yield derived from *G. soja* was also identified in the process on chromosome 17 across four environments. Experimental lines with the homozygous *G. soja* allele yielded 6 to 11% higher than the lines with the *G. max* parent allele. In both testing years, we observed a QTL for plant height on chromosome 13 Significant associations were observed between *G. soja* allele and plant maturity, plant height, lodging, and grain yield, demonstrating the potential of diverse germplasm in soybean breeding.

P-012

Introgression of novel diversity to improve soybean yield part 1: Creating high yielding soybean lines with novel haplotypes

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Modern cultivars of soybean (*Glycine max* [L.] Merr.) have been derived from few ancestors and the current North American germplasm pool is not very diverse. The objective of this research was to develop high yielding soybean lines with novel diversity that was not present in the current Corteva soybean germplasm pool. Nine lines developed from diverse germplasm (USDA-ARS breeding program at the University of Illinois) were crossed to a RM34Elite parent to develop populations and sublines for yield testing. Across 2014-2016 yield tests at 30 locations, eleven breeding lines were identified that were equivalent to or significantly higher in yield when compared to the RM34Elite parent. Among the eleven selected lines the introgressed novel haplotypes that were not present in the current Corteva soybean germplasm pool occupied an estimated 0.8% to 10.0% of the total genome. JH-2665, the highest yielding line across years, yielded 280 kg/ha more than RM34Elite and had an estimated 8.6% of the genome containing unique diversity haplotypes. JH-2665 had 229 regions of new diversity introgression ranging from 1 to 12 cM in size, with six regions over 6 cM in length. These data demonstrate how high yielding lines with large regions of novel diversity can be developed, which will be useful for expanding the germplasm pool needed to improve genetic gain in future cultivar breeding.

P-013

Introgression of novel diversity to improve soybean yield part 2: Method for early generation population screening using F2 high parent heterosis

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The germplasm pool for North American soybean (*Glycine max* [L.] Merr.) is narrow and identifying novel and useful genetic diversity is time consuming and expensive. The objective of this research was to develop an early generation population screening method to select specific combining ability of diverse x elite populations. F2 high parent heterosis (F2 heterosis) was utilized as a tool to identify populations with the greatest potential for producing high yielding lines in subsequent generations. For set1 populations, six populations had significantly positive F2 heterosis and six had significantly negative F2 heterosis. When these populations were advanced into plant row yield trials (PRYT), 5/6 populations with positive F2 heterosis had the highest average yield of the top 5% PRYT selections, and 5/6 populations with negative F2 heterosis had the lowest average yield of the top 5% PRYT selections. For set2 populations from a different germplasm pool, seven populations had significantly positive F2 heterosis and two populations had significantly negative F2 heterosis. When advanced to PRYT testing, the two populations with the highest positive F2 heterosis value had significantly higher average population yield and average top 5% selection yield when compared to two populations with negative F2 heterosis. Using F2 heterosis as an early generation population selection tool may enable the focus of resources to identify which populations have the best opportunity to develop high yielding lines with unique diversity.

P-014

Genetic variation in ozone response of Fiskeby soybeans

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Current ground-level ozone concentrations are estimated to reduce soybean yield by 10-20%. In the absence of successful international efforts to reduce air pollution, continued genetic improvement of soybean will require breeding for enhanced ozone tolerance to sustain and/or increase soybean yields. In a previous screen of 30 plant introductions, Fiskeby III [PI 438471] was identified as ozone-tolerant and Mandarin (Ottawa) [PI 548379] as ozone-sensitive based on foliar injury assessments following short-term ozone exposures in greenhouse chambers. In a two-year study reported here, open-top field chambers were used to provide a series of season-long treatments ranging from a sub-ambient charcoal-filtered air ozone control of 25 ppb (12-hour mean) to elevated ozone treatments as high as 100 ppb (12-hour mean). In both years, early season foliar injury was associated with seed yield loss. Seed yield of Mandarin (Ottawa) was reduced 40-50 % at the highest ozone concentration, but only 10-20 % for Fiskeby III. Additional plant introductions from the breeding program at Fiskeby, Sweden were also tested. Fiskeby V [PI 360955], Fiskeby 840-2-7 [PI 438475], and Traff [PI 470930] were found to have intermediate ozone sensitivities whereas yield losses for Fiskeby 840-7-3 [PI 438477] were similar to the ozone-sensitive Mandarin (Ottawa) check. For the Fiskeby genotypes, yield reductions were the result of reduced seed size whereas in Mandarin (Ottawa) reduced seed size and seed number per plant both contributed to yield loss. Overall, Fiskeby III was the most ozone tolerant genotype of the germplasm tested. Thus, selecting the correct Fiskeby line as the source of tolerance genes is critical for breeding to improve the ozone tolerance of soybean.

P-015

Korean soybean (*Glycine max*) core collection: Genetic and phenotypic diversity, population structure, and genome-wide association study

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Core collections are small populations that represent the genetic and phenotypic diversity of the entire collection within plant germplasm resources. Developing a core collection in soybean, one of the most important crop species worldwide, is an important and valuable task. Here, we developed a Korean soybean (*Glycine max*) core collection consisting of 430 accessions, using Affymetrix Axiom 180k (180,961) SoyaSNP genotyping array data. Then, we performed genetic diversity, morphological trait and population structure analyses to construct the core collection from a total of 2,872 collections. Furthermore, to evaluate the utility of the developed core collection for entire germplasm accessions, genome-wide association studies (GWAS) for several important agronomic traits were conducted and compared. Sample call rates less than 97% were excluded, along with duplicate samples with more than 99.9% similarity, according to genotype analysis using Axiom 180K SoyaSNP from the entire collections. The core collection reflected 99% of genetic diversity of the total collections. The Korean soybean core collection developed in this study was divided into five groups based on population structure analyses. Although 74% of the resources are Korean resources, groups were not divided by country. Further, morphological aspects of the Korean soybean core collection were confirmed to represent an average of 18.1% of the total collection. In addition, we attempted to validate the core collection through GWAS for highly studied traits such as days to flowering, flower color, pubescence color, and growth habit. The results of GWAS identified an identical region with already known loci. Consequentially, the Korean soybean core collection developed in this study should provide useful material for both soybean breeding programs and GWAS.

P-016

Identification of QTL underlying protein and oil content in the population derived from wild soybean

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Soybean (*Glycine max*) is a major source of protein and oil for human food, animal feed, and industrial products world-wide. Wild soybean (*Glycine soja*) is the valuable genetic resource for soybean quality improvement. The objective was to discovery QTL associated with seed protein content and oil content from wild soybean which has not been extensively explored. Two replications of a randomized block design with the 300 F5-derived RILs from cultivated x wild soybean cross and their parents "Williams 82" and "PI479752" were grown at the USDA-ARS farms in Beltsville, Maryland for two years. The population was analyzed for oil and protein content using near-infrared reflectance method. QTL analysis with composite interval mapping method and genome-wide association study approach identified four QTL on Chrs 8, 15 and 20 that were associated with oil content, and six on Chrs 8, 12, 15, 18 and 20 with protein content in both years. The QTL explained 40.7% and 28.4% of the variation for oil content, 62.0% and 53.9% of the variation for protein content in 2012 and 2015, respectively. The QTL on Chrs 8, 15, 20 controlled both protein content and oil content. All the positive allele associated with oil content were from Williams 82, but four of the five positive alleles associated with protein content were from wild soybean. We observed that the wild soybean PI479752 may carry a major protein QTL in addition to the QTL that has been reported previously. Genomic selection in the population for the protein content and oil content could reach a prediction accuracy of 0.7 when the cross-validation was increased to 8-fold. Our results will help geneticists and breeders to utilize wild germplasm for soybean seed composition improvement.

P-017

Identification and QTL mapping for soybean physiological seeds quality

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The germination and vigor seed are quantitative traits established by plant genetics constitution, the environment effect and the interaction between genotypes and environments. The purpose of this study was to identify quantitative trait locus (QTL) associated with traits related to physiological soybean seeds quality, such as vigor and germination. The experiments were carried out at The Ohio State University, Columbus, Ohio, using a population of recombinant inbred lines (RILs) F9:10 and F9:11 generations. A total of 315 lines (RILs) of F9:10 generation were harvested in 2012 and F9:11 were harvested in 2014. The emergency test, twenty-five seeds of four replicates were sowing, per seed lot (crop year) of each RIL population. For the germination test, three replicates of 50 seeds per RIL population, per seed lot were sown in a germination paper. The experimental design was in a randomized complete block design. All statistical analyzes were performed by R software. The average of phenotypic test data was used in the map construction. The QTL analysis was Performed by Inclusive Composite Interval Mapping is additive and dominance effects (ICIM) using IciMapping V4 software. The Emergence test showed that the F9:10 RIL population seeds from 2012 crop had a significantly lower emergency percentage than the F9:11 RIL population seeds from 2014 crop, averaging 84% and 98%, respectively. The Germination test revealed that F9:10 generation seeds presented a germination percentage lower than the F9:11 generation from 2014 crop season. Nine QTLs were identified for soybean seeds physiological quality in the region of the chromosomes (Ch) 2, 7, 13, 14 and 19. For seeds harvested in 2012, QTL for emergence was found on the same chromosome region for dead seeds, chromosome 19, flanked by Glyma19g41210 and Glyma19g41390 markers, showing that this is a possible area where you can identify QTL for soybean seed quality.

P-018

Estimate of genetic and phenotypic parameters associated with soybean progenies in a recurrent selection program for yield

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In the soybean breeding, several agronomic attributes have been studied by the breeders in order to release superior cultivars. Among the main traits can be highlighted grain yield and full maturity. When it is desired to perform the plant breeding for one or more quantitative traits, it is impossible to succeed one single selective cycle. The alternative is recurrent selection. In the case of soybean, there are few reports of the use of recurrent selection. Although recurrent selection has been proposed for cross-pollinated crops, it has been widely used in the autogamous plants breeding. However, a recurrent selection program aimed for soybean grain yield is unprecedented in Brazil. The purpose was to estimate genetic and phenotypic parameters associated to the progenies of the recurrent selection program for grain yield in soybean at UFLA; and to select genetically superior progenies, with good agronomic attributes for the southern region of Minas Gerais. S0:1 progenies were evaluated in the municipality of Lavras, 2015/2016 crop season and S0:2 progenies were evaluated in 2016/2017, in Lavras, Nazareno and Itutinga. Were evaluated the traits: days for flowering, full maturity, height of insertion of the first pod, height of the plant, lodging score and grain yield. The data were analyzed using the mixed model approach. The estimates of the components of variance show the existence of variability among the progenies making possible the selection of superior genotypes. The evaluated progenies present good agronomic performance. When selecting the highest grain yield progenies, there was an increase in the values of full maturity, days for flowering, height of plants and height of insertion of the 1st pod. Recurrent selection is a promising strategy for the improvement of soybeans in Brazil, not only for the ability to generate superior cultivars, but also for reducing the complexity of conventional breeding programs.

Soybean somatic embryos drying down. Photo by Lauren Lail.

P-019

Selection of early soybean inbred lines using multiple index

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The soybean crop (*Glycine max* L.) has been studied and enhanced for most of the economically important traits. Previous researches have studied the association among them and the effect of the genotype by environment interaction (GEI); however, less is known about their correlation considering the absolute maturity, as well as the use of multiple selection indexes to study the GEI and select superior cultivars. Relying this, the aim of the present study was to identify lines that associate precocity, good yield performance and high oil and protein contents in the grains, as well as to estimate the correlation among these traits and study the effect of GEI, using a standardized multiple selection index. Trials were conducted in two crop seasons in the state of Minas Gerais, Brazil, with 39 lines in 13 evaluation environments. The experiments were conducted in randomized complete blocks design with 4 replications, and the traits grain yield, absolute maturity, and oil and protein contents in the grains were evaluated. The results indicated high experimental precision and accuracy, with significant differences among lines for all traits. High magnitude correlation between evaluated traits were found, highlighting the negative correlation between absolute maturity and protein content in the grains. The GEI was also significant, and the use of multiple selection index was efficient to identify superior and stable inbred lines by the GGE Biplot method, which explained 82.23% of the GEI effect. Lines 27 and 31 stood out from the others because they associated stability and good performance for all evaluated traits.

P-020

Development and phenotypic screening of an EMS mutant population in soybean

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Soybean is an important oil-producing crop in the Fabaceae family and there are increasing demands for soybean oil and other soybean products. Genetic improvement of soybean is needed to increase its production. In order to provide genetic diversity and resources for identifying important genes, a new ethyl methane sulfonate (EMS) mutagenized soybean population was generated using the newly released germplasm, JTN-5203 (maturity group V). Treatment of soybean seeds with 60 mM EMS concentration was found to be suitable for inducing mutation. A total of 1,820 M1 individuals were produced from 15,000 treated seeds. The resulting M2 population was planted in the field for phenotyping. After harvest, seed traits including total oil, protein, starch, moisture content, fatty acid and amino acid compositions were measured by NIR. Phenotypic variations observed in this population include changes in leaf morphology, plant architecture, seed compositions, and yield. Of most interest, we identified plants with increased amounts of total protein (50% vs. 41% for control) and plants with higher amounts of total oil (25% vs. 21.2% control). Similarly, we identified plants with increases in oleic acid content and decreases in linoleic acid and linolenic acid. This EMS mutant population will be used for further studies including screening for various traits such as amino acid pathways, allergens, phytic acids, and other important soybean agronomic traits. In addition, these mutant individuals will be evaluated in the next generation to assess the heritability. Beneficial traits from these mutants can be exploited for future soybean breeding programs. This germplasm can also be used for discovering novel mutant alleles and for functional gene expression analysis using reverse genetics tools such as TILLING.

P-021

Genetic gain in Brazilian soybean over the past 50 years

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Brazilian soybean seed yields have approximately doubled over the last 50 years. Breeding contributed new cultivated varieties, and soybean growers accessed new technologies, including new fungicides, insecticides and herbicides. Also, growers improved cultivation practices, such as better recommendation of sowing dates, seeding rates, soil fertility and plant nutrition. To understand the contribution of breeding to the improvement of soybean yield, cultivated varieties released over the time must be tested in the same environment. This work evaluated 26 Brazilian soybean varieties grown over two seasons (2016/2017 and 2017/18) in Guarapuava, Parana, Brazil. The cultivated varieties were released between 1960 and 2015, and represent the Center-South region of Brazil. The management aimed to provide the best conditions for the development of plants. There was a consistent linear increase of seed yield over time, with a correlation coefficient between yield and year of release of 0.75 in both growing seasons, which highlights the important role of breeding in yield gains. The SPAD index in R1-R2 and leaf N content also increased with year of release, with a correlation coefficient of 0.50 and 0.33, respectively, for 2016/17 growing season. Yields increased in the oldest to newest varieties from 1.9 to 5.3 Mg ha-1, and from 0.74 to 5.02 Mg ha-1, respectively, for 2016/17 and 2017/18 growing season. By linear regression of the data, the average yield gain by year of release of the cultivated varieties was 43 kg per ha per year in 2016/17 growing season and 50 kg per ha per year in 2017/18 growing season. Morphological and physiological changes that occurred in the cultivars due to breeding were also investigated.

P-022
Development of soybean accession for mixed planting with corn for forage production

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The soybean [*Glycine max* (L.) Merr.], an edible legume has a high protein content in both its hay and grain, and it is often used as an intercrop with other forage crops to increase the forage yield and quality. We selected soybean accessions derived from *Glycine soja* × *Glycine max* crosses and evaluated for forage quality and yield in a mixed planting of soybean and corn. The forage yield and quality were assessed for three cropping patterns: soybean mono planting, corn mono planting, and mixed planting of soybean and corn. Mixed planting of soybean and corn produced a higher forage yield than the corn mono planting. The crude protein and crude fat content were also found to be increased with the mixed planting of soybean and corn, as compared to that in the corn monoculture. Forage quality parameters such as ADF and NDF were also found to be increased by mixed planting. The results of this study show that mixed planting of soybean and corn is an effective intercropping system to improve the forage quality and yield.

P-023
Caterpillar QTLs and the case for metabolite-mediated pest resistance

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Leaf-feeding insects such as caterpillars and beetles are costly pests of soybean production in the Southern United States and South America. Resistance to these insects is quantitative, but landraces PI 227687 and PI 229358 are highly resistant and carry the validated soybean insect resistance QTLs M, H, G and E. QTLs M and E are a particularly effective combination, and elite lines carrying M and E are currently being bred. QTLs M and E also work synergistically with Bt and are thus an important resistance management tool. Additional QTLs would enable rotations of QTL pyramids as a resistance management strategy, and QTLs N, O and F were found in Boggs by mapping in RILs of Boggs x PI 494851 (susceptible). These were validated by selecting for and against these QTLs in an F2:3 population of Boggs x Benning and checking for resistance. The next question is whether all these QTLs have the same or different modes of action. Proanthocyanidins accumulate in the leaves of lines carrying QTL M. The gene for QTL M is an isoflavone glucosyltransferase. Taken together, these results implicate the phenylpropanoid pathway in resistance. Furthermore, QTLs G, H, F, N and O co-localize with genes for known enzymes in the pathway. QTLs F, M & O co-map with reported QTLs for seed isoflavone content, while QTLs E, F, G, M & O co-map with QTLs for partial resistance to *Phytophthora sojae* and *Sclerotinia sclerotiorum*. The possible association of resistance with compounds from the phenylpropanoid pathway (flavones, isoflavones, and proanthocyanidins) provides a framework that helps guide further studies to understand the basis of resistance and help deploy durable resistance in soybean and other legumes.

P-024

Mode of action and spectrum of Cry1Ja for transgenic applications of lepidopteran pest control

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Soybean production, with over 330 million metric tons produced worldwide annually and 85% being cultivated in the Americas, is being threatened by several key pests. Lepidopteran insects can devastate the crop and substantially decrease yield. *Bacillus thuringiensis* (Bt) has been historically used by farmers to control these pests, either as a foliar spray or as a gene source for transgenic crops that express one or more Bt toxins. As the potential increases for resistance to develop as Bt technology increases, toxins with novel and diverse modes of action (MOA) will become desirable for new insect trait development. Cry1Ja has excellent efficacy against several lepidopteran insect pests both in vitro and in planta. Insect midgut binding sites are important differentiators in Bt toxin MOA. Midgut brush border membrane vesicles from *Helicoverpa zea* (Boddie), *Spodoptera frugiperda* (Smith) and *Crysodeixis includens* (Walker) were used in competitive binding assays to characterize the MOA of Cry1Ja compared to representatives of each MOA class utilized in current commercial traits. Results from these assays reveal the potential Cry1Ja may have for transgenic insect control.

P-025

GmBEHL1, a BES1/BZR1 family protein, negatively regulates soybean nodulation

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Brassinosteroids (BRs) play an essential role in plant growth, and BRI1-EMS suppressor 1 (BES1)/brassinazole-resistant 1 (BZR1) family transcription factors integrate a variety of plant signaling pathways. Despite the fact that BRs inhibit nodulation in leguminous plants, how BRs modulate rhizobia-host interactions and nodule morphogenesis is unknown. Here, we show that GmBEHL1, a soybean homolog of *Arabidopsis* BES1/BZR1 homolog 1 (BEH1), is an interacting partner of Nodule Number Control 1, a transcriptional repressor that mediates soybean nodulation. GmBEHL1 was highly expressed at the basal parts of emerging nodules, and its expression gradually expanded during nodule maturation. The overexpression and downregulation of GmBEHL1 inhibited and enhanced the number of nodules, respectively, in soybean. Intriguingly, alterations in GmBEHL1 expression repressed the expression of genes in the BR biosynthesis pathway, including homologs of *Arabidopsis* Constitutive Photomorphogenesis and Dwarf and Dwarf 4. We also detected an interaction between GmBEHL1 and GmBIN2, a putative BR-insensitive 2 (BIN2) homolog, in soybean. Moreover, BR treatment reduced the number, but increased the size, of soybean nodules. Our results reveal GmBEHL1 to be a potent gene that integrates BR signaling with nodulation signaling pathways to regulate symbiotic nodulation.

P-026

Soybean Protein-protein Interaction Prediction Engine (Soybean-PIPE): A computational approach in soybean functional genomics

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Soybean is one of the major Canadian grain crops and its production is expanding in Canada mainly Western Canada and northern regions. The list of novel factors affecting these pathways in soybean, and in model plants like *Arabidopsis*, continues to grow suggesting the presence of other novel players which are yet to be discovered. The soybean Protein-*protein* Interaction Prediction Engine (Soybean-PIPE) is a computational tool used to predict protein-protein interactions in soybean. Protein-Protein Interactions (PPIs) are essential molecular interactions that define the biology of a cell, its development and its responses to various stimuli. Theoretically, if a gene interacts with groups of genes involved in one specific pathway, that gene might also be involved in that specific pathway ("guilt by association"). Briefly, PIPE searches for re-occurring short polypeptide sequences between known interacting protein pairs and novel proteins and predicts interactions based on protein sequence information and a database of known interacting protein pairs (to achieve a specificity of 99.95%). In an independent study (Samanfar et al., 2016), we have used three different approaches; bioinformatics (Soybean-PIPE), classical plant breeding, and molecular biology (analysis of SSR and SNP haplotypes) to identify a novel gene involved in time of flowering and maturity in soybean. This strategy successfully identified a new maturity locus tentatively called "E10" and the underlying candidate gene (FT4). Identification of molecular markers tagging the PIPE-identified genes controlling flowering and maturity in soybean will allow soybean breeders to efficiently develop varieties using molecular marker assisted breeding. Allele specific markers will allow stacking of early maturity alleles to develop even earlier maturing cultivars. This bioinformatics approach (soybean-PIPE) will also help to bridge the gap in knowledge of the flowering and maturity pathway in soybean and can be applied to other important traits such as seed protein content, oil quality and host-pathogen interactions.

P-027

SACPD-C mutations uncover an impact of stearic acid in leaf and nodule structure and morphology

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Soybean [*Glycine max* (L.) Merr.] is the most widely consumed legume crop in the world, providing 56% of the world's oilseed production (Soystats 2014). Soybean cultivars contain between 3-4% seed stearic acid. Increasing stearic acid confers a higher melting temperature and oxidative stability necessary for solid fat application. Highly-saturated soybean seed oil would be suitable for this end use. Stearoyl-acyl carrier protein desaturase (SACPD-C) has been reported to control the accumulation of seed stearic acid; however, no study has previously reported its involvement in leaf stearic acid content and impact on leaf structure and morphology. A subset of an EMS mutagenized population of soybean c.v. 'Forrest' was screened to identify mutants within GmSACPD-C gene. Using a forward genetics approach, nonsense and four missense Gmsacpd-c mutants were identified to contain not only high levels of seed, but also high nodule number, in addition to increased leaf and nodule stearic acid content. The EMS nonsense F605 mutant presented the highest seed stearic acid content even reported. Homology modeling and in silico analysis of the GmSACPD-C enzyme reveals that most of these mutations were localized near or at conserved residues essential for di-iron ion coordination. Furthermore, mutations at conserved residues cause the highest stearic acid content and correlate with the presence of cell senescence and a necrotic cavity in the nitrogen fixing nodules. Interestingly, soybean plants with GmSACPD-C mutations in non-conserved residues show an increase in stearic acid content and conserving healthy nodules. Thus, random mutagenesis and mutational analysis allows the development of high seed stearic acid content soybeans with no associated negative agronomic characteristics. Finally, results obtained from the current study uncover the impact of GmSACPD-C mutations in leaf and nodule structure and morphology.

P-028

Shoot architecture traits govern canopy coverage and their genetic control in soybean (*Glycine max* L. Merr.)

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Recent evidence has revealed that shoot architecture traits such as leaf angle and branching are important traits for plant growth and productivity in crop species such as corn, sorghum, and tomato. Variation in shoot architecture likely influences canopy light interception, photosynthesis, and source-sink partitioning efficiency, and thus is related to overall yield. Very little is known about the extent of phenotypic diversity of shoot architecture traits and their genetic control in soybean. Here, we partitioned shoot architecture into various measurable traits such as canopy coverage, light penetration through the canopy, branch angle, branch number, branch density, leaf shape and size, petiole length, petiolule length, days to flowering, maturity, determinancy, number of nodes and plant height. We examined a set of 400 diverse maturity group 1 soybean accessions to study the natural variation for shoot architecture-related traits, to identify relationships between traits, and to use association mapping to identify loci that are associated with shoot architecture traits. The panel was genotyped with 32,650 SNP markers. Phenotype data was collected using drone and ground-based imagery, in addition to manual measurements. Significant QTL associated with branch angle, number of branches, branch density, leaf length/width ratio, days to flowering, maturity, plant height, number of nodes and stem termination were detected. In most cases, these QTL overlapped with previously detected genes or QTL. For example, QTL on chromosome 19 for number of branches, number of nodes, and plant height mapped to the Dt1 gene, as expected. Interestingly, we detected a major branch angle QTL located on chromosome 19 that overlaps with QTL associated with canopy coverage and light penetration, suggesting branch angle is an important determinant of canopy coverage.

P-029

Analysis of NILs for a chromosome 5 iron deficiency chlorosis tolerance QTL in soybean

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Iron Deficiency Chlorosis (IDC) is an important nutrient disease of soybean (*Glycine max*) in the upper Midwest characterized by interveinal chlorosis and substantial yield loss in calcareous and high pH soils. In these soil types iron becomes mostly unavailable for uptake by soybean plants. With limited iron available, chlorophyll production is reduced resulting in chlorosis. Annual yield losses due to IDC in Minnesota are estimated to be $260 million. Breeding efforts for IDC tolerance are limited by a lack of genetic markers for the trait and highly variable symptoms spatially and temporally which makes phenotypic screening of breeding populations difficult. In this study, a QTL for IDC tolerance was mapped to chromosome 5, and then fine mapped to an 88 kb region. The QTL was mapped in a 'Fiskeby III' (PI 438471) x 'Mandarin (Ottawa)' (PI 548379) bi-parental population, with tolerance deriving from the 'Fiskeby III' haplotype. 16 Heterogeneous Inbred Families (HIFs) were then generated from this population for fine mapping. F9:10 NILs generated from HIFs reduced the interval to 450 kb on chromosome 5 and reduced IDC severity by 1 point on average using a 1 to 9 rating scale. A subsequent F11:12 population in 2017 revealed a NIL pair with an 88kb haplotype difference that conferred IDC tolerance when the 'Fiskeby III' haplotype was present. This was confirmed both in the field and hydroponics in 2018. These NILs offer the opportunity to clone a causative gene for an IDC tolerance QTL and better understand the physiology of this trait.

P-030

Association of extracellular dNTP utilization with a GmPAP1-like protein identified in cell wall proteomic analysis of soybean roots

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Plant root cell walls are dynamic systems that serve as the first plant compartment responsive to soil conditions, such as phosphorus (P) deficiency. To date, evidence for the regulation of root cell wall proteins (CWPs) by P deficiency remains sparse. In order to gain a better understanding of the roles played by CWPs in the roots of soybean Glycine max) in adaptation to P deficiency, we conducted an iTRAQ (isobaric tag for relative and absolute quantitation) proteomic analysis. A total of 53 CWPs with differential accumulation in response to P deficiency were identified. Subsequent qRT-PCR analysis correlated the accumulation of 21 of the 27 up-regulated proteins, and eight of the 26 down-regulated proteins with corresponding gene expression patterns in response to P deficiency. One up-regulated CWP, purple acid phosphatase 1-like (GmPAP1-like), was functionally characterized. *Phaseolus vulgaris* transgenic hairy roots overexpressing GmPAP1-like displayed an increase in root-associated acid phosphatase activity. In addition, relative growth and P content were significantly enhanced in GmPAP1-like overexpressing lines compared to control lines when deoxy-ribonucleotide triphosphate (dNTP) was applied as the sole external P source. Taken together, the results suggest that the modulation of CWPs may regulate complex changes in the root system in response to P deficiency, and that the cell wall-localized GmPAP1-like protein is involved in extracellular dNTP utilization in soybean.

P-031

A protein phosphatase, GmPP2 regulates soybean lateral root growth responsive to phosphate starvation

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As one of the essential macronutrient elements, phosphorus (P) plays a crucial role in plant growth and development. Due to its physical and biochemical properties, phosphate (Pi) is easily fixed by soils, which results in low Pi availability on soils. Low Pi availability becomes a limiting factor for crop growth and production on most of soils in China. Increased acid phosphatase (APase) activities is one of adaptive strategies for plants to P deficiency. In this study, one of the acid phosphatase, GmPP2, was found to exhibit activities of protein phosphatase. GmPP2 was localized in nucleus and plasma membrane. Expression levels of GmPP2 were enhanced by Pi starvation in soybean in all tested soybean tissues, especially in lateral root tips. Then suppressed GmPP2 expression in transgenic plants resulted in a significant decrease of soybean biomass, P content and lateral root length. Furthermore, RNA-seq data show that transcription levels of 551 genes were influenced in transgenic soybean roots with GmPP2 suppression at two P levels. Among them, eight genes were involved in the auxin signaling pathway, suggesting that GmPP2 might regulate lateral growth through auxin signaling pathway. Summarily we conclude that soybean changes root architecture in response to external phosphate availability through GmPP2, providing a potential target for breeding.

P-032

Fine-tuning flowering time in the major legume crop soybean using a deletion of the E1 paralog, E1-Like-b

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Photoperiod sensitivity is the dominant factor controlling the ratio of vegetative-to-reproductive length in soybean. As such, the maturity gene E1, which controls flowering time in response to photoperiod, is a major determiner of soybean yield. However, there exists a two-week disparity in flowering time between E1-containing cultivars and those containing the partially functional mutant e1-as. In this study we seek to exploit a knockout of the as yet uncharacterized E1 paralog, E1-Like-b (E1Lb), in order to fine-tune flowering time between the major soybean maturity groups and expand soybean yield potential. Using Fast Neutron Mutagenesis on the cultivar Williams 82 (e1-as), we generated a 2.6 Mb deletion that was mapped to the region of Chromosome 4 containing the E1Lb gene. This mutagenized line (FN Bing 31) flowered 4 days before, and matured one week earlier, than the Williams 82 control. We are currently introgressing this deletion into a functional E1 background, and intend to test its effect on yield at different latitudes within the US. These initial results highlight the potential of the E1Lblocus as an unexplored resource for fine-tuning photoperiod sensitivity in the major soybean maturity groups.

P-033

Development of an mPing-based activation tagging system for soybean

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Development of a powerful mutagenesis system will facilitate the identification of agronomically important genes in soybean. An efficient mutagenesis system requires high rates of germinal mutation, the ability to produce overexpression phenotypes (activation tagging), and the ability to readily identify the location of the mutations. We previously used the mPing transposable element from rice to create a mutagenized soybean population. This strategy showed that mPing induces mutations and preferentially inserts into gene-rich regions. While these original mPing mutagenesis lines were successful at generating some germinal insertions, they relied on the native mPing element, which generally produces recessive knockout phenotypes. In addition, the transposition rate in this populations was limited because it relied on the native transposase proteins from rice. In order to improve the system, we have developed a second-generation mutagenesis population that incorporates an mPing-based activation tag. This activation tag carries the enhancer region from the Fig Mosaic Virus, allowing for upregulation of nearby genes. Thus, this population should contain higher rates of altered gene expression, resulting in larger numbers of dominant phenotypes. In addition, these lines contain modified Pong transposase proteins designed to induce higher rates of transposition. Evaluation of the transposition in these lines has shown that transposition is occurring. The line showing the highest rate of germinal transposition is being developed into an advanced mutagenic population in preparation for phenotypic screening.

P-034

Assembly and annotation of a draft genome sequence for *Glycine latifolia*, a perennial wild relative of soybean

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[*Glycine latifolia* (Benth.) Newell & Hymowitz (2n=40), one of the 27 wild perennial relatives of soybean, possesses genetic diversity and agronomically favorable traits that are lacking in soybean. To better understand its genomic characteristics, we conducted whole genome assembly and annotation of *G. latifolia* using exclusively Chromium linked reads sequenced from a single barcoded library. The Supernova assembler yielded a scaffolded assembly of 989.63 Mb (scaffold N50=853.56 Kb), which was subsequently organized into 20 chromosome-scale pseudomolecules using genetic maps and the soybean genome sequence. Several assessments of the genome assembly, including genome completeness, gene content completeness, and assembly accuracy, all indicated a high quality scaffolded assembly which covers most of the *G. latifolia* genome and gene set. High copy numbers of putative 91-bp centromere-specific tandem repeats were observed in consecutive blocks within predicted pericentromeric regions on several pseudomolecules. No 92-bp putative centromeric repeats, which are abundant in *G. max*, were detected in *G. latifolia* or *G. tomentella*. Annotation of the assembled genome and subsequent filtering yielded a high confidence gene set of 54,475 protein-coding loci. A total of 304 putative nucleotide-binding site (NBS)-leucine-rich-repeat (LRR) genes were identified in this genome assembly. Different from other legume species, we observed a scarcity of TIR-NBS-LRR genes in *G. latifolia*. The genome sequence and annotation of *G. latifolia* will provide a valuable source of alternative alleles and novel genes to facilitate soybean genetic diversity improvement. This study also highlights the efficacy and cost-effectiveness of the application of Chromium linked-reads in diploid plant genome de novo assembly.

P-035

RNA-seq profiling of two contrasting drought-responsive soybean cultivars in leaves tissue under normal and drought stress at early development stage

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Growth, development, and thus yield in soybean, are severely affected by various environmental stresses, especially drought which can cause yield loss by approximately 11–50%. In our study, we screened soybean cultivars for their behavior against drought stress. We treated 12 soybean cultivars with drought stress at V3 stage and measured the leaf water content, chlorophyll content, and their growth condition. Among them, two cultivars showed tremendous water loss differences under drought treatment: SS2-2 and Taekwang, were drought resistant and susceptive, respective. To identify genes that response for drought resistance, transcriptomic profiling of these two cultivars was compared under both normal and drought conditions by RNA-seq. RNA samples extracted from young leaves at V3 stage were sequenced and, on average, 6.7 GB reads were produced for each sample. Differentially expressed genes (DEGs) were detected between SS2-2 and Taekwang and between treated and non-treated conditions, and gene ontology (GO) analysis was carried out. Some genes were up-regulated in both cultivars under drought stress. However, some genes are only increased in SS2-2, a drought resistant cultivar. Among these DEGs, we focused on abiotic response gene family, such as NAC, ZIP, and WRKY and the expression changes were confirmed by qRT-PCR. These results revealed the mechanism of drought stress response in soybean.

P-036

Exploring transcriptograms: the flooding tolerance in soybean Brazilian cultivars

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Flooding is one of the most damaging plant stresses and it is caused by strong and/or continuous precipitation in areas with limited drainage capacity. Here, we have used the Transcriptogramer tool to analyze transcriptome data in a genome-wide scale, providing a wide biological scenario. This is a robust and powerful approach to identify temporal gene expression profiles which provide an important characterization of dynamic biological systems. Two RNA-seq experiments were analyzed, where soybean Brazilian cultivars with contrasting genotypes were submitted to flooding. In the first experiment TECIRGA 6070 (flood-tolerant) and FUNDACEP 62 (flood-sensitive) were grown in concrete tanks filled with a lowland gleysolic soil and the stress was imposed when plants were at V6 growth stage. Total RNA was extracted from leaves 24 hours after the stress beginning. Libraries were constructed from two genotypes, 2 experimental conditions and sequenced in HiSeq™ 2000. In total, both genotypes presented 421 induced and 291 repressed genes. TECIRGA presented 284 and 460 genes up- and down-regulated, respectively, under flood condition. From these, 100 and 148 genes were exclusively up and down-regulated, respectively, in the tolerant genotype. Between cultivars, TECIRGA showed less up and down-regulated genes than FUNDACEP 62. Noteworthy GO categories in DEGs were mainly related to ethylene mediated signaling, starch biosynthetic process, photosynthetic electron transport, structural constituent of ribosome, acireductone dioxygenase [iron (II)-requiring] activity and cofactor binding. Physiological and agronomical parameters were also evaluated. The second experiment consists of RNA-seq public data in which roots of EMBRAPA 45 (flood-tolerant) and BR4 (flood-sensitive) were analyzed at V1 stage under hypoxia stress. Considering the 4 cultivars, the response of the two organs is more different than the one between the contrasting genotypes. Leaves presented more evident responses than roots probably due to the experimental condition more similar to that of the field.

P-037

Phylogenetic gene family analysis reveals a history of gene duplication and deletion in soybean and related legumes

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Polyploidy and gene duplication are some of the most important drivers of evolution and diversification among flowering plants, and legumes (*Fabaceae*) offer an invaluable model for studying how polyploidy events affect genome evolution as a large, diverse, economically important family. With high quality reference genomes available for soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), cultivated peanut (*Arachis hypogaea*), diploid peanut (*Arachis duranensis* and *ipaensis*) and alfalfa relative *Medicago truncatula*, a thorough study of how ancient polyploidies have shaped these legume genomes is possible. Two shared paleopolyploidy events are present in these legumes, at approximately 130 My and 58 My old, with a newer 8-13 My old duplication event specific to the *Glycine* lineage. To estimate the probability of duplicate gene deletion along the evolutionary history of these legumes, gene families or orthogroups were built using complete protein sequences for these legumes and grapevine *Vitis vinifera* as an out-group. 25,147 gene families were constructed from 250,514 genes, with 83.5% of all genes being represented. 15 different phylogenetic trees were then modeled which each represented a possible loss of a gene at every stage of the evolution of these species. These gene deletion model trees were mathematically compared against all 25,147 observed gene family trees to calculate the probability of losing a duplicated gene at each branch along the species tree. The soybean lineage showed a low rate of gene deletion along with most of the *Arachis* branches, while a gene loss at the base of the Faboideae directly after the duplication event 58 Mya was quite common. A gene ontology (GO) term analysis also revealed terms like "protein dimerization" and other putatively dosage-sensitive genes to be involved in gene families prone to duplicate deletion, suggesting dosage effects are a crucial component of duplicate gene evolution.

P-038

Leveraging historic and modern soybean uniform regional trial results to train genomic prediction models

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Genomic prediction is being utilized by plant breeders in multiple crop species as a method to accelerate genetic improvement. Initial examples of genomic prediction being used for crop improvement focused on large populations with simple family structures and relatively large amounts of phenotypic data per line. In reality, typical breeding programs utilize more complicated breeding designs with varying amounts of phenotypic data per line. In a typical breeding program pipeline, many lines from a family are screened on a limited basis early in development, and only a few lines per family are broadly tested later in development. This data structure leaves breeders with a limited number of lines with the best estimates of phenotypic value, and in addition, these lines often have less direct relatedness to each other, which can make sharing data between individuals challenging. Despite these challenges and limitations, breeding programs need to utilize genomic prediction to accelerate genetic improvement in order to meet future production demands. This project is focused on developing a community resource by utilizing public cooperative regional trial data from MG 00 – 4 and genotypic data from available lines to train genomic prediction models. We are interested in analyzing this data using a genomic prediction approach to both help breeders calculate genomic prediction values for selection purposes as well as to help breeders select the best parents to generate populations with favorable population means and variances.

P-039

Transposon-induced gene-silencing for gene discovery

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Glycine max has over 30,000 genes, many with unknown function. One tool used for gene discovery is the use of transposable elements. A non-autonomous transposable element from rice, mPing, preferentially inserts near genes. However, the rate of germinal transposition for mPing averages at just one heritable insertion per generation. To further increase the heritability and transposition rate, we redesigned mPing vectors to contain non-pest-derived, meristem-expressing promoters, along with tissue culture to induce transposition. We used soybean, pea, and arabidopsis promoters and terminators with germinal expression patterns to drive the gene expression of Transposase and ORF1, genes necessary for transposition. These types of promoters and terminators were used to construct a traditional insertional mutagenesis vector as well as a gene-silencing vector that uses trans-acting small interfering RNA sequences nested within mPing to silence homologous genes. Both of these constructs were biolistically transformed into soybean (cv. Jack). Each construct produced plants showing active transposition. Additionally, lines previously transformed with mPing and its transposase and ORF1 were passaged through tissue culture to induce increased transposition, which was then assessed with qPCR to measure copy number. The process is being repeated for the lines with the highest copy number. The results suggest our alternative strategy, using tasi sequences for gene silencing, may be more fruitful in producing phenotypes, as it works with duplicated genes. Confirmed insertions will be uploaded to SoyBase to facilitate gene discovery efforts in the soybean breeding community.

P-040
Monitoring Ds transposition in the soybean genome

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The maize Ac/Ds transposon system was introduced into soybean as a means to create random activation tags in the genome. Towards this goal a T-DNA element that carries the cassava vein mosaic virus promoter (CaVMV), delineated by Ds was assembled. The binary vector harboring this T-DNA element is designated pPTN999. To estimate germinal transposition frequencies the transgenic allele in a set of pPTN999 events was mapped, and subsequently stacked with an Ac transposase cassette under control of the 35s CaMV promoter. Genotyping of derived F2 populations revealed an estimated Ds germinal transposition frequency of approximately 3%, with most residing in unlinked locations relative to the original mapped locus. To date 233 Ds germinal transpositions have been mapped, of which 42 were mapped via a TAIL-PCR strategy and 130 mapped using Nanopore-based sequencing method, with remaining 61 sequence analysis on going. Moreover, 90 independent events out of approximately 400 soybean events originally developed that carry the T-DNA element from pPTN999 were also mapped via TAIL-PCR and the Nanopore-based sequencing method. Efforts are ongoing to further improve the Nanopore-based throughput genotyping platform as a means to reliably and cost-effectively map transgenic alleles in higher plants.

P-041
Genome-wide association study to dissect underlying loci for four correlated growth period traits in soybean

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Soybean (*Glycine max* (L.) Merri) is a typical short-day and photoperiod-sensitive crop. Vegetative growth period (V), reproductive growth period (R), whole growth period (V+R) and the ratio of growth period structure (R/V) are important photoperiod-related traits that are critical to adaptability and yield in soybean. To fully dissect the genetic basis of these four growth period traits and their correlations, a panel of 277 soybean accessions were genotyped with a single nucleotide polymorphisms (SNPs) chip and were phenotyped in three locations (planting in Spring and Summer in Changping and Shunyi district of Beijing respectively, and winter in Sanya) over three years. A genome-wide association study was conducted using the method of FarmCPU, and 30 SNPs over fifteen chromosomes were identified harboring 40 significant marker-trait associations (MTAs), 10, 9, 6 and 9 for V, R, V+R and R/V respectively. The significance tests for the 40 MTAs were conducted using the phenotypic variation of accessions carrying different alleles of the SNPs. Twenty-seven of the 40 MTAs displayed significant differences in the panel and seven in a subpopulation that was derived from the population structure analysis. Three SNPs, SNP1, SNP2 and SNP3, showed pleiotropy in two or three traits. SNP1, associated with V, R and V+R, was a synonymous SNP of the GmA, a member of PEBP family and was reported to relate to flowering time. Nineteen candidate genes were identified near the significantly associated SNPs. These results provide underlying genes and SNP markers for trait correlations information for further gene functional experiments and to promote molecular marker assisted breeding.

P-042
Generating high density, low cost genotype data in soybean

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Obtaining genome-wide genotype information of millions of SNPs in soybean often involves completely resequencing a line at 5x to 20x coverage. This depth of sequencing allows researchers to both call and genotype SNPs in a line. Currently, this type of resequencing has been done for hundreds of soybean lines with the data deposited in the NCBI short read achieve. This data has been used to call over 10 million SNPs in soybean. Using SNPs called from this data will make it is possible to obtain high density SNP information at an economical price at low coverage of genome sequencing data. Using a reference panel of 99 soybean lines resequenced at an average of 17x, over ten million SNPs were called using GATK's Haplotype Caller tool first. Whole genome resequencing at approximately 1x depth was performed on 114 ungenotyped experimental soybean lines. The low coverage reads were aligned to the genome and SNPs discovered in the reference panel were called in the experimental lines. Once SNPs were called, imputation using Beagle 4.1 was performed on all experimental lines. Sequencing depth coverages of the experimental lines ranging from 0.1x - 1x were evaluated and found to be up to 98.6% accurate. Genotype accuracy was inversely related to minor allele frequency, and highly correlated with marker linkage disequilibrium. The high accuracy and low cost of low coverage sequencing combined with imputation provides a low cost method for obtaining dense genotypic information that can be used for various genomics applications.

P-043
Molecular studies of mutations induced by proton beam irradiation in soybean using genotyping-by-sequencing

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Ionization radiation causes DNA single strand breaks (SSB), double strand breaks (DBS), damage or loss of bases, and intramolecular / intermolecular crosslinking. Since there is no report on the mutation rate according to the proton beam irradiation in soybean, the present study investigates the genetic variation in the soybean chromosome induced by the proton beam irradiation. Total of 22 plants including each 10 M2 plants induced from 118Gy and 239Gy of proton beam, respectively, and 2 wild-type plants (Deapungkong) were sequenced using GBS technology. Total of 7,453 SNPs were observed in proton beam irradiated M2 plants. Of these, 3,569 SNPs were observed in the genic regions. Among 5,829 union SNPs, transition and transversion were 47% and 53%, respectively. The highest substitution ratio was for transversion A / T with 37%. Most SNPs (82%) were distributed in one gene, while the rest (12%) had 2 to 5 SNPs per gene. The SNPs induced by proton beam were uniformly distributed in most of the chromosomes. This study will establish a framework for constructing a pool of mutant genetic resources using the proton beam.

P-044

Soil microbe pool and plant genotypes cooperatively shape soybean rhizosphere microbiome assembly

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Plants have evolved intimate interactions with soil microbes, which are essential for plant function by increasing nutrient availability, improving plant growth and enhancing plant health. To leverage those benefits in agroecosystems, we need to develop a foundational understanding of the factors that consistently drive plant microbiome assembly. In this study, we comprehensively characterized and compared soybean rhizosphere microbiome composition, function and microbe-microbe interactions between two soil types and six cultivars using 16S rRNA gene sequencing. The results suggest a strong rhizosphere effect on microbial community both compositionally and functionally. Proteobacteria and Actinobacteria were consistently enriched in soybean rhizosphere in both soil types, while Acidobacteria and Verrucomicrobia were steadily depleted. However, the recruitment pattern of microbes is not always consistent between soil type, with *Rhizobium*, *Streptomyces*, *Bacillus*, *Novosphingobium* and *Pantoea* being selectively enriched in the soybean rhizosphere when grown in agriculture soil, while *Dyella*, *Mucilaginibacter* and *Burkholderia* were accumulated when grown in forest soil. Soil type strongly impacted soybean rhizosphere microbial composition, indicating the indigenous microbe pool and local soil environment largely drive the assembly process. Differences between soybean cultivars were significant though smaller, and mainly exemplified by discriminant enrichment of several bacteria taxa. Microbe-microbe interactions examined by co-occurrence network analysis showed similar patterns between rhizosphere and bulk soil. However, when highly interacted taxa were taken into account, the pattern differed between cultivars, further suggesting cultivar specific preference of key microbe-microbe interactions. Taxonomic information-based function analysis demonstrated different pathways in rhizosphere, with xenobiotic and aromatic degradation, flagellar assembly, bacteria secretion and phosphotransferase system pathways being significantly enriched. This study expands our understanding of rhizosphere microbe assembly in general and contributes significant new knowledge in terms of legume-specific rhizosphere microbiome assembly.

P-045

A phosphorylation-based regulatory mechanism of G-protein signaling during nodule formation in soybean

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Heterotrimeric G proteins act as molecular switches regulating signal transduction pathways in all eukaryotes. While in animals the G-proteins are linked almost completely with GPCRs with 7TM domains, plants there are numerous examples of interactions between plant G-protein components and single TM domain containing RLKs. In this study, we have identified physical interaction between few symbiosis related receptor-like kinases and Gα and RGS proteins in soybean. Interestingly, the serine/threonine kinase domain of the receptors phosphorylated Gα and RGS proteins. Analysis of phosphorylated Gα protein identified specific amino acids which, when phosphorylated, resulted in significantly reduced GTP-binding activity. Furthermore, the phosphorylated Gα lost its ability to physically interact with the Gβ subunits. On the other hand, phosphorylated RGS resulted in significantly higher GTPase accelerating activity, favoring conversion of active Gα to its inactive form. RLK-dependent phosphorylation of Gα and RGS proteins had important physiological consequences as overexpression of phosphomimetic version of Gα and RGS proteins enhanced production of root nodule in soybean likely by modulating the signal transduction pathway. These results reveal a new phosphorylation-based regulatory mechanism of G protein-mediated signaling controlled by receptor-like kinases.

P-046

Assessing feasibility of vegetable soybean (edamame) production for the fresh market in southwest Virginia

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Feasibility is an important aspect of production and has yet to be elucidated in Virginia for fresh market vegetable soybean (edamame). The purpose of this study is to simulate fresh market production of edamame and investigate producer feasibility by developing a budgetary decision aid for Virginia growers. In 2017, fresh edamame were processed into either stripped pods or bundles of trimmed stalks, and distributed to local retailers. Data on production expenses, yield, harvest and post-harvest labor, and returns were used to develop customizable enterprise budgets. The total cost to produce edamame was $24,565/ac for 11,197 lbs of pods, and $25,744/ac for 11,745 lbs of bundles. Break-even price for pods and bundles were both $2.19/lb, but profit margin for pods ($0.97/lb) was considerably higher than that of bundles ($0.19/lb). Therefore, fresh pods may be a profitable end-product to supply retailers with than bundles. Consistent with previous literature, harvest and post-harvest labor were the biggest expense in edamame production, comprising approximately 72% of total expenses for pods and 76% for bundles. Thus, it is necessary for producers to examine feasibility of employing manual labor for harvest.

P-047

Radio sensitivity of cowpea plants after gamma-ray and proton-beam irradiation

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Cowpea (*Vigna unguiculata* L.) is rich in vitamins (B1 and B2), lysine and polyphenols, which are good for muscular growth and development, anticancer and anti-aging in human. In addition, this crop is suitable for cooking with rice, rice cakes, soups and stews. In this study, morphological responses were investigated in cowpea plants with two different types of radiations, the proton-beam and the gamma-ray. Seeds of Okdang cultivar were exposed to 100, 200, 300, 400 and 500 Gy of gamma-ray and proton-beam, respectively. After 5 days of sowing, the germination rate tended to decrease with increasing dose regardless of the type of radiation, but all treatments showed more than 90% germination rate after 10 days of sowing. The survival rate decreased significantly over 300 Gy. The survival rates of proton beam and gamma ray at 500 Gy were 35% and 27%, respectively. The half-lethal dose (LD50) of Okdang was 327 Gy in gamma ray and 330 Gy in proton beam. The plant height and the fresh weight of shoot tended to decrease with increasing dose in both radiations with a significant difference from the control group except 100 Gy of gamma-ray in fresh weight. This study will be valuable as a basic research to compare the mutagenic effects of two different types of radiation in cowpea.

P-048

Genome-wide association study reveals novel loci for SC7 resistance in a soybean mutant panel

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Soybean mosaic virus (SMV) is a member of Potyvirus genus that causes severe yield loss and destroys seed quality in soybean (*Glycine max* (L.) Merr.). It is important to explore new resistance sources and discover new resistance loci to SMV, which will provide insights to improve breeding strategies for SMV resistance. Here, a genome-wide association study (GWAS) was conducted to accelerate molecular breeding for the improvement of resistance to SMV in soybean. A population of 165 soybean mutants deprived from two soybean parents was used in this study. There were 104 SNPs identified significantly associated with resistance to SC7, some of which were located within previous reported quantitative trait loci. Three putative genes on chromosome 1, 9 and 12 were homologous to WRKY72, eEF1Bβ and RLP9, which were involved in defense response to insect and disease in *Arabidopsis*. Moreover, the expression levels of these three genes changed in resistance and susceptible soybean accessions after SMV infection. These three putative genes may involve in the resistance to SC7 and be worthy to further research. Collectively, markers significantly associated with resistance to SC7 will be helpful in molecular marker-assisted selection for breeding resistant soybean accessions to SMV, and the candidate genes identified would advance the functional study of resistance to SMV in soybean.

P-049

Co-regulation of the *Glycine max* soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE)-containing regulon occurs during defense to a root pathogen

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Heterodera glycines also known as Soybean Cyst Nematode is a major pathogen of soybean (*Glycine max*) that causes nearly a billion-dollar loss in U.S. annually. Various strategies such as plant breeding, crop rotation, biocontrol, nematicides are in practice to control this pathogen but the success is minimum. Therefore, focusing on mechanism of resistance at cellular level could provide species specific control through genetic resistance. Closer study of the infected cells in resistant variety *G. max* [Peking/PI548402] and the susceptible variety *G. max* [Williams 82 (PI518671)] through laser microdissection have revealed various unique genes that are present in *G. max* [Peking/PI548402]. Transcripts mapping of the major resistance locus, rhg1 led to the identification of alpha soluble N-ethylmaleimide sensitive fusion protein (alpha-SNAP) being present within the locus. Overexpression of these genes in susceptible cultivar *G. max* [Williams 82 (PI518671)] resulted resistance by inducing incompatible reaction and RNA interference of these genes in resistant genotypes resulted susceptible reaction by inducing compatible reaction. In this approach we have overexpressed the components of the Soluble N-ethylmaleimide-sensitive fusion (NSF) Attachment Protein (SNAP) REceptor (SNARE) complex that helps in docking of the vesicles to the membrane and subsequent release of the vesicular contents to the apoplast. The core components of this study are syntaxin 121 (SYP121), Synaptosomal-associated protein 25 (SNAP-25), Synaptotagmin (SYT), Synaptobrevin (SYB), Secretion 1/mammalian uncoordinated-18 (Munc18/[SEC1]), and N-ethylmaleimide-sensitive fusion protein (NSF). Syntaxin 121, *Glycine max* homolog of *Saccharomyces cerevisiae* Suppressor of sec1 (SSO1) identified genetically in *Arabidopsis thaliana* as PENETRATION1 (PEN1), function in resistance to *H. glycines*. Co-expression of SYP121 with SNARE homologs results elevated transcripts in infected cells inducing resistance. Thus, studying actual cellular mechanism of resistance and implicating host resistance against this devastating pathogen can help minimize yield loss thereby saving billions of dollars loss worldwide.

P-050

Enhancing soybean resistance to *Phytophthora sojae* by engineering the glyceollin gene regulatory network

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Phytoalexins are numerous and diverse antimicrobial plant metabolites that are biosynthesized in response to pathogens. Glyceollins are the major isoflavonoid phytoalexins of soybean that are suggested to have roles in providing resistance to a wide range of microbes including *Phytopthora sojae*, a pathogen responsible for 1-2 billion dollars in soybean yield loss per year worldwide. To identify transcription factors (TF) genes for the regulation of glyceollin biosynthesis, we used a comparative transcriptomics approach of pathogen- and abiotic stress-treated soybean tissues that had either elicited or suppressed glyceollin biosynthesis. While previous reports have identified only disparate, non-homologous TFs for the regulation of different phytoalexin biosynthesis in various plant species, our data suggested that several of those TFs were involved in regulating glyceollins. We confirmed the role of three of these in directly activating glyceollin biosynthesis. Overexpression of the each of the TFs in soybean hairy roots (HRs) increased glyceollin biosynthesis 4.5- to 11.7-fold. Yet, overexpression of each of the TFs alone in the absence of an elicitor could not activate the entire glyceollin biosynthesis pathway, suggesting that multiple TFs work together in a GRN to coordinate phytoalexin biosynthesis. Overexpressing a single TF of the GRN in hairy roots of the universally susceptible variety Williams resulted in incompatibility with race 1 P. sojae, whereas RNAi silencing of the TF gene in the incompatible variety Williams 82 resulted in compatibility. The results confirm that glyceollins have a major role in mediating incompatibility with P. sojae. They also provide evidence for a conserved phytoalexin GRN in plants and exemplify a novel strategy for enhancing plant resistance to economically devastating pathogens.

P-051

Phenotypic characterization of a major QDRL associated with partial resistance to *Phytophthora sojae*

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Phytophthora root and stem rot is major yield-limiting disease of soybean [*Glycine max* (L). Merr.] and the widespread deployment of race-specific resistance (Rps) genes has led to a shift in pathogen virulence across the Midwest. This rise in pathotype complexity increases the need for multigenic partial resistance. Previously, a major quantitative disease resistance locus (QDRL) was identified on chromosome 18 in two plant introductions – PI 427105B and PI 427106. Major QDRL are rare in this pathosystem and may serve as a valuable source of partial resistance in breeding programs. Thus, our objectives were to determine the allelic effect of this major QDRL on resistance to *P. sojae* and yield, as well as its isolate-specificity using near isogenic lines. Resistant QDRL alleles R105B and R106 increased partial resistance to *P. sojae* up to 28% and 47% based on growth chamber and greenhouse-based disease assays, respectively. Yield was also increased up to 18% and 29% under disease conditions. However, a real-time quantitative PCR assay showed no allelic difference in the relative amount of *P. sojae* DNA in infected root tissue 3, 24, and 48 hours after inoculation. Furthermore, the QDRL was found to be effective against seven *P. sojae* isolates of varying pathotype complexity and no interaction between isolate and allele was detected. This information will allow soybean breeders to make informed decisions regarding the deployment of this major QDRL in their respective breeding programs.

P-052

Integration of sudden death syndrome resistance loci in the soybean genome

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Soybean resistance to sudden death syndrome (SDS) is composed of foliar resistance to phytotoxins and root resistance to pathogen invasion. There are more than 80 quantitative trait loci (QTL) and dozens of single nucleotide polymorphisms (SNPs) associated with soybean resistance to SDS. The validity of these QTL and SNPs is questionable because of the complexity in phenotyping methodologies, the disease synergism between SDS and soybean cyst nematode (SCN), the variability from the interactions between soybean genotypes and environments, and the inconsistencies in the QTL nomenclature. This review organizes SDS mapping results and proposes the Rfv (Resistance to *Fusarium virguliforme*) nomenclature based on supporting criteria described in the text. Among ten reproducible loci receiving our Rfv nomenclature, Rfv18-01 is mostly supported by field studies and it co-localizes to the SCN resistance locus rhg1. The possibility that Rfv18-01 is a pleiotropic resistance locus and the concern about Rfv18-01 being confounded with Rhg1 is discussed. On the other hand, Rfv06-01, Rfv06-02, Rfv09-01, Rfv13-01, and Rfv16-01 were identified both by screening soybean leaves against phytotoxic culture filtrates and by evaluating SDS severity in fields. Future phenotyping using leaf- and root-specific resistance screening methodologies may improve the precision of SDS resistance, and advanced genetic studies may further clarify the interactions among soybean genotypes, F. virguliforme, SCN, and environments. The review provides a summary of the SDS resistance literature and proposes a framework for communicating SDS resistance loci for future research considering molecular interactions and genetic breeding for soybean SDS resistance.

P-053

Genetics characterization of a dominant susceptible phenotype to Asian Soybean Rust disease

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Most plant disease resistance genes (R genes) are inherited in a dominant fashion and their corresponding susceptible alleles are inherited recessively. However, a specific R-gene reversal of this dominance/susceptibility was discovered during the breeding process for soybean resistance to Asian Soybean Rust (ASR). The R gene Rpp1 is normally dominant over susceptibility, but an unexpected soybean line named TMG06-0011 exhibited dominant susceptibility when crossed with two resistant Rpp1 carrying lines, PI 594760B and PI 561356. Evidence suggests that the dominant susceptible "gene" in the TMG06-0011 genome is at the Rpp1 locus, or at least close enough to co-segregate with the Rpp1 locus (Garcia et al. Crop Science 2011). Two hypotheses on the mechanisms of dominant susceptibility were proposed. One is that the Rpp1 locus in TMG06-0011 may make siRNAs that target the Rpp1 gene, thus interfering the transcription or translation of Rpp1. Another is that RPP1 proteins may function as a dimer or as a polymer, and that TMG06-0011 may carry a non-functional allele of the Rpp1 gene, that results in non-functional Rpp1 protein complexes. In order to evaluate these hypotheses, we sequenced the whole genomes of TMG06-0011, PI 594760B and PI 561356 using the 10X Genomics Chromium technology and their Rpp1 loci were assembled using Supernova2 software. RNA sequencing and small RNA sequencing were used to measure expression levels. However, due to the repetitive nature of NBS-LRR gene clusters, the whole genome sequencing-based assembly might not be accurate for these regions. Therefore, we also are using fosmid libraries derived from these three genotypes for the Rpp1 locus, to verify and correct the assemblies. An update of the project will be provided.

P-054

Unraveling the potential use of tolerance as a defense strategy against Asian Soybean Rust

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Asian Soybean Rust (ASR) is the major disease affecting soybean production in Brazil. In spite of existing alternative options for its control, fungicide remains the leading strategy. Genetic resistance has been modestly used to cope with ASR while researches on tolerance continue scarce. Tolerance is defined as the host's ability to maintain fitness regardless of the pathogen load. Here, we investigate the tolerance as a strategy to sustain fitness (i.e., grain yield) in the presence of ASR using germplasm from a recurrent selection program. Therefore, this research aimed to understand the important features of this defense mechanism, how the disease affects important traits and the relationship among them and discuss the applications of tolerance for soybean breeding. The plant-based selection was done in 64 single-cross F5 populations in two contrasting locations under high disease pressure, totaling 768 F5:6 inbred lines. Lines were evaluated in preliminary yield trials using augmented block design in two rust conditions (high pressure and absence of ASR). Agronomic traits were measured and seed shape parameters were assessed using a high-throughput phenotyping approach based on RGB imagery. The adjustments for local and global spatial trends in the field trials were done using a spatial approach based on two-dimensional P-Splines and mixed models. The spatial variation was more intense in the ASR trial, requiring more parameters to model the field trend and the generalized heritability was negatively impacted in 10 out of 15 traits, compared to the ASR-free trial. We found a negative correlation (-0.87**) between tolerance and fitness in the absence of the pathogen, which implies "allocation costs". In this scenario, the biological costs to sustain tolerance may not be translated into increased fitness in the absence of ASR. Despite this, sufficient genetic variability was found, allowing the selection of agronomic superior lines even under high pathogen burden.

P-055

A study of the genetic architecture of quantitative disease resistance towards *Phytophthora sojae*

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Phytophthora sojae is one of the most damaging soybean pathogens causing greater than $200 million in losses domestically, and $50 million in losses in Ohio annually. Reduction of losses can be achieved through integrated disease management plans including agronomic practices and deploying host resistance. Historically, single dominantly inherited Rps-genes were the focus of *P. sojae* resistance breeding, though selection and pathogen adaptation have resulted in these genes becoming less effective. Complementing Rps-mediated resistance with quantitative disease resistance (QDR), can improve breeding programs. QDR is generally conferred through the multiple QDR loci (QDRL), each contributing to the overall resistance phenotype. Furthermore, QDR is expected to remain effective when *P. sojae* populations adapt. Unfortunately, QDR is not as well understood as Rps-mediated resistance, and purposeful introgression of QDRL has occurred infrequently in breeding programs. The overarching goal of this research is to understand the genetic architecture of QDR towards P. sojae. Specific objectives include, (1) mapping and validation of QDRL using a genome-wide association (GWA) analysis and (2) assessment of a Genomic Prediction (GP) model. A collection of 1200 soy cultivars representing diverse germplasm was phenotyped for QDR. Phenotypic analyses indicate a statistically significant amount of genetic variation in QDR for these lines. A GWA analysis will be used to map this variation to the genome to identify and/or confirm QDRL. Based on current analyses GP accuracies of between 0.37 and 0.62 for four measurements of resistance were achieved. To our knowledge this project is the first GP study targeting QDR toward P. sojae, with the results providing formative data on which traits can be most successfully used to predict P. sojae resistance levels.

P-056

Meloidogyne incognita resistance impacts on soybean yield

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Southern Root-knot nematode [*Meloidogyne incognita*] (RKN) causes major damage in soybean in southern United States. Occurring mainly in sandy soils, RKN can cause yield losses up to 75%. Besides crop rotation, management relies on the use of resistant cultivars. Few resistance QTLs were found in PI 96354, PI 408088, PI 417444, and PI 438489B. PI96354 represents the most popular source of resistance used in breeding programs. In this experiment, 100 high-yielding lines from the advanced yield trial (AYT) of the University of Missouri - Fisher Delta Research Center Soybean Breeding program were tested in 4 different environments in Clarkton and Portageville, Missouri. Clarkton is on sandy soil with high RKN pressure, and Portageville consists of 2 on loam soil and 1 on clay soil, both without RKN pressure. The maturity groups of selected lines ranged from 4-early to 5-early. They were previously phenotyped for RKN resistance at the University of Georgia based on number of galls, and resistance was reported in a scale from 1 (resistant) to 5 (susceptible). Yield (bu/ac) of RKN resistant lines across all maturity groups was 14.75 bu/ac higher than susceptible lines under RKN pressure, while no significant difference was observed between resistant and susceptible lines without RKN pressure (2.14 bu/ac difference). Further analysis showed significant difference among resistant lines under RKN pressure, but no significant difference was observed without RKN pressure. This possibly indicates that different genetic backgrounds for RKN resistance may have an impact on the level of resistance. The identification and implementation of resistance genes in breeding programs stands as a powerful tool to manage RKN. Potential new sources of resistance may contribute to superior levels of resistance and lower yield suppression under RKN pressure.

P-057

Identification of novel SCN resistance strategies in wild soybean

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The domestication process of crop plants often involves selection for agronomic traits against the plant's intrinsic resistance strategies. Thus, domestication processes decrease genetic variation, making crop plant varieties more susceptible to pests than their wild relatives. Domestication of the wild soybean (*Glycine soja*) accounts for a major loss is genetic diversity. The G. soja gene pool is indisputably more diverse than the cultivated soybean (*Glycine max*) due to a primary loss of nucleotides in domestication and continued loss due to selection and modern breeding practices. Therefore, we dissect the diversity contained in the wild soybean population, which has been going through differential stress from varying environments, as a naturally adapted source of resistance. In this study, resistance to Soybean Cyst Nematodes (SCN) is investigated in a newly identified SCN resistant ecotype, s100. In order to investigate the global gene expression changes, we compare RNA seq-based transcriptomes of the novel SCN-resistant wild soybean ecotype (s100) vs SCN-susceptible ecotype (s67), SCN-resistant cultivar (Peking), and SCN-susceptible cultivar (Williams 82) under both control and nematode-treated conditions. All accessions were inoculated with SCN HG type 2.5.7. High-throughput Illumina total RNA sequencing of root tissue is used to produce expression profiles to compare transcriptomes of all samples from treatment and control experiments. This project identified candidate genes involved in SCN resistance and advances the long-term goal to develop SCN resistant soybean cultivars, which has crucial significance to agriculture and environmental sustainability. Many of the differently expressed genes were found to be functionally comparable with other wild soybean resistance strategies, but not with the cultivated soybean.

P-058

Confirmation of additional quantitative trait loci that underlie resistance to soybean sudden death syndrome using NILs and SNPs

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Soybean (*Glycine max* (L.) Merr.) cultivars vary in their partial resistance or susceptibility to sudden death syndrome (SDS), caused by *Fusarium virguliforme* (Aoki). Breeding for improved response has been challenging. There are 42 resistance loci reported. The aims here were to compare the inheritance of those loci for resistance to SDS in a near isogenic line (NIL) population. That NIL population was fixed for resistance to SCN but segregated at two additional loci (cqRfs1 and cqRfs). Used were; a NIL population derived from residual heterozygosity in an F5:9 recombinant inbred line EF77 (lines 1-40); SDS response data (2) from each of two locations in different years; four segregating microsatellite and 1,500 polymorphic SNP markers. Polymorphic regions were found from 18,033 Kbp to 18,447 Kbp and 45,768 Kbp to 48,582 Kbp on chromosome (Chr. 6), a new region on Chr. 13 between 27,527 Kbp and 33,689 Kbp and a known region between 35,776 Kbp to 38,239 Kbp on Chr. 19 that were significantly ($P<0.001$) associated with resistance to SDS. The loci Rfs4, qRfs8, and qRfs12 were previously reported in these regions, one novel QTL was identified. Rfs5 was not segregating. This study indicates that using the SoySNP6k chip, QTL that underlie SDS resistance could be mapped with much greater accuracy.

P-059

Development of transgenic soybean against mungbean yellow mosaic India virus (MYMIV) using RNA interference approach

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Edible oil is an important food item for everyday use. Pakistan cannot meet its domestic requirement and imports 74% of its vegetable oil. Imports of soybean meal are continuously increasing to feed the growing livestock industry in Pakistan. These facts highlight the importance of soybean, which is a leading oilseed crop in the world, to Pakistan. In Pakistan, one of the major constraints to the yield and productivity of soybean is biotic stresses such as viruses. Among viruses, mung bean yellow mosaic India virus (MYMIV) is a major constraint to legume production. The MYMIV is a bipartite begomovirus which belongs to family Geminiviridae. In Pakistan, MYMIV frequently infects legumes. In order to control MYMIV in soybean, a transgenic approach will be used. Transgenic soybean lines were produced in order to control MYMIV using both siRNA and tasiRNA approaches. The MYMIV genes coding for replication associated protein (Rep) and transcriptional associated protein (TraP) were targeted. For this purpose, primers were designed to amplify the highly conserved regions of Rep and TraP genes. The amplified products were cloned in pFGC5941 and pG31514H (transformation vectors). The construct(s) were transformed into soybean embryos using biolistic transformation. The aim of the study is to devise better control strategies for the control of begomoviruses infecting soybean.

P-060

Analysis of the RLK LRR domain and its synthetic mutants and preparatory binding studies to some of its peptide ligands

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The Rfs2 region on chromosome 18 of soybean encodes, among other genes, a receptor like kinase (RLK) protein known to mediate resistance to soybean cyst nematode (SCN) *Heterodera glycines* (I.) and Fusarium virguliforme (Aoki) causal agent of sudden death syndrome (SDS). In this present study, the extracellular leucine rich repeat (LRR) domain of RLK and its mutants were cloned in *Escherichia coli* and its expression optimized. The mutants of the RLK-LRR domain (H143D,N,Y) was generated using site-directed mutagenesis. Previously, the amino acid residues at that site was shown to be involved in mediating crucial interactions for the stability of the RLK-LRR homodimer and thus its function. One of our long term goal is to correlate the effect of the synthetic mutations to the binding of LRR domain to its cognate CLE (CLV3/ESR) peptide ligands found in soybean plant secretome and SCN. CLE peptides mediate signaling in developmental and disease resistance pathways in soybean plants. Fluorescein isothiocyanate (FITC) labeling of several of the peptides were optimized to be used in fluorescence polarization (FP) based binding studies. Immunohistochemistry assays were performed to show the localization of the RLK protein in root tissues of soybean plants. Although RLK-LRR expression was optimized, almost 100 % of the protein formed inclusion bodies and attempts to refold the protein to native functional state was unsuccessful thereby limiting its use in functional binding assay. The work presented here motivates future studies of LRR domain interaction with its binding partners and relate it to its function.

P-061

Current effectiveness of Rps genes against *Phytophthora sojae* pathogen populations in Ohio and Indiana

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Phytophthora root and stem rot of soybean continuously causes significant yield losses when soybeans are grown on poorly drained soils. Annual yield losses from *Phytophthora* root and stem rot was most recently estimated at 24 million bushels of soybean across the North Central region of the United States. Historically, resistance to *Phytophthora sojae* (Rps) genes have been incorporated into soybean germplasm to provide R-gene mediated resistance against the pathogen. However, the pathogen population has adapted over time so that certain commercially deployed Rps genes are no longer effective at providing disease resistance. A survey of the current *P. sojae* population was conducted by collecting soils from 78 locations across Ohio and Indiana with a history of Phytophthora root and stem rot. Isolates of *P. sojae* were recovered from the soil through baiting with a moderately susceptible soybean cultivar, Sloan. To date, 192 *P. sojae* isolates have been recovered from 340 soil samples from 6 and 9 counties in Ohio and Indiana, respectively. A set of 16 differential cultivar lines, each containing a single Rps gene, were used to pathotype each isolate. Differential lines containing Rps1a, Rps1b, Rps1c, Rps1d, Rps1k, Rps3a, Rps6 and Rps 8 were susceptible to 91%, 97%, 67%, 41%, 97%, 27%, 7%, and 17% of the P. sojae isolates, respectively. On average, each isolate had a mean complexity of 6.46 and a Shannon diversity index of 3.69, with 72 distinct pathotypes identified. This is an increase from a previous survey conducted in 2015, where isolates of P. sojae had a mean complexity of 5.7 and a Shannon diversity index of 3.37. These results highlight the importance of periodic surveys to assess the increasing virulence in pathogen populations.

P-062

Evaluating soybean ancestral cultivars for resistance to Southern Stem Canker (*Diaporthe aspalathi*)

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Diaporthe aspalathi is the causative agent of Southern Soybean Stem Canker, which is a major disease in soybean production in the Southern United States. The disease can cause up to 80% yield loss. Soybean (*Glycine max*) has a limited genetic base with 35 ancestral cultivars providing 95% of the genes found in modern soybeans. Evaluating these 35 cultivars for resistance to Southern Stem Canker could help understand the resistance that is seen in modern cultivates. The 51 genotypes, including the 35 ancestors, 6 known resistant cultivars, 4 susceptible checks, and 6 parents of mapping populations were evaluated in Griffin, GA using two strains of D. aspalathi (BL 3-15 and BL22). Genotypes were inoculated with D. aspalathi infected toothpicks method 4 weeks after planting. Plants were scored on scale of 1 to 5 with 1= no symptoms and 5 = premature plant death. The internodes of infected plants were counted to evaluate the spread up the stem of the plant. Thirty-three genotypes were identified as being resistant to southern stem canker, which included 6 known resistant lines, 25 ancestral cultivars, and 3 of the parents of the mapping populations. 18 genotypes were identified as susceptible, including the 4 susceptible checks and 3 parents of the mapping population. Resistance to southern stem canker was not significantly correlated to the maturity of the genotypes. Eighteen resistant and 7 susceptible genotypes were Northern germplasm (MG000 to IV), while 15 resistant and 11 susceptible genotypes were Southern germplasm (MGV to X). The evaluation of these genotypes provides understanding of the genetic diversity of soybean ancestors and selection of populations for fine mapping work.

P-063
Gene stacks and rotation to combat soybean cyst nematode virulence

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Soybean cyst nematode (SCN) is the most important pathogen of soybean causing more than $1 billion in yield losses annually. Planting SCN resistant soybean is a primary management strategy. Plant introduction (PI) 88788 (rhg1-b) and Peking (rhg1-a and Rhg4) are the main sources of resistance that are currently available. PI 88788 is the most widely used source of resistance in over 95% of SCN resistant varieties in the north central US. Consequently, the over reliance on a single resistance source has selected for SCN populations that have overcome this resistance. Limited options for rotating resistance sources are available, however, new sources such as PI 468916 (cqSCN-006 and cqSCN-007) and PI 567516C (chromosome 10 QTL) have been identified. Our objective was to evaluate unique resistance gene stacks that have these new resistance sources to determine what would be beneficial in rotation to combat the increase in virulent SCN and limit nematode adaptation to resistant cultivars. In a greenhouse experiment, eight SCN populations were developed by selecting an SCN field population (HG type 1.2.5.7) on a single resistance source or on a rotation of resistance gene stacks. Egg density data were collected after each generation and HG type tests were conducted after eight generations. Continuous use of rhg1-b (887) or 006/007 (468) had limited effectiveness for reducing SCN density. Continuous use of rhg1-a/Rhg4 resistance reduced SCN density but retained virulence on PI 88788-type resistance and selected for increased SCN virulence (HG type 1.2.3.5.6.7) on Peking-type resistance. Rotation of rhg1-a/Rhg4 and a stack of rhg1-b (887) + 006/007 (468) + CHR 10 (567) was the most effective at reducing population density and minimized selection pressure. Our results suggest that implementation of a strategic rotation plan will be needed to manage the widespread virulence on PI 88788 and enhance durability of SCN resistance.

P-064
Identification of molecular biomarkers associated with reniform nematode (*Rotylenchulus reniformis*) resistance in soybean

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Reniform nematode (*Rotylenchulus reniformis*) is a yield-limiting pathogen of soybean in the Southeastern region of the United States. Several studies have identified soybean germplasm with resistance to reniform nematode and recent studies have explored the soybean genome for quantitative trait loci (QTL) linked to reniform nematode resistance. Our objective for this study was to identify high resolution single-nucleotide polymorphism (SNP) biomarkers that correlate with reniform nematode resistance in soybean using genotyping-by-sequencing (GBS). A population of 250 F2:8 recombinant inbred lines (RIL) developed from a cross between reniform nematode-resistant soybean cultivar, 'Forrest', and susceptible cultivar, 'Williams 82', was utilized to correlate reduced nematode reproduction to SNP biomarkers localizing specific QTL regions in the soybean genome. The phenotype of each RIL was determined by inoculating three replicates of each line with 2000 vermiform reniform nematodes and quantifying nematode populations 8 weeks after inoculation. Reproduction factor for each line was transformed by log10. DNA collected from each line was digested using specific restriction enzymes MseI and PstI to prepare gene libraries, then sequenced on the Illumina HiSeq platform. Several annotation tools were used to align and filter the sequences and call SNP markers. A genetic linkage map was constructed with 558 SNP markers and significant markers were identified using GModel software. We report SNP markers that correlate to the resistant phenotype observed in the developed lines. The characterized genetic markers can be used by soybean breeders in marker assisted selection to enhance their efforts in selecting and employing lines with known resistance to reniform nematode.

P-065

Identification of soybean germplasm with resistance to *Pythium ultimum* var. *ultimum* and other *Pythium* species detrimental to soybean production in North America

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Pythium ultimum var. *ultimum* and several other *Pythium* species are important soil-borne pathogens in North America, causing seed rot, root rot, and pre- and post-emergence damping-off in soybean. Many *Pythium* spp. thrive in cool, moist soils, so earlier planting dates increase the risk of infection. A series of experiments was conducted to identify soybean germplasm with resistance to an aggressive isolate of *P. ultimum* var. *ultimum* and to other *Pythium* species and isolates. Several important ancestors of North American soybean varieties were found to be resistant, including the Canadian cultivar Maple Isle and two Swedish ancestors of numerous cultivars adapted to Canada and the northern United States. These ancestral lines and certain other early maturity group germplasm accessions were also resistant to isolates of P. irregulare, *P. sylvaticum*, and several other *Pythium* spp. While seed rot losses can be reduced by planting seeds treated with certain fungicides, *Pythium* resistance would help to protect a plant as its roots grow beyond the zone of fungicide activity surrounding the germinating seed.

P-066

Identification of known and unknown viral genomes in soybean using RNA-seq

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Several viruses are able to negatively impact soybean production and may be responsible for hidden problems such as poor seed quality and yield loss. Furthermore, it is likely that some soybean diseases caused by unknown pathogens are due to viral infections. We phenotypically identified plants in the field with virus-like symptoms during the 2016 and 2017 growing seasons across the US. Using RNA-Seq, we identified several viruses in both growing seasons known to infect soybean, but that are not considered to be major pathogens. Viruses such as Bean pod mottle virus (BPMV) and Soybean mosaic virus (SMV), which are generally considered to be the major viral pathogens of soybean, were not found. However, Clover yellow vein virus (ClYVV), a member of the genus Potyvirus was found in soybean in both growing seasons in Iowa. To our knowledge, ClYVV has not previously been reported to infect commercial soybean fields in the US. Our results also indicate that mixed virus infections are common. Overall, our RNA-Seq data suggests that the profile of soybean viruses affecting soybean production has evolved significantly since the late 1990s/early 2000s. More research is needed to understand the importance, impact, and ecology of individual and mixtures of viruses, including viruses that were previously not known to affect soybean.

P-067
Korean elite soybean cultivars are potentially threatened by *Phytophthora sojae*

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Phytophthora root and stem rot (PRSR) of soybean is a major disease that causes economics losses in major soybean-growing regions. The causal agent, *Phytophthora sojae*, is a soil-borne oomycete that causes pre- and post-emergence damping-off of soybean under wet and warm condition. In South Korea, PRSR has not been considered as a severe problem, while a few isolates of *P. sojae* were first reported 20 years ago. The incidence of PRSR is more frequently reported as cultivation of soybean in paddy fields increases recently. Interaction between *P. sojae* and Korean soybean cultivars was not largely unknown. R-gene mediated resistance is known as a primary strategy for management of this disease. The objective of this study was to evaluate major Korean soybean cultivars for R-gene resistance to *P. sojae*. Tens of soybean cultivars widely grown in S. Korea were tested against 4 *P. sojae* isolates originated in S. Korea using modified hypocotyl inoculation technique. Spore suspension (105 spores/ml) was inoculated on the hypocotyl of 7-day-old seedlings, and the responses of the cultivars to each isolate were observed 7 days after inoculation. The disease responses of cultivars varied by isolate, while the majority of the tested cultivars exhibited susceptible reaction to two isolates or more. Nine cultivars were susceptible to all the four isolates. Only three cultivars were resistant against two isolates. This study revealed that many of Korean soybean cultivars are susceptible to multiple *P. sojae* isolates and potentially threatened by this pathogen in fields. These results imply that the four isolates may be genetically different and some of the cultivars may have different kinds of R-gene effective to specific P. sojae isolates. This study will be a framework for soybean researchers in S. Korea to improve the susceptible cultivars by deploying R-genes that are effective to existing *P. sojae* isolates.

P-068
Evaluation of Korean soybean cultivars for resistance to *Phytophthora sojae*

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Phytophthora root and stem rot (PRSR) of soybean is a major disease that suppresses soybean yield in major soybean-growing regions over the world. The causal agent, *Phytophthora sojae*, is a soil-borne oomycete pathogen, and causes pre- and post-emergence damping-off at early growth stages and root and stem rot at later growth stages. In South Korea, PRSR has not been considered as a severe problem, while a few isolates of *P. sojae* were first reported 20 years ago. Thus, interaction between *P. sojae* and Korean soybean cultivars was not much studied. Recently, the incidence of PRSR is more frequently reported as cultivation of soybean in paddy fields increases. The objectives of this study is to evaluate major Korean soybean cultivars for resistance to *P. sojae*, and to identify resistant soybean cultivars. Twenty-one soybean cultivars widely grown in S. Korea were tested against four *P. sojae* isolates originally isolated in S. Korea using modified hypocotyl inoculation. Spore suspension (105 spores/ml) was inoculated on the hypocotyl of 7-day-old seedlings, and the responses of the cultivars to each isolate were observed 7 days after inoculation. The disease responses of cultivars varied by isolate, while the majority of the tested cultivars exhibited susceptible reaction to two isolates or more. Of the twenty-one, nine cultivars were susceptible to the four isolates. Only three cultivars were resistant against two isolates. The results imply that the four isolates of *P. sojae* may be genetically different and some of the cultivars may have different kinds of R-gene effective to specific *P. sojae* isolate. This study will be an informative framework for soybean breeders in S. Korea to improve the susceptible cultivars by deploying R-genes that are effective to existing P. sojae isolates.

P-069

Molecular characterization of soybean osmotins and their involvement in the drought stress response

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Osmotins are stress responsive multifunctional proteins, belonging to thaumatin-like family, that have been used in biotechnology engineering to confer plant tolerance to abiotic and biotic stresses. Drought is one of the most relevant environmental factors that dramatically limits soybean yield, an important commodities in the world. To better understand the functions of soybean osmotins in the drought stress response, the current study presents the characterization of four previously described proteins and a novel putative member of soybean osmotins. Gene and protein structure analyses were carried out, as well as, gene expression data mining. *Nicotiana tabacum* and *Solanum nigrum* osmotins, previously characterized as providers of drought tolerance were used as references. Phylogenetic analysis allowed the identification of a new soybean osmotin (Gm600). Whole genome duplication, as well as, tandem and proximal duplications contributed to the osmotin genes expansion in soybean genome. Almost all sequences did not present introns and showed a signal peptide that drives the protein to the secretory pathway. *N. tabacum*, *S. nigrum* and GmOLPb also present a C-terminal elongation that mediates their transport to the vacuole. The nine osmotin sequences analyzed share the conserved amino acids signature and 3D structure of the thaumatin-like family. Despite their similarities, some differences were observed in the conserved regions of protein sequences and in the electrostatic potential surface. P21-like presented the most similar electrostatic potential to the Solanaceae osmotins. Expression data mining showed that P21 is expressed in leaves and flowers, while P21-like and GmOLPa are expressed in roots and flowers in an unstressed situation. Under drought situation, during reproductive stage, P21-like and GmOLPa could also be identified in leaves and overexpressed when compared to P21. The analyses suggest different contributions of soybean osmotins in plant response to drought.

P-070

Ozone effects on soybean roots

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High concentrations of tropospheric ozone (O3) during the growing season leads to significant reductions in global soybean yields. Research on the impact of O3 on soybean has generally focused on above-ground tissues, not on roots, which are major tissues supporting plant fitness. Mandarin (Ottawa) (O3-sensitive) and Fiskeby III (O3-tolerent) soybean genotypes provide contrasting materials with which to investigate how O3 alters root morphology. In this study, we analyzed root initiation, biomass, and diameter respectively within root classes of 16-day-old (approximately V2 stage) Mandarin (Ottawa) and Fiskeby III grown in greenhouse with charcoal-filtered (CF) air followed by treatment with CF or 75 ppbv O3 for 7 h/day in continuously stirred-tank reactors (CSTR) for 0, 4, and 7 days. The results showed that O3 significantly decreased the biomass of rapidly developing basal roots in Mandarin (Ottawa) during the O3 treatment; root initiation rates were not affected. Biomass accumulation was not significantly affected in either basal or tap roots present before the O3 treatment began. However, root diameter was reduced by O3 treatment in Mandarin (Ottawa) with a reduction of 0.0726 mm in the developing basal roots and 0.0181 mm in lateral roots of developing basal roots, basal roots, and tap roots. There were no impacts of O3 on the root biomass or root diameter of O3-tolerant Fiskeby III. Our study provides robust evidence that O3 alters root architecture complexity in O3-sensitive soybeans by decreasing the biomass and root diameter of developing basal roots along with a general reduction in root diameter of all lateral roots. Our findings uncover a morphological impact of O3 on below-ground tissues and potentially identifies O3-tolerent root trait assessments for soybean breeders.

P-071

Physiological and metabolic responses of soybean plants to global climate changes

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According to the United States Environmental Protection Agency, in absence of explicit climate change policy, atmospheric CO_2 concentrations ([CO_2]) are projected to reach 800 µmol.mol-1 by the end of this century. The continuous increase in atmospheric [CO_2] is leading to changes in temperature and precipitation that can result in severe consequences in crop growth and productivity. For instance, drought is expected to decrease yield and seed quality whereas elevated [CO_2] is anticipated to partially compensate the effects of drought, especially in C3 plants. We have performed a comparative and comprehensive study of the responses of whole soybean plants to global climate changes. For this purpose, the group at the University of São Paulo provided samples of organs (roots, stems, seeds, and leaves) from soybean plants that have been subjected to drought and/or elevated [CO_2]. Interestingly, physiological data indicated that elevated [CO_2] seems to be capable to buffer all the effects of drought. More specifically, a strong impact was observed in the number and biomass of fruits and seeds produced. To underline the metabolic changes due to drought and/or elevated [CO_2] in whole soybean plants, biomass components - oil, protein, and carbohydrates – were sequentially extracted and quantified from the different organs at different stages of development. Additionally, the quantification of intracellular metabolites such as sugars-sugar alcohols, amino acids, organic acids, phosphorylated compounds, and phenolics was achieved by liquid chromatography tandem mass spectrometry. The present research focuses on integrating the results of these analyses and attempts to highlight the metabolic changes due to drought and/or elevated [CO_2]. Ongoing experiments are expanding this study and combining the metabolomic data obtained here with transcript levels. The long-term goal is to design possible strategies to transform soybean plants on the basis of the metabolic routes that are altered due to global climate change.

P-072

Identification of factors controlling expression of purple color in hypocotyl of soybean sprouts

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Soybean sprouts have good digestibility, high isoflavone content compared with soybean seeds, and large amounts of aspartic acid, which is effective in eliminating hangovers. However, the region between the cotyledon and hypocotyl in soybean sprouts appears purple, the product value of soybean sprouts reduces. To date, the scientific correlation among factors such as growth temperature, spray water temperature, and cultivation periods for soybean varieties related to purple color expression between the cotyledon and hypocotyl of soybean sprouts are unknown. The purpose of this study was to identify the factors regulating the expression of purple color between the cotyledon and hypocotyl of soybean sprouts. Of 15 Korean soybean varieties with purple color in the hypocotyls and flowers, 9 varieties, including Sowonkong, Wonhwang, Sinhwa, Eunhakong, Pungsannamulkong, Paldonamulkong, Kwangankong, Shingang, and Jangki showed purple color between the cotyledon and hypocotyl of sprouts. However, the remaining six cultivars, including Dachae, Myeongjunamulkong, Sobaeknamulkong, Sojinnamulkong, Anpyeong, and Jonam, did not show purple color. The proportion of soybean sprouts with purple hypocotyls was the lowest at 26βand there was no significant difference at the other three cultivation temperature conditions (17°Cβ 20°C, and 23°Cβ. Similar to the results of the cultivation temperature experiment at five different spray water temperatures (10°C, 17°C, 20°C, 23°C, and 26°C), the proportion of soybean sprouts with purple hypocotyls was the highest at the lowest spray water temperature (10°C), and lowest at the highest temperature. These results suggested that cultivation temperature and spray water temperature for purple color expression in soybean sprout hypocotyls are the most important factors in the sprout cultivation environment.

P-073

Variation in the phenology and yield component traits of late-planting soybean in the Upper Midwest

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There is a growing interest to develop double cropping production for improving the water quality conditions in the Upper Midwest. Double crop soybean following the harvest of a winter cash crop could provide agroecosystem services to the landscape, in addition to generate diversified income to growers. However, there is a lack of knowledge about late planting soybean outside of the Southeast region. Our research objective is to examine the difference in phenology and yield component traits of soybean between a normal (May) and a late planting date (late June-early July). The late-planting soybean needs to mature by early October, while maintaining sufficient yield and quality. We conducted a pilot study in 2017, and selected extremely early soybean genotypes so they could fit in a double cropping system. We found an average of 27% reduction in yield, and the majority of genotypes matured by our expectation. Differences in the relative importance of yield components as contributors to overall yield between late planting and normal planting conditions were not observed. Regarding phenology, results from this study suggest that days to flowering is the strongest predictor of yield, whereas the length of the post-flowering stages is the strongest predictor for the normal planting date. Additional results need to be obtained to better establish relationships between yield and these traits under various planting dates intended for different cropping systems. Knowledge of these relationships will help guide soybean breeding efforts towards developing new soybean varieties adapted to double cropping systems in the Upper Midwest.

P-074

Looking beyond the obvious-parameterizing the soybean canopy architecture to measurable traits

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Canopy architecture (CA) is a composite of many traits that contribute to varying degrees to the entire structure of the plant. Canopy architecture is an important determinant of light interception and consequently photosynthesis, and ultimately yield in crop species. In Soybeans, most studies of CA have focused on just a handful of traits. In addition, few studies have focused on trait combinations which can be more powerful than individual traits in describing the overall architecture of the plant. Our aim is to deconstruct CA into distinct measurable traits and trait combinations to study their correlation to each other as well as their contribution to light interception and canopy coverage. Towards this end, a set of 40 genotypes that displayed variation in canopy architecture were selected from a pool of fast neutron mutants as well as several maturity group I lines from the USDA Soybean Germplasm Collection. CA was parameterized into more than 50 different CA related traits that were grouped into multiple trait combinations. We have observed variation in both populations for several individual traits and trait combinations such as lateral branch angle, branching density, phyllotaxy, branch orientation, canopy characteristics and overall plant shape characteristics. Canopy coverage and light penetration measurements were also made concurrently. Analysis is currently under way to examine how each of these traits and various trait combination effects canopy coverage as well as light interception by plants. Association mapping and biparental mapping are also underway to identify genes controlling key traits identified in this study.

P-075
Towards 13C-metabolic flux analysis of developing Thorne embryos

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Understanding the restrictions that rule carbon distribution during embryo development is a major goal of the plant breeding/engineering industry. 13C-metabolic flux analysis provides a rigorous approach to identify targets responsible for those restrictions. This approach requires the application of isotope tracers to map the flow of carbon through the different metabolic pathways, and therefore culture conditions that mimic the development of the embryo in planta. Then, flux map analysis relies in a mathematical model of biochemical reactions. The construction of flux maps in *Glycine max* cv. Thorne is especially attractive as this variety is less recalcitrant for transformation than others with similar seed composition. Therefore, the identified metabolic targets could be evaluated in vivo in the same cultivar. Only a few fluxes, such as the accumulation of biomass components, can be measured directly. For this purpose, we performed a complete study of Thorne embryo biomass during its development in planta. The rates of accumulation of dry weight, oil, protein, and carbohydrates were measured, and found to be linear for 18 days. The other fluxes have to be determined after feeding soybean embryos with 13C-labeled compounds. In order to define an appropriate culture media, we identified the substrates received by the embryo in planta. For this purpose, funiculi and endosperm tissues were collected, and the amino acids, sugars, organic acids, and hormones concentrations were quantified. Finally, several culture conditions were tested considering those previous results, where substrate levels were further adjusted according to their effect in protein and oil accumulation. We anticipate that optimized Thorne embryo cultures will permit the construction of embryo´s flux map, the identification of critical control points that govern protein and oil content and their evaluation through the construction of transgenic lines.

P-076
Analysis of volatile compounds in high-yielding soybean varieties using electronic nose

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Soybean (*Glycine max* L.) is the world's most important seed legume, which contributes to 25% of the global edible oil, and about two-thirds of the world's protein concentrate for livestock feeding. One of the factors that limit soybean's utilization as major source of protein for humans is the characteristic soy flavor. This off-flavor can be attributed to the presence of various chemicals such as phenols, aldehydes, ketones, phenols, furans, alcohols, and amines. In addition, these flavor compounds interact with protein and causes formation of new off-flavors. Hence, studying the chemical profile of soybean seeds is an important step in understanding how different chemical classes interact and contribute to the overall flavor profile of the crop. In this study, we utilized gas chromatography electronic nose (eNose) for identification and characterization of different volatile compounds in high-yielding soybean varieties, and study their association with off-flavors. With aroma profiling and chemical characterization, we aim to determine the quantity and quality of volatile compounds in these varieties and understand their effect on the flavor profiles. The study would help us to understand soybean flavor characteristics, which in turn would increase soybean use and enhance profitability.

P-077

Implications of shoot architecture resultant of secondary growth of *Glycine max* on fruit yield using ImageJ-Fiji

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Shoot diameter has been found to influence yield and, along with other architectural components such as branching angles, correlated to structural stability. Persimmon trees with thicker branches are able to produce a higher yield. A larger diameter gives the branches more structural stability to maximize fruit loading capabilities. Loading capabilities are resultant of secondary growth. This study was conducted to determine the significance of shoot architectural components resulting from new secondary growth and the production of new cell walls containing cellulose and lignin in soybeans to determine the effect on fruit yield. We studied six cultivars that varied in canopy breadth. This variance in breadth indicates differing branching characteristics. We hypothesized that a cultivar that has a narrower branching angle and a greater branch diameter will yield more fruit. We examined the branching diameters of the main stem and the lowest branch on the stem and the branching angle of that branch off of the main stem using ImageJ-Fiji. Measurements were taken for six cultivars from two field sites in Virginia during the seed filling stages. Across the six cultivars planted in two locations, there was a difference in branching angles and diameters between the cultivars. Cultivars also displayed differences in these traits based on location. This is evidence that there are cultivar and locale effects that may influence yield. These differences may be the result of reaction wood formation to support higher fruit loads.

P-078

Stem strength versus seed production: Are soybeans making resource allocation decisions?

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Soybeans are the second most harvested crop in the US. Crop production has fallen recently and could be due to pods not being able to completely fill, forming aborted pods. Known causes of aborted pods are drought periods during critical seed filling stages, or type of cultivar. Previous research has found that plants continue to grow radially in secondary growth during their reproductive growth stages, this growth could be drawing nutrients away from the fruits being produced. To better understand this, a field study was conducted comparing four different cultivars. We compare the cell wall biochemistry of main stems of soybeans from the R6 stage, the beginning of seed filling stages to the R8 stage, the plant at full maturity, in order to determine what resources are going to the fruit production and what is going towards an increase in stem secondary growth of xylem and phloem tissues. The changes in stem chemicals during seed filling stages are targeted specifically in two determinate cultivars (Hutcheson and Essex). Our results illustrate that the cultivar that has more aborted pods as percent total pods, Essex, has higher lignin accumulation and lower cellulose accumulation throughout their growth periods. Lignin occurs predominantly xylem cell walls, and can suggest that plants with new xylem growth could have competition with developing fruits, leading to aborted pods. The formation of more lignin and xylem growth may be needed to support the new growth of the plants structurally, but the resources used are taken away from fruit production and cause a decrease in yield.

P-079

Plasticity in branching architecture as an interference in canopy cultivar selection

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Farmers select soybean cultivars by referencing annual yield trials in combination with desired traits to maximize growth in local environments. Discrete cultivar traits such as flower color and determinacy are easily scored. Continuous traits, however, show varying levels of expression and are often scored on a relative scale, which may vary from year to year. Such traits include resistance to herbivores and pathogens, lodging resistance, and field emergence. One trait that is included in the Pioneer seed selection guide is canopy width, a lower score presumably indicates a narrower canopy with fewer branches. We investigated expression patterns of the canopy width trait to determine whether the relative scoring scale would accurately predict branching architecture in soybean canopy. Six cultivars, representing determinate & indeterminate and ranging from canopy width 4 to 7 were grown in two Virginia field sites in 2017, one as a single crop and the other as a double crop planting. Long branches reaching 75% of the main stem height were assessed visually in the field and using ImageJ-Fiji to assess de-leaved stems through image analysis. Canopy width scores provided by Pioneer did not predict branching architecture. In addition, all cultivars produced a significant portion of plants producing no long branches. Cultivars also adopted one of two branching composition phenotypes, either favoring lower numbers of branches or showing even dispersion among low, medium, and high numbers of branches. Since higher branching (i.e. canopy width) leads to greater numbers of seeds, it may be beneficial to select cultivars showing even dispersion. Additional influence of branching plasticity on yield traits will be discussed.

P-080

Response of soybean accession having salt tolerance on reclaimed land in Korea

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Approximately 20% of the world's total land area is affected by salt. Salinity not only affects the plant health but also overall crop yield. Soybean [*Glycine max* (L.) Merr.] is considered as a salt-sensitive crop. Therefore the development of salt tolerance soybean is one of important breeding programs for land with a high amount of salinity. Due to the shortage of cultivation land or territory, Korea has a long history for land reclamation. From the land reclamation project, Korea has been expanded around 190,000 ha. Development of salt tolerant soybean cultivars has become one of important research projects to expand the utilization of reclaimed land in Korea. In one of the previous studies, we developed a recombinant inbred population from a cross between PI 483463 (*G. soja*, salt tolerant) and Hutcheson (*G. max*, salt susceptible). This population was phenotyped with 100mM NaCl in greenhouse conditions. To confirm the salt reaction from greenhouse test, we planted this population in a reclaimed field having ~0.3% salinity levels in the land. These results of the salt reaction in the reclaimed land were in accordance that observed in the greenhouse condition for each of the RILs tested. This suggested that screening the soybean plants for salt tolerance in the greenhouse conditions reasonably provides an early indication of the salt response and the method can be applied to screen larger population. Additionally, the agronomic performance of salt tolerance genotypes was also assessed on the reclaimed land and normal soil conditions. The salt tolerant genotypes showed 76% of plant height, 79% of a number of pods, 70% of seed sized, and 60% of seed yield, as compared to that in the normal soil conditions. Therefore, selected breeding lines or tolerance accessions will be used as a breeding material to develop the cultivars suitable for reclaimed land.

P-081

Altering concentration of omega-6 and omega-3 by improving fatty acid bio-synthesis system in soybean

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Soybean seed has about 20% oil content at maturity with the oil being the world's most widely used vegetable oil. Soybeans contain five predominant fatty acids, 12% palmitic acid, 4% stearic acid, 23% oleic acid (ω-9), 54% linoleic acid (ω-6, LA) and 8% ω- acid (ω-3, ALA). Linolenic acid or ω-3 was reported to have anticancer and anti-inflammatory effects and a role in preventing cardiovascular diseases. In contrast, oils high in ω-6 were reported to have negative effects on health in humans. Studies have shown that minimizing the ω-6/ω-3 ratio in edible oils could have human health benefits. Therefore, reducing the ω-6/ω-3 ratio of soybean fatty acids has become a goal in breeding programs. Normal soybeans have a ω-6/ω-3 ratio of 6 to 7. Wild soybeans contain ~15% ω-3 in seed oil whereas cultivated soybeans have ~8%. Therefore, progeny having different ω-6/ω-3 ratios can be derived from interspecific crosses between a wild and cultivated soybeans. A cross between S08-14717 with high oleic genes FAD2-1A and FAD2-1B having ~80% oleic acid and ~5% ω-6 and ω-3 each was made with wild soybean, PI 483463, with ~14% oleic acid and 55% ω-6 and 15% ω-3. There was large variation in fatty acid composition in F2:3 seed oil from each of 1500 F2 plants from the population. Lines having FAD2-1A or FAD2-1B mutations had 33.5 – 47.1 % oleic acid, 26.5 – 37.8 % ω-6, and 13.9 – 15.7 % ω-3. Therefore, several lines were identified with ω-6/ω-3 ratios as low as 2-3:1. Lines with low ω-6/ω-3 ratios have been advanced to the F5 generation with the ω-6/ω-3 ratios stable in every generation. The ratio between ω-6 and ω-3 is reduced by using FAD2-1A or B mutations to increase oleic acid and reduce ω-6 while increasing the ω-3 from wild soybean. This system will be useful to improve fatty acid composition in soybean.

P-082

Regulation of seed oil accumulation in soybean

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Seed oil is a major agronomical trait of soybean targeted by domestication in breeding. Although multiple oil-related genes have been uncovered, the knowledge of regulatory mechanism of seed oil biosynthesis is currently limited. Through analysis of transcriptomes in developing soybean seeds and identification of gene co-expression networks, we demonstrate that seed-preferred gene GmZF351 encoding a tandem CCCH zinc finger protein is selected during domestication. Further analysis shows that GmZF351 facilitates oil accumulation by directly activating WRI1, BCCP2, KASIII, TAG1 and OLEO2 in transgenic Arabidopsis seeds. Overexpression of GmZF351 in transgenic soybean also activates lipid biosynthesis genes, thereby accelerating seed oil accumulation. ZF351 haplotype from *Glycine max* group and *Glycine soja* subgroup III correlates well with high gene expression level, seed oil contents and promoter activity, suggesting that selection of GmZF351 expression leads to increased seed oil content in cultivated soybean. We also identified other factors including GmNFYA and GmDREBL that play roles in oil accumulation. Overexpression of the two genes in stable transgenic soybean promotes fatty acid accumulation. Mutual regulation of these genes was also examined. Our study provides novel insights into the regulatory mechanism for seed oil accumulation and manipulation of these genes may have great potential in improvement of oil production in soybean and other related crops.

P-083
Development of heart-healthy soybeans through mutation breeding

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Soybean (*Glycine max* (L.) Merrill) is considered a key crop of modern agriculture due to its seed's high oil and protein content. However, the high percentage of polyunsaturated fatty acids in soybean oil limits its stability and shelf life. To reduce these fatty acids, soybean is usually subjected to partial hydrogenation that results in the formation of trans fatty acids that are linked to coronary heart disease. In order to improve stability and shelf life of soybean oil without hydrogenation, soybean with increased oleic acid or reduced linolenic acid content should be developed and characterized through mutation breeding or genome editing techniques. This study aimed to develop heart-healthy-soybeans through the identification of high oleic/low linolenic acid mutants, and to screen for mutations in the crucial genes in oil biosynthesis genes including fatty acid desaturase (FAD2) and microsomal omega-3-fatty acid desaturase (FAD3). JTN-5203 (MG V) soybean mutant population was generated using an induced ethyl methane sulfonate (EMS) mutagenesis. Optimum concentration of EMS was used to treat 15,000 bulk seeds producing a total of 1,820 M1 individuals. Fatty acid profiles such as oleic, linoleic, and linolenic acid were measured in the M2 lines using near-infrared spectroscopy. Oleic acid content in some mutants was increased by up to 40% from 25% in wild type (WT). Linoleic acid in five mutants was reduced to 35% from 49% in WT. Mutants with reduced linolenic acids to 2.9% (WT:7.6%) were recovered. 295-2 mutant line has been found to have a high oleic, low linoleic, and low linoleic acid content. Sequencing will be performed using both Sanger and Illumina platforms in a paired-end multiplexed library. Through mutagenesis and high-throughput sequencing, the novel alleles underlying the mutations observed in mutants with reduced polyunsaturated fatty acids will be identified, thereby developing improved and heart-healthy soybeans.

P-084
High oleic, non-GMO soybean breeding

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High oleic soybean oil is a new food product which is projected to grow extremely rapidly over the next years. Unlike conventional soybean oil, high oleic soybean oil does not require partial hydrogenation to have a high oxidative stability and long shelf life, and is therefore free of trans fats. The projected growth of this new product is due to many factors, including an FDA ban on trans fats in effect beginning in June 2018. High oleic soybean has been developed using several different methods by several different groups. DuPont Pioneer and Monsanto have used transgenic RNAi approaches to develop GMO high oleic soybeans, Plenish® and Vistive® Gold, respectively. Calytx™, a publicly traded biotech startup, has released a gene-edited high oleic soybean as their first commercial product. Many public soybean breeders are breeding non-GMO high oleic soybean varieties using FAD2 mutants developed by Kristin Bliyeu at the USDA. Most public breeders are using a winter nursey, marker-assisted backcross procedure towards introgression of the high oleic trait from FAD2 mutants into elite varieties. The Soybean Breeding Program at Michigan State University has used a single-seed descent approach with early generation, phenotypic selection. Compared to marker-assisted backcrossing, this approach allows us to improve yield and oil quality simultaneously. It also allows us to select for low linolenic acid and low saturated fat using the same GC phenotyping data. Essential to this strategy is a high-throughput oil extraction procedure used to screen over 10,000 breeding lines every year. Commercialization partnerships, frying tests, genetic mapping, as well as past, current, and future research will also be discussed.

P-085

Identification of a haplotype and improved associated molecular marker for the soybean seed ultra-low RFO phenotype

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Soybean is an important annual crop. The raffinose family of oligosaccharides (RFOs) raffinose and stachyose are anti-nutritional carbohydrates. Consumption of soybean seed products with low RFOs reduced flatulence in humans and increased metabolizable energy efficiency in chickens, pig and dog. Many researchers have an interest in the development of soybean lines producing high metabolizable energy with low anti-nutritional factors in the seed. Soybean lines with significant reductions in RFO beyond the observed low RFO levels in soybean seeds were discovered through traditional plant breeding, and the phenotype was designated as the Ultra-Low RFO phenotype. The patented Ultra-Low RFO phenotype was associated with a Raffinose synthase 2 mutant allele (RS2, Glyma.06g179200, rs2W331-) from PI200508 plus a naturally occurring variant of the raffinose synthase 3 gene (RS3, Glyma.05g003900, rs3snp6) on chromosome 05. For the present study, we utilized SNPViz software to survey the number of major haplotypes of RS3 alleles and determined a SNP position that defined a unique RS3 haplotype. We developed independent populations in our research inventory with rs2W331- alleles and each of the three major RS3 haplotypes (A, B, C) to associate the RFO phenotypes with a marker that distinguished the RS3 haplotypes. A one base pair difference, termed rs3snp6, between wild type and variant RS3 alleles in haplotype C was found in intron 1 of the RS3 gene. Although no causative mutant polymorphisms were discovered that explained the molecular basis for the haplotype C variant RS3 alleles to associate with the ultra-low RFO phenotype, this work improved the selection potential for the phenotype with the development and validation of the rs3snp6 molecular marker assay. We determined that the RS3 haplotype C alleles are present in modern breeding lines that can be utilized in combination with rs2W331- alleles to produce the Ultra-Low RFO phenotype in soybean seeds.

P-086

Use of ImageJ to identify stem tissues that predict changes in chemical composition within the seed-filling stages of soybeans

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Cellular activities and compositions change within each of the different stages of plant development. To be able to identify these changes within stems our research group uses ImageJ-Fiji to analyze colorimetric images of plant tissues. The different stains allow us to distinguish between living and non-living cells and between cells containing secondary walls with cellulose or lignin. We then use these fluorescent images of stem tissues (xylem, phloem, cortex, pith, and cambium) to determine the differences between a variety of soybean cultivars with different branching characteristics. Our goal is to determine how branch growth influences pod abortions using these images to determine changes in chemical composition which influence nutrient remobilization within the seed-filling stages. We conducted a field study with six cultivars at two field sites (Suffolk, VA and Orange, VA) and harvested plants for biomass measurement at R5 and R7. Internode segments harvested from the main stem and lowest branch were collected for image analysis. Cultivar variations in branching and stem composition are related to mature and aborted pod and seed yield. This research is important for the farmers of Virginia since soybeans are one of the state's top farming commodities. It will also help improve seed yield by identifying the resource constraints that comes with plant growth, which influences pod abortions.

P-087

Increased soybean seed size and seed protein content due to a fast neutron-induced mutation

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Seed size (or single seed weight, SSW) and chemical composition are key determinants of soybean yield and seed quality. Phenotypic screening of our fast neutron (FN) mutant population identified a line, K83, that produced bigger seeds and vegetative aerial tissues compared to unmodified Williams 82. Microscopic examination of cotyledon epidermal cells showed more cells per unit area and higher stomatal index in K83 compared to Williams 82, indicating that the increased seed size is due to increased number of cells rather than due to increased cell size. Seed composition analysis indicated that K83 seeds showed consistently higher protein content compared to Williams 82 over three growing seasons. Comparative Genome Hybridization (CGH) analysis identified four deletions, encoding a total of 318 genes, in the K83 genome. Segregation analysis of BC1F2 plants indicated that the big seed phenotype is due to a single dominant locus. Moreover, the big seed phenotype co-segregated with the 361 Kb deletion in Chr. 17, indicating that a knock-out mutation in a gene(s) encoded within the deleted region is responsible for the seed phenotype. Interestingly, the deletion in Chr.17 overlaps qSw17-1, a major and stable QTL associated with seed weight in soybean. Similar to the FN K83 mutant, the increased seed weight phenotype associated with qSw17-1 is due to a dominant allele. Therefore, it is very likely that the big seed phenotype is due to the same gene in K83 and qSw17-1. A large number of seed trait QTLs have been defined in soybean (https://soybase.org/), but so far very few genes controlling seed size and quality traits have been identified to date. A main goal of our research is to complement traditional breeding approaches with induced mutagenesis technologies, including targeted gene editing, to facilitate gene discovery and seed trait improvement in soybean.

P-088

A quantitative HPLC method to determine trypsin inhibitor concentration in soybean seeds

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Trypsin inhibitor (TI) is an important anti-nutritional factor present in soybean seeds that prevent animal's protein digestibility. Determining seed trypsin inhibitor concentration is essential for screening and selecting soybean germplasm and breeding lines with low TI. Currently, a colorimetric bioassay that measures TI activity (TIA) is widely used to measure TI in soybeans. This bioassay is time consuming, expensive and has repeatability issues. This study developed a high performance liquid chromatography (HPLC) method, based on quantifying Kunitz trypsin inhibitor (KTI), as a high throughput, less expensive and more reliable assay to quantify TI in soybean seeds. The HPLC method was evaluated on 100 lines with various TI concentration and compared to the modified colorimetric bioassay. For the bioassay, TI was extracted by mixing soybean seed powder in 9 mM HCl and TIA was determined based on Kakade et al., 1974. For comparison, the TIA was converted into weight-based unit assuming that KTI is a dominant TI contributor. For the HPLC method, KTI was extracted by using 0.1M NaOAc, separated on a Poros R2/H perfusion column and detected at 254 nm. Each method was performed by duplicate. KTI from the bioassay ranged from 3.69 to 8.43 mg/g with an average of 5.21 mg/g, and the KTI from HPLC method ranged from 0.61 to 11.21 mg/g with an average of 5.25 mg/g. Data from both methods were strongly correlated ($r = 0.78$, $p<0.0001$). The coefficient of variation (%) of KTI data (66% HPLC and 19% bioassay) suggests that the HPLC method reveals a wider range of KTI data that may have a better sensitivity to detect KTI than the bioassay. In addition, the colorimetric bioassay is experimentally tedious and labor intensive. Therefore, the HPLC method is highly preferred to provide a simpler, faster, more sensitive and reliable quantification of TI in soybean seeds.

P-089

Genome-wide SNP selection associated with amino acid content in core collection of wild soybean using the elastic-net method

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Soybeans are one of the most important food crops because they contain all of essential amino acids. In this study, we identified genome-wide SNP markers associated with amino acid content in core collection of wild soybeans genotyped with Affymetrix Axiom 180k SoyaSNP array. A total of 375 wild soybean accessions were used to determine amino acid content with amino acid auto-analyzer. The contents of total amino acids ranged from 33,843 to 50,819 (mg/100g), and all amino acids was positively correlated with each other ($P \leq 0.05$). As a result of SNP estimation using elastic-net method, total of 59 SNPs associated with amino acids content were identified across soybean chromosomes. The identification of SNP markers associated with amino acids contents are expected to be helpful for the development of molecular markers that can be used for soybean breeding.

P-090

Identification and characterization of fast-neutron induced mutations underlying altered seed composition phenotypes for improvement of soybean seed composition

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Soybean is a major component of feed, food, and fuel. Protein, oil, amino acids, fatty acids, and sugars in seeds affect the efficacy of soybean for end use. Improving soybean seed composition is an essential goal for soybean breeders. However, negative relationships between protein content and yield as well as protein and oil contents present challenges for soybean breeders improving both seed quality and yield. Fast neutron radiation introduces genomic deletions, duplications, and translocations resulting in novel genetic and phenotypic variation for traits of interest. Two elite soybean lines were irradiated with fast neutrons and screened for altered seed composition using near-infrared (NIR) spectroscopy. Twenty-three lines with altered protein, oil, or sucrose content were selected based on NIR data from five environments over two years and yield tested at five locations. Protein contents of these mutants were 0.6 to 2.9% higher than those of the parents, oil contents were 0.1 to 7.9% lower than the parents, and high sucrose mutants had 0.6 to 3.7% higher sucrose than the parents across environments. Comparative genomic hybridization (CGH) was performed on four of these mutants and identified putative mutations resulting in these phenotypes. Whole genome sequencing (WGS) of two mutants was conducted to assist in marker development at genomic mutation regions. Two F2:3 populations were developed from two seed composition mutants to determine association between genomic changes and altered phenotypes. Bulked segregant analysis of these populations using the SoySNP50K Infinium BeadChip identified deletions on chromosomes 12 and 16 putatively responsible for elevated protein content and for increased sucrose and decreased oil contents, respectively. Deletion regions were confirmed with both CGH and WGS. Mutants with altered seed composition are a new resource for gene function studies in soybean and provide elite breeding materials for development of varieties with improved seed composition.

P-091

Introgression of a high protein allele into an elite variety results in a high-protein near-isogenic line with yield parity

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Soybean meal is the largest source of protein meal consumption worldwide. Soymeal is a high-protein product with all the essential amino acids for animal growth and development. Soybeans are valued for their suitability for use in feed, but in recent years the protein content of soybeans in the United States has been decreasing. Soybean seed protein content is negatively correlated with both yield and oil content. These negative relationships complicate the simultaneous improvement of seed protein and selection for high-yielding varieties. A major protein QTL on chromosome 20 has been identified in many GWAS and bi-parental population mapping studies. The effects of various sources of the chromosome 20 high-protein allele have been studied in several Northern and Midwestern backgrounds. The high-protein allele is often associated with a significant increase in protein and decreases in oil and yield. The chromosome 20 high-protein allele from the Korean tofu cultivar, Danbaekkong (PI619083), was introgressed into an elite MG-VII line, Benning (PI595645), at the University of Georgia. A near-isogenic line (NIL), designated Benning HP, was developed through backcrossing. The Benning HP NIL was entered into yield trials in 2015 at three locations, seed was increased in 2016, and Benning HP was again entered into 2017 UGA Advanced Yield Trials. Results from eight environments over two years revealed yield parity between the recurrent parent, Benning, and the NIL, despite a 3-4% increase in protein in the NIL. This NIL may be used to develop high protein soybean varieties in the Southern U.S. without sacrificing yield.

P-092

Evaluating an isoflavone synthase gene as a potential caterpillar resistance gene

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Over the years, farmers all across the world have been experiencing dramatic losses in their soybean yield. One cause of this problem is due to caterpillars, however, there is a range of damage seen across different cultivars. One of the potential genes that could be important for making plants resistant to caterpillars is isoflavone synthase (IFS). The purpose of our work is to examine the levels of expression of IFS in soybean lines Boggs (partially-resistant) and Benning (susceptible). We know that both cultivars contain IFS, but we don't know how much of the gene is being expressed. The levels of expression between leaves with caterpillar feeding and control leaves will tell us whether or not IFS is important for soybean plants being resistant to caterpillars. To test expression levels, RNA was extracted from leaves of insect infested plants and non-infested control plants of Boggs and Benning and converted into cDNA. Real-time polymerase chain reaction (RT-PCR) was used to test expression levels for the IFS gene. Our data show that there is a trend between the levels of gene expression between lines that may be connected to resistance, but more samples will be required to investigate this trend. Further work at UGA will follow up on these findings and evaluate this IFS as a potential resistance gene.

P-093

An analysis of the relationship between test weight and seed composition of soybean

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Test weight of grain has been established as an effective measure to broadly assess overall grain quality. The present study aimed to identify correlations between test weight and protein and oil contents of soybean [*Glycine max* (L) Merr.]. The protein and oil contents of seed samples were obtained by near- infrared reflectance (NIR) spectroscopy. Test weight analysis was performed using the Dickey John GAC 2500-UGMA grain analysis computer. The sample set consisted of 200 lines selected for high protein content and 200 lines selected for low protein content. Of the 400 lines, analysis of 100 lines of the highest protein content to that of 100 lines of the lowest protein content yielded a non-significant positive correlation between test weight and protein ($r = 0.12$, $p = 0.083$) and a negative correlation between test weight and oil ($r = 0.22$, $p = 0.0015$). Results show a non-significant positive correlation between test weight and protein content in the sample set consisting of the remaining 200 lines ($r = 0.00095$, $p = 0.99$) and a non-significant negative correlation between test weight and oil content ($r = - 0.015$, $p = 0.83$). The second set of 200 had a much smaller spread in protein and oil compared to the first set of 200. The entire sample set (n=400) displayed a non-significant positive correlation between test weight and protein content ($r = 0.077$, $p = 0.17$) and a negative correlation between test weight and oil content ($r = -0.14$, $p = 0.0042$). The results of this study indicate the degree of variation in protein content of seed samples influences the test weight to protein and oil correlation. Further testing is needed to validate these findings. Two replicates of the 400 lines are currently being grown at two locations in NC to provide the necessary data points.

P-094

Molecular characterization of HPPD inhibitor herbicide resistance in waterhemp

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Waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) is a problem weed commonly found in the Midwestern United States that can cause crippling yield losses of up to 56% in soybean (*Glycine max* L. Merr) production systems. Since the discovery of p-hydroxyphenylpyruvate-dioxygenase (HPPD, EC 1.13.11.27) inhibitor herbicide resistance, studies have identified the likely mechanism of resistance and described the inheritance of the herbicide resistance, however causal genes remain unknown. To date, no studies have examined genome-wide gene expression changes in response to HPPD herbicide treatment in herbicide resistant and susceptible waterhemp. We conducted RNA-seq analyses of two waterhemp populations (HPPD-herbicide resistant and susceptible), from herbicide-treated and mock-treated leaf samples at three, six, twelve, and twenty-four hours after treatment (HAT). We performed a de novo transcriptome assembly using all sample sequences. Following assessment of our assembly, individual samples were mapped to the de novo transcriptome allowing us to identify transcripts specific to a genotype, herbicide treatment, or time point. Our results indicate that the response of HPPD-herbicide resistant and susceptible waterhemp genotypes to HPPD-inhibiting herbicide is rapid, established as soon as three hours after herbicide treatment. There was little overlap in gene expression between resistant and susceptible genotypes, highlighting dynamic differences in response to herbicide treatment. Further, mapping differentially expressed genes allowed us to identify clusters of herbicide-responsive genes suggestion coordinated regulation. Using stringent analytical methods we identified candidate single nucleotide polymorphisms (SNPs) that distinguish the resistant and susceptible genotypes. The waterhemp transcriptome, herbicide-responsive genes and SNPs generated in this study provide valuable tools for future studies by numerous plant science communities. This collection of resources is essential to study and understand herbicide effects on gene expression in resistant and susceptible weeds. Additionally, increased understanding of the prolific traits intrinsic in weed success could lead to crop improvement.

P-095

Identifying miRNAs and their target mRNAs to understand the temporal and spatial regulation of gene expression during soybean seed development

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Soybean seeds are utilized widely for agricultural and commercial purposes due to its high oil and protein content. Understanding the molecular mechanisms that underlie soybean seed development will be useful to increase soybean seed yield. Soybean seeds consist of three major regions: embryo, endosperm and seed coat. The seed regions are further differentiated into subregions. Each subregion undergoes unique temporal and spatial developmental programs. micro RNAs (miRNA) are a class of small RNAs that are involved in developmental processes in plants. In recent years, miRNAs in plants, including soybean, have been extensively studied. However, little is known about miRNA accumulation at the tissue level during soybean seed development. We profiled small RNAs from 37 different subregions of soybean seeds at four different developmental stages by using Laser Capture Microdissection. Of over 200,000 miRNA candidates, 276 miRNAs were identified during soybean seed development by our stringent miRNA evaluation method based on stemloop formation, the unique characteristics of miRNA biogenesis, and miRNA abundance. The majority of miRNAs accumulates in one specific subregion or stage, suggesting miRNAs may play important roles in regulating spatial and temporal developmental processes. To understand miRNA function in seed development, we analyzed 14 publicly available parallel analysis of RNA ends (PARE) datasets to identify the mRNAs cleaved by miRNAs. We identified 720 miRNA-mRNA target pairs by using stringent filtering criteria. Based on our PARE dataset analyses, miRNAs are predicted to regulate a wide range of biological processes including development, hormone, and defense. Understanding the functions of miRNAs at the tissue level at different developmental stages will provide new insights into the molecular mechanisms controlling soybean seed development.

P-096

Identification of recombination hotspots throughout the soybean [*Glycine max* (L.) Merr.] genome

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The identification of recombination hotspots will increase plant breeders power to introgress favorable traits, thus increasing the rate of genetic gain. However, it can be difficult to detect the low recombination frequency without a dense genetic map and a sufficient number of offspring. In soybean [*Glycine max* (L.) Merr.] a high-density genotyping array, SoySNP50K, was employed on two biparental populations: Williams 82 x Essex consisting of 922 RILs with 11,922 polymorphic SNPs and Williams 82 x PI479752 (*G. soja*) containing 1038 RILs and 21,478 polymorphic SNPs. Here we used two methods to identify recombination hotspots and coldspots in the biparental RIL populations. The first method is a pairwise comparison between the statistical genetic map and physical map. The second method a linkage disequilibrium (LD) approach that directly relates LD to recombination patterns by considering all loci simultaneous and remains computationally tractable for an entire chromosome. These populations will be used to identify genomic features such as gene rich regions, GC content, transposable elements, and motifs associated with variation in recombination rate.

UGA Advanced Yield Trial at Iron Horse Farm. Photo by Zenglu Li.

P-097

SNP marker associations with soybean fatty acids

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The five-fatty acids (FA) - palmitic, stearic, oleic, linoleic, and linolenic acid - in soybean seed are essential FAs, and these FAs determine proportions of fat production and oil quality. These FAs associated genetic variants have been widely studied. FA is a quantitative trait which can also be affected by environments such as growing conditions and temperature, thus identifying correct genetic variants is crucial for successful development of new soybean lines. We used genome wide association study (GWAS) to analyze the genetic variations in seed FAs. GWAS was performed by using a 34,014 single nucleotide polymorphism (SNP) for 621 of USDA germplasm accessions in maturity groups I - IV. This study builds upon our previous study using GWAS for analyzing the environmental effects. To detect FA-environment association, the accessions were grown in five different environments in the U.S. The phenotypic data were acquired by using gas chromatograph for each FA content analysis. Genomic association and prediction integrated tool was used to identify the significant SNPs related to the FAs. We determined appropriate significance level of α=5% and α=25%, the adjusted significance thresholds were $-\log10(P) > 5.44$ and $-\log10(P) > 4.74$, respectively, which is different from our previous study. In this study, we identified two, six, two, and two genomic regions significantly associated with seed palmitic, stearic, oleic, and linoleic acid contents, respectively. On the other hand, there was no genomic region significantly associated with linolenic acid. The most significant SNPs for palmitic, oleic, and linoleic acid were on chromosome 5, and for stearic acid was on chromosome 14. We also identified six of common SNPs which have effects on both oleic and linoleic acids on chromosome 5. These findings will be useful to understand the complexity of FA related genetic mechanism.

P-098

Early detection and quantification of *Pythium* seed rot in soybean through hyperspectral imaging

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A severe dilemma faced by soybean [*Glycine max* (L.) Merr] farmers is the increasing occurrence of biotic stresses such as *Pythium* Seed Rot disease (caused by *Pythium sylvaticum*), which is prevalent in most production areas and commonly causes poor stand establishment leading to lower seed yield. The most promising disease management approach is to develop Pythium sylvaticum resistant soybean cultivars, and the identification of genetic sources of resistance is a precursor for the development of resistant varieties. Quick and accurate phenotyping is essential to identify novel genetic loci successfully and for cultivar development approaches. Unfortunately, most current *Pythium* Seed Rot phenotyping protocols rely heavily on visual rating methods, which can be error-prone and tedious. Remote sensing techniques such as hyperspectral imaging (HSI) is a viable alternative to visual disease phenotyping as it can capture consistent and accurate phenotypic data in a non-destructive manner. HSI research for the detection and quantification of plant disease relies on the identification of differentiating spectral signatures across the electromagnetic spectrum to distinguish the plant's disease status. The primary objective of this project is to use HSI to identify significant disease signatures for the detection and quantification of *Pythium* Seed Rot disease on soybean cultivars for eventual use in a high throughput phenotyping scheme as well as for genetic studies. To identify *Pythium* Seed Rot spectral signatures, we used a circular seed plate assay technique in an incubator to investigate inoculated and non-inoculated treatments consisting of resistant and susceptible soybean genotypes. Preliminary results show the effectiveness of HSI to accurately distinguish inoculated and healthy plant tissue at a temporal scale that can be deployed in phenomics and genomics studies as well as in production fields for disease diagnosis

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P-099

Insight into the genetic variation of test weight and its impact in soybean

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Soybeans are the world's largest source of animal protein feed and the second largest source of vegetable oil. The United States is the leading soybean producer and exporter. Current grading standards list six grade-determining factors for yellow soybeans which are test weight (TW, bulk density), splits, total damaged soybeans, heat damaged, foreign material and soybeans of other colors. Of those, TW is a primary indicator of grain quality and measure of bulk density. The objectives of this research were to understand the genetic variation of TW among genotypes across environments and between planting dates, and to determine its relationship with agronomic traits and seed composition. Thirty-six genotypes were grown at four locations three replicates per location in GA and NC and 85 genotypes were grown at two locations in GA in 2017. Yield, TW, seed composition, maturity, and seed size were collected from all plots. Another panel of 88 genotypes were grown in Athens at two planting dates and same set of traits except yield were also recorded. Significant variations ($p<0.001$) of TW were observed among genotypes across locations, ranging from 703 to 765g/L. Locations and G x E interactions had significant effects on TW ($p<0.001$). Seed oil content and size are negatively correlated with TW across locations, while protein content are positively correlated with TW ($p<0.05$). No significant difference of TW among the genotypes was observed between planting dates. At early planting date, similar results were observed for the TW. However, positive correlation between maturity and TW ($p<0.05$) was observed only at late planting date. The results could be used to guide the genetic improvement of TW.

P-100

Optimizing resources for predicting phenotype using canopy coverage image and genotypic information in soybean

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Genomic prediction (GP) techniques became an important part of plant breeding programs due to their advantages compared to traditional phenotypic or pedigree based selections. GP is a technique where plants' genotypic and phenotypic information - called training set - are used to predict plant's phenotypic performance for which only marker information is available, also known as testing set. GP models can be extended to include high-throughput phenotypic information in the hope of increasing predictive ability. Herein we introduce an algorithm to predict a trait using marker and canopy information collected from 5600 recombinant inbred lines of a soybean nested association mapping panel, which utilizes a hybrid matrix for the inclusion of marker and canopy information for the purpose of predicting the trait. The model shows improved predictive ability compared to the models when only marker and only canopy information is included.

P-101

Increasing predictive ability by modeling interactions between environments, genotype and canopy coverage image data for soybeans

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Phenomics is a new area that offers numerous opportunities for its applicability in plant breeding. One possibility is to exploit this type of information obtained from early stages of the growing season by combining it with genomic data. This opens an avenue that can be capitalized by improving the predictive ability of the common prediction models used for genomic prediction. Imagery (canopy coverage) data recorded between days 14–71 using two collection methods (ground information in 2013 and 2014; aerial information in 2014 and 2015) on a soybean nested association mapping population (SoyNAM) was used to calibrate the prediction models together with the inclusion of several types of interactions between canopy coverage data, environments, and genomic data. Three different scenarios were considered that breeders might face testing lines in fields: (i) incomplete field trials (CV2); (ii) newly developed lines (CV1); and (iii) predicting lines in unobserved environments (CV0). Two different traits were evaluated in this study: yield and days to maturity (DTM). Results showed improvements in the predictive ability for yield with respect to those models that solely included genomic data. These relative improvements ranged 27–123%, 27–148%, and 65–165% for CV2, CV1, and CV0, respectively. No major changes were observed for DTM. Similar improvements were observed for both traits when the reduced canopy information for days 14–33 was used to build the training-testing relationships, showing a clear advantage of using phenomics in very early stages of the growing season.

P-102
Molecular tools for reducing yield losses due to pod shatter

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Pod shattering is an ancestral trait that promotes seed dispersal; however, shattering can have substantial yield losses in cultivated soybean. During the improvement process, American soybean breeders virtually eliminated the shatter phenotype for released varieties, but in other countries, shatter persists. The objective of our research was to find a molecular tool to implicate genetic shatter susceptibility, validate its usefulness, and apply this knowledge to identify shattering potential in parental lines. Previous research revealed the gene Pdh1on chromosome 16 plays a crucial role in determining the shatter phenotype. We developed a perfect molecular marker assay to detect alleles of the Pdh1 gene. We also performed a Genome Wide Association Analysis Study using the Pdh1 allele status as a phenotype and identified a highly associated marker in the SoySNP50K array. Soybean accessions from the GRIN National Plant Germplasm System (GRIN-NPGS) with shatter score and SoySNP50K data were evaluated to determine the impact of the predicted Pdh1 alleles on early and late pod shattering. We developed an online tool to enable researchers to query the GRIN collection for the predicted Pdh1 allele status. Soybean breeding programs that access germplasm from the GRIN collection can utilize these resources to eliminate the Pdh1 effects on pod shatter and thus improve yield potential.

P-103
Deciphering composition of root microbiome of soybean genotypes with different traits

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Microbial community is one of key drivers for plant growth and development including plant health and decomposition of complex substances to absorbable nutrients. Hence, identifying beneficial microbiomes which improve plant productivity is a promising approach for cultivar development and plant cultivation. With development of high-throughput sequencing, studies of microbiome have been conducted in various crops. Recent studies indicated some bacteria including *Pseudomonas aeruginosa*, *Bradyrhizobium* spp., *Rhizobium* MRPI affected positively to plant immunity and nodulation of nitrogen fixation. Here, we focused on discovering root-associated bacteria across different soybean genotypes using 16s rRNA sequencing. Six soybean genotypes with contrasting traits: high/ normal protein content and resistant/ and susceptible to soybean cyst nematode (SCN) and root-knot nematode (RKN) were grown at the UGA Iron Horse Farm with three replications per genotype. Root and soil samples were collected for bacterial 16s analysis. Sequencing of the 16s gene indicated that soybean root and soil samples overlapped and had difference in bacterial communities. There was a transition pattern of some bacterial communities between root and soil samples, revealing that soybean had an ability to select bacterial from soil and combine with the bacteria in roots to have their bacterial niche. However, by comparing between different traits, soybean genotypes did not show significant differences in bacterial composition. This could be due to using a limited number of soybean genotypes and evaluating at one sampling site. Yet, this preliminary served as a platform to further study on soybean root-associated bacteria with different traits.

P-104

Genome-wide association analysis pinpoints additional major genomic regions conferring resistance to soybean cyst nematode

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Soybean cyst nematode (SCN) is the most destructive pest affecting soybean in the U.S. Two major resistance loci, Rhg1 and Rhg4 on chromosome (Chr) 18 and 8, have been reported in many sources of SCN resistance. PI 88788 (rhg1) and 'Peking' (rhg1/Rhg4) have been widely used to develop resistant cultivars in the U.S. However, this has become a major concern and it is essential to identify new resistant genes before nematodes develop immunity to these two sources. To discover new sources of resistance, we screened 462 soybean accessions using a greenhouse bio-assay and genotyped them with three SNPs developed at Rhg1 and Rhg4 loci. Of 462, 50 accessions were classified as the 'Peking'-type resistance, while 30 accessions classified as the PI88788-type resistance. There were 58 accessions that were rated as SCN resistance in greenhouse phenotyping that do not carry either 'Peking' or PI88788 resistance alleles at Rhg1 and Rhg4 loci. The result indicated that these 58 accessions might possess novel SCN resistance genes or alleles. The genome-wide association study (GWAS) was performed on 461 accessions using ~35,820 SNPs from the Soy50KSNP Infinium Chip data. It identified 12 SNPs representing four genomic regions on Chrs 7, 8, 10 and 18 were significantly associated with SCN resistance. Three of 12 SNPs were located at two known major QTLs: Rhg1 and Rhg4 on Chr 18 and 8, respectively. Twenty-four predicted genes were found near the significant SNPs on Chr 7 and 10 that encoded various types of protein kinase, receptor-like protein suggesting that they could be the genes associated with SCN resistance. The identified SNPs and candidate genes from this study might be beneficial for development of DNA markers to be used for marker-assisted breeding and to aid in developing soybean cultivars with novel sources of SCN resistance.

P-105

Soybean iron deficiency chlorosis high throughput phenotyping using an unmanned aircraft system

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Iron deficiency Chlorosis (IDC) is an abiotic stress in soybean [*Glycine max* (L.) Merr.] that causes significant yield reductions. Symptoms of IDC include interveinal chlorosis and stunting of the plant. While there are management practices that can overcome these drastic yield losses, the preferred method to manage IDC is to grow tolerant soybean varieties. To develop these varieties, breeders may phenotype thousands of soybean lines every year for IDC severity, a task traditionally done with a 1-5 visual rating scale. The visual rating system is subjective and time consuming and can typically only be accomplished once or twice during a growing season. The goal of this project is to use an unmanned aircraft system (UAS) to improve field screening for tolerance to soybean IDC. During the summer of 2017, 3,386 plots were visually scored for IDC stress on two different dates. In addition, images were captured with a DJI Inspire 1 platform equipped with a modified dual camera system which simultaneously captures digital red, green, blue images as well as red, green, near infrared (NIR) images. A pipeline was created for image capture, orthomosaic generation, processing, and analysis. Plant and soil classification was achieved using unsupervised classification resulting in 95% overall classification accuracy. In addition, a total of six predictors were extracted from the imagery of each plot including spectral and canopy properties. Random forest and neural network algorithms were used on two separate dates of visual rating collection and resulted in an accuracy of 0.73 for random forest and 0.77 for neural network. The normalized difference vegetation index was significantly different across the phenotypic classes and had the highest contribution to the fit of the models. Overall, the UAS was able to capture differences in IDC stress and may be used for selection in a breeding program.

P-106

Characterizing LEC1 transcriptional regulatory networks controlling soybean seed development

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The transcription factor LEAFY COTYLEDON1 (LEC1) is a central regulator of seed development. It was previously shown that LEC1 has the ability to regulate the expression of several genes during the development of the soybean seed, such as establishment of embryo morphology and accumulation of storage compounds. Despite its importance, knowledge of the mechanisms that underlie LEC1 function is still limited. Soybean seed quality can be increased through modifications in developmental traits, such as increases in embryo size and nutritional quality. In that sense, understanding LEC1 regulatory networks is crucial to develop strategies to improve soybean seed quality. In order to identify target genes that are transcriptionally regulated by LEC1 and three other transcription factors involved with seed development (ABI3, AREB3 and bZIP67), we performed chromatin immunoprecipitation – DNA sequence analyses and differential gene expression analyses during soybean seed development. Detailed analysis of target genes uncovered complex transcription factor regulatory networks in which LEC1 associates with other transcription factors to control distinct biological programs in soybean embryos, such as seed maturation, photosynthesis and embryo morphogenesis. DNA sequence motif enrichment analyses suggested that the formation of distinct LEC1 complexes is determined by the presence of unique combination of cis-regulatory elements in the promoters of target genes. For instance, a significant enrichment of G-box (CACGTG) and RY (CATGCA) elements was only identified in the promoter regions of genes that are regulated by LEC1 in association with ABI3, AREB3 and bZIP67. We also observed that distinct sets of LEC1 complexes formed due their ability to physically interact to each other, and this interaction was essential to determine LEC1 function. The results obtained in this study are helping us to elucidate the LEC1 regulatory networks that control soybean seed development.

P-107

Novel alleles of FAD2-1A induce high levels of oleic acid in soybean oil

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Soybean plays an important role in seed oil production for foods and industrial products in the United States. Chemical hydrogenation of commodity soybean oil increases functionality, but unavoidably creates trans-fat as a byproduct which is linked to many health issues in humans. An alternative to using hydrogenation of the oil to enhance oxidative stability is to genetically increase the level of oleic acid in the seed oil. Our goal was to create high oleic acid and low linolenic acid soybean germplasm with increased stearic acid content utilizing new and existing variant alleles of key fatty acid desaturase genes. We hypothesized that novel alleles of FAD2-1A identified from a mutant population containing missense SACPD-C alleles that elevate the stearic acid content would increase the oleic acid seed oil content when combined with existing mutant alleles of FAD2-1B; and that incorporating existing FAD3 mutant alleles with the novel FAD2-1A and FAD2-1B combination would create new genetic sources of high oleic acid soybean germplasm. The effects of the new FAD2-1A alleles had not been characterized before, and changes in stearic acid content were previously demonstrated to be quite variable. We observed an increase in oleic acid seed oil content to ~81% in one of our populations. Stearic acid increased to ~10-11% in lines containing the mutant alleles; however, it was associated with a decrease in the oleic acid content and did not meet our target of 20% stearic acid. One F3 germplasm line was homozygous for all five mutant alleles. This study is beneficial for improving the quality of soybean oil based on nutritional value and oxidative stability.

P-108

Role of FANCM in soybean: A strategy to increase meiotic crossovers

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The ability to manipulate meiotic recombination or crossovers (CO) will provide a tool that will allow breeders to combine needed genetic diversity more efficiently to select for desired alleles to improve genetic gain. The genetic underpinnings that govern this process are coming into focus. For example, a mutation in the AtFANCM allele was observed to have CO frequencies increase by 3-fold in *Arabidopsis*. The goal of this project is to characterize soybean homologs of known gene calls that have been assigned function associated with CO in *Arabidopsis*. To this end a suite of vectors have been assembled that carry regulatory regions of soybean homologs of FANCM, MHF1, MHF1a and MHF2 fused to the visual marker YFP as a means to monitor promoter activity of the respective promoter elements. In addition, ectopic expression cassettes were constructed for GmMHF2, along with a hair-pin element designed to down-regulate GmFANCM. The promoter/reported cassettes have been introduced into Arabidopsis and the GmFANCM silencing allele introduced into soybean. Successful transformations were recovered from all except for GmMHF2 fused to YFP introduced in Arabidopsis. Soybean transformations with the ectopic expression cassettes are in progress. Current results characterize the Arabidopsis events carrying the promoter-YFP fusions and soybean events harboring the GmFANCM silencing allele. This work will determine if the FANCM gene affects recombination in soybean.

P-109

Accumulation of iso-flavonoids and phenolic acid conjugates in response to soybean cyst nematode in wild soybean (*Glycine soja*)

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Plants produce a wide range of biologically active metabolites to protect themselves against attacking pests. Elucidating the key metabolites and associated pathways underlying defense responses is critical in understanding the molecular mechanisms of plant chemical defense. Non-targeted metabolomics analysis has emerged as a useful strategy to increase our understanding of the resistance-related (RR) metabolites and pathways in plant-pathogen interactions. In this study, we performed a non-targeted metabolomic analysis to determine and compare the roles of key metabolites and pathways in response to infection by the soybean cyst nematode (SCN, Heterodera glycines) in wild soybean (Glycine soja). SCN is the most devastating pest causing significant losses in soybean yield. A comparison of the metabolic profiles among SCN-resistant (S54) and susceptible (S67) genotypes showed clear differences, mirroring the effects of isoflavonoids (daidzein, daidzin, malonyl daidzin, formononetin, and iso-formononetin), as well as phenolic acids and phenolic acids-derived hydroxyl and methylated glucoside esters, in defense. To the best of our knowledge, these findings uncover the first metabolomics-based network for defending against SCN HG type 1.2.5.7 (SCN-2). The results of the present research can facilitate the future metabolic engineering to develop novel and diverse soybean cultivars with enhanced SCN resistance and/or improved nutraceutical value.

UGA Soybean Yield Trial at Iron Horse Farm. Photo by Ethan Menke.

P-110

Screening for SCN resistance and field evaluation of soybean recombinant inbred lines

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Soybean Cyst Nematode (SCN) is the most damaging pathogen in the US and in the world. Annual yield losses due to SCN are estimated to cost over $1.2 billion in the US. Breeding for resistance remains the main strategy to control SCN and maintain yields. However, the sole use of a single source of resistance from PI88788 caused a shift in virulence of SCN populations and the latter became adapted, breaking the resistance of most of the available commercial cultivars. Moreover, there are reports associating SCN resistance to a considerable yield drag in sites with no or few SCN inoculum. Therefore, there is a need to continuously develop cultivars with multiple sources of SCN resistance. We evaluated 115, F5 derived recombinant inbred lines (RILs), resulting from a cross between TN09-029 and NCC05-1168. The population was segregating for SCN and resistance to other diseases, yield as well as other agronomic and seed quality traits. The lines were evaluated over two years in 5 environments across Tennessee, each with two replications in a completely randomized block design. Statistical analyses were done with SAS 9.4. The 115 lines were screened for resistance to SCN race 2 (HG 1.2.5.7) with each line in 5 replications in the greenhouse at USDA-ARS Jackson Tennessee. Female Indexes (FI) were calculated based on susceptible standards (Hutcheson or 5601T). Lines with FI£ 10% were recorded resistant and FI£10% susceptible. Differences were registered in yield ($P<0.001$) and other traits. The RIL top yielders (SCNepi-038= 4096 kg ha-1, SCNepi-095=3991 kg ha-1) were found in the same group as the check (Ellis = 4057 kg ha-1). No significant differences were found between the mean seed yield of the resistant lines (3,075 kg ha-1) and the susceptible lines (3,076 kg ha-1), suggesting that resistance to SCN race 2 does not necessarily compromise yield.

P-111

Transcriptome, spliceosome, and methylome analyses of soybean nodules at various developmental stages

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Nodule formation by nitrogen-fixing soil rhizobia on the roots of leguminous plants is a sophisticated biological process that involves a successive cascade of regulatory events, which remain largely unknown. In this study, we investigated the transcriptome, spliceosome, and methylome of soybean nodules at three developmental stages compared with root tissues. RNA-seq analysis of 12-, 22- and 36- day-old nodules resulted in the identification of core nodule genes that were similarly regulated during all developmental stages. In addition, stage-specific differentially expressed genes (DEGs) were also identified. Spliceosome analysis revealed active alternative splicing particularly in the developing nodules as more than 2,000 genes were identified as differentially spliced. Thus, alternative splicing seems to increase proteome diversity and hence, plays a key role in nodule identity and function. Methylome analysis indicated that DNA methylation contributes significantly to the regulation of gene expression in the nitrogen-fixing nodules as more than 1,000 DEGs were also found to be differentially methylated. Gene Ontology and pathway analyses revealed the molecular functions, biological processes, and signaling pathways that are critical for nodule formation, development, and senescence.

P-112

Comparative RNA-seq analysis uncovers a complex regulatory network for soybean cyst nematode resistance in wild soybean (*Glycine soja*)

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Soybean cyst nematode (SCN) is the most damaging pest of soybean worldwide. The molecular mechanism of SCN resistance remains largely unknown. We conducted a global RNA-seq comparison between a resistant genotype (S54) and a susceptible genotype (S67) of Glycine soja, the wild progenitor of soybean, to understand its regulatory network in SCN defense. The number of differentially expressed genes (DEGs) in S54 (2,290) was much larger than that in S67 (555). A number of defense-related genes/pathways were significantly induced only in S54, while photosynthesis and several metabolic pathways were affected in both genotypes with SCN infection. These defense-associated DEGs were involved in pathogen recognition, calcium/calmodulin-mediated defense signaling, jasmonic acid (JA)/ethylene (ET) and sialic acid (SA)-involved signaling, the MAPK signaling cascade, and WRKY-involved transcriptional regulation. Our results revealed a comprehensive regulatory network involved in SCN resistance and provided insights into the complex molecular mechanisms of SCN resistance in wild soybean.

P-113

Rapid NIL development through identification of genetic heterogeneity within accessions in the USDA Soybean Germplasm Collection

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Soybean is a valuable crop for the United States and the rest of the world. The genetic diversity of cultivated soybean is narrow due to a strong domestication bottleneck and intense selection in modern breeding. This narrow genetic base limits the identification of genetic loci responsible for traits of interest to breeders. A major approach to identify the genomic basis of desirable traits is through near-isogenic lines (NILs). While NILs are a useful approach for trait mapping and characterization, creating such lines are costly and time-consuming. Thus, other resources and techniques for NIL development have arisen for trait mapping and validation alongside rapidly evolving genomic tools. NILs are now often derived from heterogeneous inbred families (HIFs) instead of repeated backcrossing, allowing for faster, cheaper development, as well as QTL mapping of more complex phenotypes with molecular markers. Despite the long-held assumption that elite varieties are highly homogeneous, within-accession variation has been observed in elite germplasm of various crop species, including the soybean reference cultivar 'Williams 82'. However, the investigation of within-accession heterogeneity has been limited to select cultivars, and has not been evaluated on a germplasm-wide scale. The USDA Soybean Germplasm Collection, containing over 20,000 wild and cultivated soybean accessions, has been recently genotyped with over 42,000 single nucleotide polymorphism markers. In this study, this SoySNP50K genotyping data was used to develop a pipeline for detecting genetic heterogeneity within accessions throughout the entire Soybean Germplasm Collection. Accessions in the collection with detected heterogeneity in a genomic region of interest could be used as HIFs for rapid NIL development for virtually any region of the genome. This heterogenous accession and interval detection can be a valuable new tool for trait mapping and gene validation to uncover untapped variation and advance efforts in soybean breeding.

P-114

Re-engineering the soybean flower to capture hybrid vigor

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Hybrid vigor (also known as heterosis) is a phenomenon in which crosses between genetically diverse individuals produce offspring that outperform either parent. Heterosis has been used for over a century to increase crop yields, improve abiotic and biotic stress tolerance, and enhance the nutritional quality of seeds. Soybean, one of the top-produced crops in the US, is a self-fertilizing plant that does not readily hybridize with other plants, and therefore lacks the natural benefits of heterosis. Manually produced soybean hybrids exhibit a 10-20% increase in yield over their inbred parents, indicating that heterosis is an untapped source of increased yield in this crop. A projection based on soybean harvests from 2017, demonstrates that US farmers could be harvesting an additional 12-24 million tons of soybean per year if they had access to hybrid seeds. To address the need for hybrid breeding in soybean, we are testing a biotechnology approach that offers an environmentally sustainable, effective, and efficient approach for producing hybrid seeds. Our strategy is to: (1) block self-fertilization by engineering single-generation male sterility into soybean; and (2) re-engineer soybean flowers with key visual and biochemical traits to attract bees that will participate in delivering pollen between plants. The combination of these two alterations will enable breeders to identify and efficiently implement hybrid crosses for increased yield, enhanced seed quality traits, and improved stress tolerance in soybean, supporting a sustainable solution for meeting our future agricultural needs. Moreover, our modifications will fundamentally alter the reproductive strategy of soybean, changing this crop from a self-pollinating into a bee pollinated plant. This change could offer new sources of refuge for bee populations that are currently under severe decline, and thus have a transformative (and positive) effect on our agricultural ecosystems.

P-115

Pyramids of QTLs enhance host-plant resistance to leaf-chewing insects in soybean

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Plant resistance to leaf-chewing insects minimizes the need for insecticide applications, reducing crop production costs and pesticide concerns. In soybean [*Glycine max* (L.) Merr.], resistance to a broad range of leaf-chewing insects is found in PI 229358 and PI 227687. PI 229358's resistance is conferred by three quantitative trait loci (QTLs): M, G, and H. QTL-M is the major source of resistance in PI229358, and is required for the expression of QTL-H and QTL-G. PI 227687's resistance is conferred by QTL-E. The near-isogenic lines (NILs) Benning^{ME}, Benning^{MGHE}, and Benning$^{gME+cry1Ac}$ were developed to determine if novel QTL pyramids would enhance soybean resistance to leaf-chewing insects, and if pyramiding these QTLs with Bt (cry1Ac) enhances resistance against Bt-tolerant pests. In field-cage conditions, Benning^{ME}and Benning^{MGHE} suffered 61% less defoliation by soybean looper [SBL, *Chrysodeixis includens* (Walker)] than Benning. In detached-leaf assays Benning$^{ME+cry1Ac}$ was more resistant than Benning^{ME} and Benning^{cry1Ac} against the Bt-resistant Southern armyworm [SAW, *Spodoptera eridania* (Cramer)]. To determine the QTL introgressions in BenningME and Benning^{MGHE}, high-density SNP genotypes were obtained using the SoySNP50K iSelect BeadChip (Illumina, San Diego, USA). To facilitate selection of lines carrying a specific QTL pyramid, KASP markers were developed for high- throughput genotyping. These NILs are valuable genetic resources for breeding host- plant resistance to insects in soybean. The combination of QTL-M and QTL-E provides agriculturally relevant levels of resistance, and with only two loci, the use of this pyramid is feasible in a breeding program.

P-116

An allelic series of CRISPR-derived mutations reveals CPR5 role in soybean trichome development

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A short-trichome soybean mutant was identified in a fast-neutron mutant population at the University of Minnesota. This mutant phenotype was mapped to a genetic interval on chromosome 6 that contains a deletion encompassing several genes, including Glyma.06g145800, a homolog of Constitutive Pathogen Response 5 (CPR5). The CPR5 homolog in *Arabidopsis thaliana* is implicated in the Unfolded Protein Response, ploidy control, effector triggered immunity, and cell proliferation, along with a mutant trichome phenotype. For validation, Glyma.06g145800 was targeted with a single gRNA CRISPR gene-editing construct using human codon optimized *Streptococcus pyogenes* Cas9. Cell lines from one transgene introduction were identified and sampled throughout T0, demonstrating the progression of editing over time. Differential editing at the T0 generation of separate lineages, all derived from the initial event, created seven different alleles containing both frame-shift and non-frame-shift mutations. Edited plants phenocopy the reduced trichome cell size of the *Arabidopsis* mutant. Finally, some T1 populations segregated for phenotypic severity, depending on the edited alleles inherited.

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Expression of candidate genes in insect resistance QTL regions upon feeding by soybean looper caterpillars

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Caterpillars are among the most important pests that feed on soybean foliage in the Southern United States and damage the supply of feed and food, as well as the incomes of soybean growers. Previously mapped QTLs for insect resistance co-map with key genes in the flavonoid-associated pathways. Our objective focused on helping validate candidate genes found in two QTLs, known as N and O. Resistant alleles for both are found in 'Boggs' variety. Primers were designed for sequences specific to the chalcone isomerase (CHI) and phenylalanine ammonia-lyase (PAL) genes, which co-locate with QTL N and QTL O respectively. Expression of the CHI and PAL candidate genes was quantified in the leaves of 'Benning' (susceptible) and the resistant Boggs under both insect-infested and uninfested conditions. The mean Boggs expression for CHI declined by 44 percent after insect feeding, whereas the Benning expression rate more than doubled. CHI sits near the top of the flavonoid pathway, and lower expression rates for CHI suggest that either the metabolites downstream from QTL-N are needed for caterpillar health, or its precursors may be accumulating and be toxic. Expression of PAL from the resistant Boggs increased by 160 percent upon feeding, but PAL from the susceptible Benning also increased. Increased expression might mean the cause of resistance is associated with increased production of metabolites downstream from PAL. Sequencing resistant and susceptible alleles will be necessary to determine if both alleles are translated into protein. Ultimate confirmation would require knocking out and/or overexpressing these alleles. Understanding the cause of resistance found in QTL N and QTL O would be a major step forward in the production of a defoliator-resistant commercial soybean.

Soybean plant regenerated from tissue culture. Photo by Lauren Lail.

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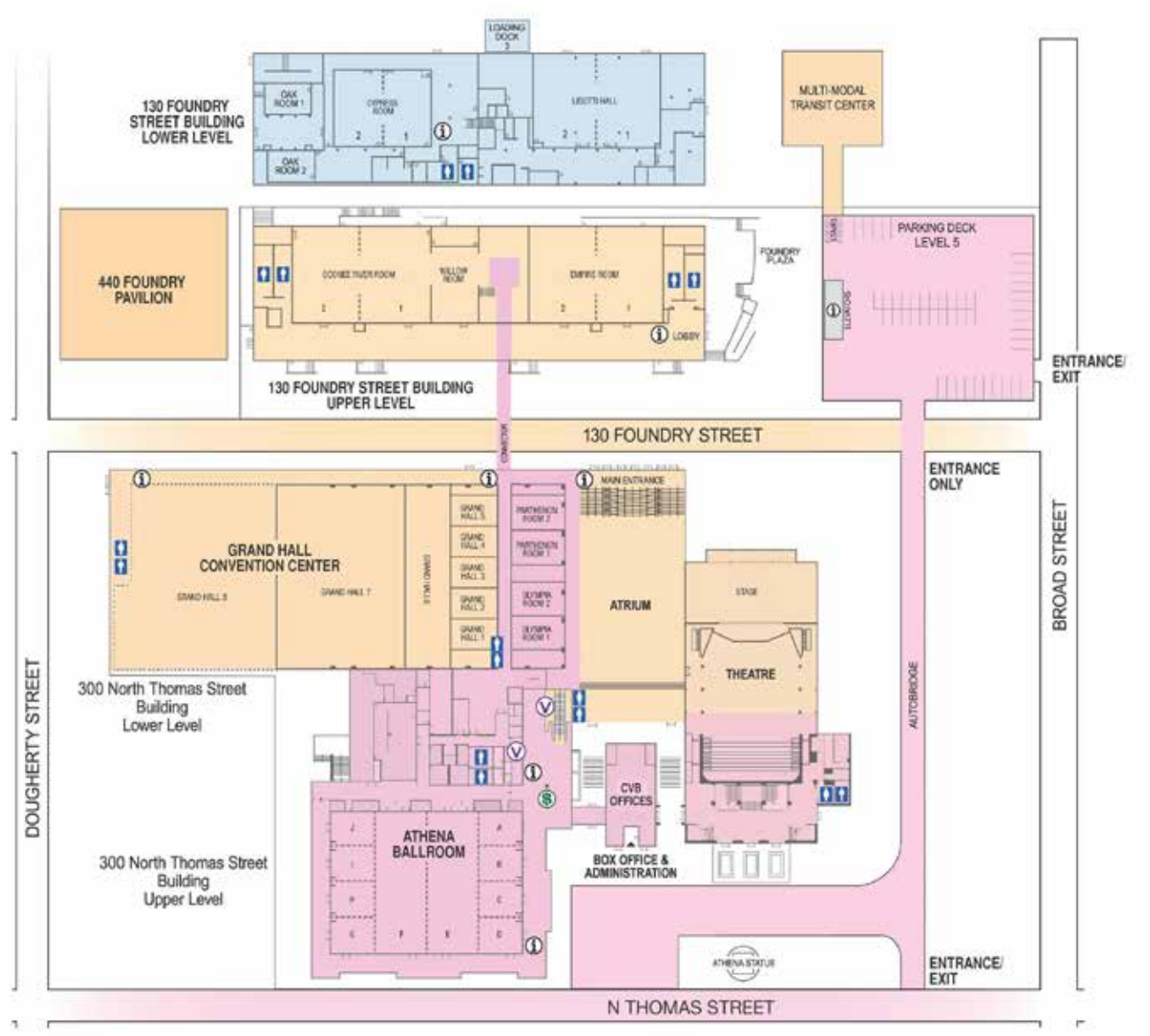
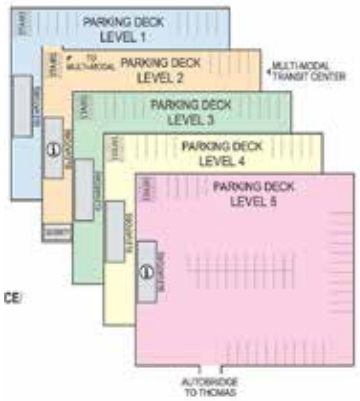
The Classic Center maintains a guest network for its patrons.

In addition, the conference network is **SOY2018**.The password is **KingSoy** and is case-sensitive.

THE CLASSIC CENTER





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  Loading Deck 1
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  130 FOUNDRY STREET BLDG.–UPPER LEVEL
  Theatre-Orchestra Level
  Parking Deck Level 2
  Loading Deck 2 & 4
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CLASSIC CENTER
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SMITHONIA FARM

As soon as the Civil War was over – 150 years ago – James Monroe Smith started digging in the soil. With one mule, he began turning Northeast Georgia red clay. And when his crops were in the barn, he hitched that same mule to a peddler's wagon to sell tinware. In time, Smith's vision, guts and hard work paid off. He owned one of the largest farms in Georgia, encompassing 30-square miles.

The center of his agricultural empire was in Smithonia. James Monroe Smith built 17 miles of railroad tracks to haul his products to market. The rail lines, his personal rail car, sawmill, fertilizer plant, brickyard, cotton gin, schools, post office and hundreds of other structures are gone, but six historic structures – his mansion, the milk house, the hotel, the plantation's commissary and three massive brick barns- still stand in the center of his Oglethorpe County community.

Dinner will be hosted in one of the three magnificent barns. The bricks were handmade on Smithonia Plantation. The gigantic 65 ft. heart-pine beams and trusses were cut from the farm.

Guests are welcome to walk down to the third barn, where the beams are most visible. A hand-dug well, 12 ft. in diameter and 61 ft. deep, taps into four natural springs, and is found in the pasture between the second and third barn.

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SOY2018

17th Biennial Conference of Molecular & Cellular Biology of the Soybean

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