Dear Soy2016 attendees:

Thank you for your attendance and participation in the 16th Biennial Conference on the Molecular and Cellular Biology of the Soybean. Over the preceding years, the soybean biennial meeting has been held in eleven states across the north central and southern US. This is the first time that this meeting has been held in Ohio and we are truly honored to host the event.

We are pleased to welcome two plenary speakers to kick off the event. On Sunday evening, Dr. Blake Meyers, a leader in bioinformatics and plant functional genomics, will delve into the world of small RNAs in soybean. Monday morning, Dr. Erich Grotewold will provide his perspectives and experiences on the application of systems approaches to understanding gene regulatory networks. The sessions that follow will continue to highlight the cutting-edge research by members of the soybean research community working in areas that include functional genomics; genome biology; translational genomics; resistance to abiotic stresses, pests, and pathogens; symbiotic interactions and the phytobiome; as well as seed composition and nutrition.

This event would not have been made possible without a number of people and organizations. In particular, we would like to thank our sponsors for their generous support which goes towards defraying the registration fees of graduate students and post docs. We would like to thank Robert Stupar and Michelle Graham for their guidance and advice on the organization of this meeting as well as Karin Samoviski and Eva Dale for their expertise and time put into the design of conference materials. We would also like to give special thanks to Amanda Gutek, and numerous graduate students that helped in the organization of this meeting!

Thank you for joining us in Columbus, Ohio for this meeting. We hope you enjoy the program and your stay!

Sincerely,

The Soy2016 Organizing Committee
Leah McHale, Chair, The Ohio State University
Anne Dorrance, The Ohio State University
John Finer, The Ohio State University
Tom Fontana, The Ohio Soybean Council
Joanna Gardner, The Ohio State University
Andy Michel, The Ohio State University
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Peg Redinbaugh, USDA-ARS, The Ohio State University
Donnalyn Roxey, The Ohio State University
Feng Qu, The Ohio State University
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MEETINGS OF THE BIENNIAL CONFERENCE ON MOLECULAR & CELLULAR BIOLOGY OF THE SOYBEAN

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)
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)

THANK YOU TO OUR SPONSORS

Our sponsors have graciously provided financial contribution to make this meeting a success. These contributions lowered the costs of the meeting, and provided for food and refreshments. Please thank them for their generosity.

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SOY2016 AGENDA

SUNDAY, AUG. 7, 2016

3 p.m.  Registration opens (Hyatt Regency Columbus)

6 p.m.  **Plenary Address** (Regency Ballroom)
        Blake Meyers, The Donald Danforth Plant Science Center
        Everything you always wanted to know about small RNAs in soybean – and more!

7:30 p.m.  **Opening Reception** (Ballroom South Foyer)
        Sponsored by the Ohio State University College of Food Agriculture and Environmental Sciences

8:30 p.m.  Dinner on your own

Continued on next page
**MONDAY, AUG. 8, 2016**

7 a.m.  Registration and Continental Breakfast (Ballroom South Foyer)

8 a.m.  **Plenary Address** (Regency Ballroom)
  Erich Grotewold, The Ohio State University  
  *Systems Approaches to Unravel Plant Gene Regulatory Networks*

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**PEST AND DISEASES** (Session 1, Regency Ballroom)  
Chairs: Bret Cooper, USDA-ARS, Beltsville, Maryland and Aardra Kachroo, University of Kentucky

9 a.m.  **Roger Innes, Indiana University**  
*Expanding the Recognition Specificity of a Plant Disease Resistance Gene Using Decoys*

9:20 a.m.  **Colin Davis, Virginia Tech University**  
*Identifying Novel Resistance Genes against Phytophthora sojae using an Effector-Directed Approach*

9:40 a.m.  **Wenbo Ma, University of California-Riverside**  
Phytophthora sojae Uses Effectors to Manipulate Small RNA Pathways in Soybean

10 a.m.  Break (Ballroom South Foyer)

---

**PEST AND DISEASES** (Session 2, Regency Ballroom)  
Chairs: Bret Cooper, USDA-ARS, Beltsville, Maryland and Aardra Kachroo, University of Kentucky

10:20 a.m.  **Bret Cooper, USDA-ARS, Beltsville, Maryland**  
*Nuclear Proteins Controlling Soybean Rust Resistance*

10:40 a.m.  **Mark Tucker, USDA-ARS, Beltsville, Maryland**  
*Determining the Role of IDA (INFLORESCENCE DEFICIENT IN ABSCISSION)-like Genes in Root-Knot Nematode Infection of Roots*

11 a.m.  **Ling Li, Iowa State University**  
*A Molecular Tool to Increase Protein Content and Broad Disease Resistance in Soybeans*

11:20 a.m.  **Vincent Klink, Mississippi State University**  
*A Developmental Genomics Analysis Identifies Expressed Genes Functioning in Defense to Root Pathogens*

11:40 a.m.  **Aardra Kachroo, University of Kentucky**  
*Molecular Analysis of Microbial Defense Signaling Components in Soybean*

12 p.m.  Lunch (Ballroom South Foyer)

*Continued on next page*
MONDAY, AUG. 8, 2016

ABIOTIC STRESS (Regency Ballroom)
Chairs: Kent Burkey USDA-ARS, Raleigh, North Carolina and Henry Nguyen, University of Missouri

1:30 p.m. Clinton Steketee, University of Georgia
Toward Genetic Improvement of Soybean Drought Tolerance

1:50 p.m. Anna Locke, USDA-ARS, Raleigh, North Carolina
Soybean Hydraulics and Water Use: FACE-ing the Future

2:10 p.m. Heng Ye, University of Missouri
Genetic Improvement of Flooding Tolerance and Understanding the Underlying Mechanism in Soybean

2:30 p.m. Gunvant Patil, University of Missouri
Genomics-Assisted Haplotype Analysis and Marker-Assisted Selection for Salinity Tolerance in Soybean

2:50 p.m. Jennifer Robison, Indiana University Purdue University Indianapolis
Ethylene signaling negatively impacts cold stress responses in soybean

3:10 p.m. Break (Franklin Room)

SYMBIOTIC INTERACTIONS AND PHYTOBIOME (Regency Ballroom)
Chairs: Gary Stacey, University of Missouri and Hongyan Zhu, University of Kentucky

3:30 p.m. Hongyan Zhu, University of Kentucky
Genetic Control of Nodulation Specificity in Soybean

3:50 p.m. Katalin Toth, University of Missouri
Plant Immunity Plays an Important Role in Legume-Rhizobium Symbiosis

4:10 p.m. Yaowei Kang, Novozymes
Development of Next Generation Soybean Inoculants

4:30 p.m. Chiling Chen, Iowa State University
Soybean-Associated Bacterial and Fungal Microbiota: Effects of Drought and Crop

5-5:45 p.m. Poster Reception (Franklin Room)
Odd numbered posters presented

5:45-6:30 p.m. Poster Reception (Franklin Room)
Even numbered posters presented

6:30 p.m. Dinner on your own

Continued on next page
TUESDAY, AUG. 9, 2016

7 a.m.   Registration and Continental Breakfast (Ballroom South Foyer)

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COMPOSITION AND NUTRITION (Regency Ballroom)
Chairs: Tom Clemente, University of Nebraska and Zenglu Li, University of Georgia

8:15 a.m.  Glenn Bowers, Calyxt
Efficient Genome Editing for Crop Improvement

8:35 a.m.  George Graef, University of Nebraska
Identifying Unique Phenotypes and Genotypes for Protein, Oil, and Carbohydrate Concentration in Soybean Seeds

8:55 a.m.  Hari Krishnan, USDA-ARS, Columbia, Missouri
Development and Characterization of Hypoallergenic Soybeans

9:15 a.m.  Sungwoo Lee, North Carolina State University
Genome Wide Association Study of Soybean Seed Composition in Maturity Groups II to IV

9:35 a.m.  Edgar Cahoon, University of Nebraska
Development of a Soybean-based Feedstock for Aquaculture

---

FUNCTIONAL GENOMICS (Regency Ballroom)
Chairs: Robert Stupar, University of Minnesota and Steven Whitham, Iowa State University

10:15 a.m.  Eliot Herman, University of Arizona
Engineering Altered Soybean Protein Composition and Content

10:35 a.m.  Tarek Hewezi, University of Tennessee
Soybean Kinome: Identification and Functional Classification

10:55 a.m.  John Harada
Dissection of Gene Networks that Govern Seed Development

11:15 a.m.  Mingsheng Qi, Iowa State University
Functionally Characterizing Soybean Genes Involved in Defense by Using BPMV VIGS

11:35 a.m.  Cuong Nguyen, University of Missouri
Use of Crispr/Cas Genome Editing Demonstrates a Critical Role for Uricase and Xanthine Dehydrogenase in Soybean Nitrogen Fixation and Nodule Development

12 p.m.   Lunch (Ballroom South Foyer)

Continued on next page
TUESDAY, AUG. 9, 2016

GENOME BIOLOGY (Session 1, Regency Ballroom)
Chairs: Michelle Graham, USDA-ARS, Ames, Iowa and Matthew Hudson, University of Illinois

1:30 p.m.    Keith Slotkin, The Ohio State University
Movement of siRNAs into Arabidopsis Sperm Cells Directs Transposable Element Repression

1:50 p.m.    Robert Schmitz, University of Georgia
Combinatorial Application of FANS with ATAC-seq to Identify Open Chromatin in Plant Genomes

2:10 p.m.    Robert Stupar, University of Minnesota
Inheritance Patterns of Transgenes and Targeted Mutations in a Soybean CRISPR-based Mutagenesis System

2:30 p.m.    Jamie O’Rourke, USDA-ARS, Ames, Iowa
Nutrient Deficiencies: Beyond the QTL

2:50 p.m.    Break (Franklin Room)

TRANSLATIONAL GENOMICS (Session 1, Regency Ballroom)
Chairs: Aaron Lorenz, University of Minnesota and Wayne Parrott, University of Georgia

3:10 p.m.    Zenglu Li, University of Georgia
Insight into the Genomic Regions under Breeding Selection and Genomic Selection for Yield in Soybean

3:30 p.m.    Brian Diers, University of Illinois
Impact of Rhg1 Copy Number and Type on SCN Resistance

3:50 p.m.    Rima Thapa, Purdue University
Identifying Novel Alleles for Soybean Meal Composition Traits

4:10 p.m.    Gustavo de los Campos, Michigan State University
Leveraging Genomic & Environmental Data for Predicting the Distribution of Yield of Candidate Varieties in Target Locations

4:30 p.m.    Adjourn
Optional Soybase tutorial session (Champagne Room)

6:30 p.m.    Reception at the Boat House Restaurant

6:15 p.m.    Banquet Dinner at the Boat House Restaurant
(Sponsored by the Ohio Soybean Council)

Continued on next page
WEDNESDAY, AUG. 10, 2016

7 a.m. Registration and Continental Breakfast (Ballroom South Foyer)

GENOME BIOLOGY (Session 2, Regency Ballroom)
Chairs: Michelle Graham, USDA-ARS, Ames, Iowa and Matthew Hudson, University of Illinois

8:30 a.m. Steven Cannon, USDA-ARS, Ames, Iowa
Drivers of Soybean Genome Size Change, and Other Insights from Multi-Species Legume Genome Comparisons

8:50 a.m. Lila Vodkin, University of Illinois
A Mutation in an Argonaute Protein Explains the Epistatic Interaction of the K and I Loci Controlling Seed Color Patterns

9:10 a.m. Jianxin Ma, Purdue University
Genetic Basis and Process of Soybean Domestication

9:30 a.m. Mohammad Belaiff, University of Illinois at Urbana-Champaign
A study of Molecular Evolution Patterns in the Genus Glycine

9:50 a.m. Break (Ballroom South Foyer, sponsored by Ricerca)

TRANSLATIONAL GENOMICS (Session 2, Regency Ballroom)
Chairs: Aaron Lorenz, University of Minnesota and Wayne Parrott, University of Georgia

10:10 a.m. Michelle Graham, USDA-ARS, Ames, Iowa
Genomic Approaches for Soybean Improvement

10:30 a.m. Benjamin Campbell, University of Minnesota
The Long and Short of Soybean Petioles: The Effect of a 3-bp Insertion on Plant Architecture and Harvest Index

10:50 a.m. Asheesh (Danny) Singh, Iowa State University
Integrating Image Processing and Machine Learning to Decipher the Genetics of Iron Deficiency Chlorosis

11:10 a.m. Joseph Byrum, Syngenta
Advanced Analytics for Agricultural Product Development

11:30 a.m. Concluding Remarks
SPEAKER ABSTRACTS
S01  
**Everything You Always Wanted to Know about Small RNAs in Soybean – and More!**

Blake Meyers (bmeyers@danforthcenter.org), Donald Danforth Plant Science Center

Small RNAs are ubiquitous, versatile repressors and include (a) microRNAs (miRNAs), processed from mRNA forming stem-loops, and (b) small interfering RNAs (siRNAs), the latter derived in plants by a process typically requiring an RNA-dependent RNA polymerase. My lab, with collaborators, has conducted extensive genome-wide analyses of soybean small RNAs; from these data we have found that soybean contains the highest number of loci generating 21-nt ‘phased’ siRNAs (phasiRNAs, from PHAS loci) of any eudicot yet examined - over 500 loci, of which 483 overlapped annotated protein-coding genes. The miRNA triggers for many of these PHAS loci were detected, and molecular analyses provide insights into the mechanisms by which these small RNAs are generated. The primary class of PHAS loci (>40% of the total) corresponded to NB-LRR genes; some of these small RNAs preferentially accumulate in nodules. Of particular interest due to the abundance of small RNAs in the anthers of many plant species is a non-coding PHAS locus, triggered by miR4392. The miRNA trigger and resulting phasiRNAs accumulate preferentially in anthers; the phasiRNAs are predicted to target transposable elements (TEs), with their peak abundance during soybean reproductive development. Thus, our observations from soybean demonstrate that miRNAs and phasiRNAs show tremendous diversity in eudicot plant species.

S02  
**Systems Approaches to Unravel Plant Gene Regulatory Networks**

Erich Grotewold (grotewold.1@osu.edu), The Ohio State University

Establishing the architecture of gene regulatory networks and linking system components to agronomic traits is an emerging theme in plant systems biology. We have combined gene- and transcription factor (TF)-centered approaches in maize and Arabidopsis to establish how transcriptional regulatory networks are wired, what are the emerging properties of the network, and how genome size influences gene network behavior. For the gene-centered approach, we used the yeast one-hybrid system with a collection of 2,000+ maize TFs together with 175 regulatory regions of genes involved in phenolic metabolism. These studies revealed several thousand previously unknown gene-TF interactions, providing unique insights on how branches of complex metabolic pathways are regulated. For the TF-centered approach, we combined chromatin immunoprecipitation (ChIP)-based approaches (e.g., ChIP-Seq and ChIP-chip) with gene expression analyses (e.g., microarray, RNA-Seq) contrasting mutant and wild type plants to identify genome-wide TF targets. These studies provided unique insights on how single TFs can activate and repress hundreds of genes, while showing that TFs often recognize very different DNA motifs in vivo and in vitro. In addition, TF target characterization permitted the identification of missing gaps in agronomically important metabolic pathways. The web-accessible knowledge bases GRASSIUS (http://grassius.org/) and AGRIS (http://arabidopsis.med.ohio-state.edu) integrate information on network components (TFs, promoters, interactions) for the grasses and Arabidopsis, respectively.

S03  
**Expanding the Recognition Specificity of a Plant Disease Resistance Gene Using Decoys**

Roger Innes (rinnes@indiana.edu), Indiana University  
Sang-Hee Kim, Indiana University  
Dong Qi, Indiana University  
Tom Ashfield, Indiana University  
Matt Helm, Indiana University

Genetically determined disease resistance is one of the most effective and environmentally sustainable approaches to protecting crops from disease. Although significant progress has been made toward understanding the mechanistic basis of plant-pathogen interactions, a remaining challenge is to expand the recognition specificity of endogenous plant resistance (R) proteins to confer entirely new specificities. Many R proteins detect pathogens by an indirect mechanism in which the R proteins sense modification of specific host proteins that are targeted by pathogen effectors. How modified host proteins are sensed by R proteins, however, is poorly understood. We have been investigating this question using the Arabidopsis R protein RPS5, which detects proteolytic cleavage of the protein kinase PBS1 by the Pseudomonas syringae effector protein AvrPphB. We have recently shown that the AvrPphB cleavage site within PBS1 can be replaced with cleavage sites for other proteases, which then enables RPS5 to be activated by these proteases instead of by AvrPphB. Thus, the specificity of RPS5 can be changed simply by altering the protease cleavage sequence within PBS1. We are now using this technology to engineer resistance in soybean to Soybean mosaic virus (SMV). Soybean contains three co-orthologs of PBS1, and all three can be cleaved by AvrPphB. Furthermore, most soybean varieties recognize AvrPphB, thus likely contain a disease resistance protein that detects PBS1 cleavage. We hypothesize that we will be able to engineer durable resistance to SMV by using genome editing to introduce a cleavage site for SMV Nia protease into a soybean PBS1 ortholog.
S04
Identifying Novel Resistance Genes against Phytophthora sojae Using an Effector-Directed Approach

Colin Davis (cdavis12@vt.edu), Virginia Tech
Michael Fedkenheuer, Virginia Tech
Kevin Fedkenheuer, Virginia Tech
Neelam Redekar, Virginia Tech
Rachel Matthiesen, Iowa State University
Alison Robertson, Iowa State University
Bret Tyler, Oregon State University
Saghai Maroof, Virginia Tech
John McDowell, Virginia Tech

Phytophthora sojae (P. sojae), the causal agent of soybean root and stem rot disease, is an oomycete pathogen responsible for over $400 million dollars of soybean crop damage annually in the US, and over $1 billion dollars worldwide. For successful P. sojae infection, the pathogen must secrete effector proteins into the host, which function to repress natural defense systems. To date, P. sojae has been managed through the inclusion of P. sojae resistance (Rps) genes and quantitative trait loci (QTL) into commercial lines. Rps genes are able to recognize specific pathogen effectors inside the host and up regulate the defense response in turn. However, the effectiveness of current R-genes is decaying as certain strains of P. sojae evolve to overcome the resistance. The reduction of Rps gene effectiveness can be attributed to P. sojae’s loss or manipulation of recognized effectors. This project seeks to identify novel genes conferring durable resistance. Resistance gene screening, targeting core P. sojae effectors would provide durable resistance, as a loss of these effectors by the pathogen would result in a loss of pathogenicity. An effector-based screening assay has been developed utilizing Pseudomonas fluorescens to rapidly screen soybean germplasm for resistance genes. Our strategy is to use the effector-based screening assay, along with a pathogen assay, to develop genetic maps of the novel genes conferring resistance to P. sojae.

S05
Phytophthora sojae Effectors Manipulate Small RNA Pathways in Soybean

Wenbo Ma (wenbo.ma@ucr.edu), University of California-Riverside

During the co-evolutionary arms race with the hosts, microbial pathogens have evolved a large repertoire of secreted virulence proteins, called effectors, to facilitate colonization and infection. Many effectors are believed to directly manipulate targeted processes inside the host cells; and a fundamental function of the effectors is to dampen immunity. Phytophthora sojae is an important soybean pathogen that causes the root and stem rot disease. Genome sequence analysis leads to the prediction of several hundreds of effectors from P. sojae. Although the majority of the P. sojae effectors are functionally uncharacterized, a few of them have been investigated for their virulence functions. In particular, two effectors, named PsPSR1 and PsPSR2, were found to suppress RNA silencing in plant hosts by inhibiting the accumulation of small RNAs. Importantly, ectopic expression of the PsPSRs in plants resulted in hypersusceptibility, supporting a profound impact of plant small RNAs on Phytophthora-soybean interactions. Here, we will discuss the molecular mechanisms underlying the virulence function and the RNA silencing suppression activity of PsPSRs during P. sojae infection.

S06
Nuclear Proteins Controlling Soybean Rust Resistance

Bret Cooper (bret.cooper@ars.usda.gov), USDA-ARS

The soybean immune system is not well-characterized and a better understanding of it is needed to develop resistant plants to the soybean rust fungus Phakopsora pachyrhizi. To find soybean proteins that contribute to resistance, the susceptible Williams 82 cultivar was compared to a resistant Williams 82 inbred isolate harboring the Rpp1 rust-resistance gene. The goal was to examine nuclei where transcription factors and other proteins accumulate to govern transcriptional and other biochemical changes controlled by Rpp1. The abundances of approximately 2,300 proteins observed in both susceptible and resistant plants were measured by mass spectrometry, and clustering was performed to reveal sets of differentially accumulating proteins linked to Rpp1-mediated resistance. Among the proteins found were transcription factors, chromatin-associated proteins, DNA polymerases, DNA repair enzymes, nucleolar proteins, spliceosome components, nuclear pore proteins, and other proteins with likely activity in the nucleus. Genes for candidate proteins linked to disease resistance were cloned and expressed by a plant virus to test gene functionality by virus-induced gene silencing. After silencing, the normally-resistant Rpp1 plants developed rust symptoms and accumulated rust fungal RNA and protein. Silenced plants also had reduced amounts of RNA for the soybean Myb84 transcription factor and soybean isoflavone O-methyltransferase, both of which are important to phenylpropanoid biosynthesis and lignin formation. These data reveal that rust infection leads to the accumulation of transcription factors and other proteins in the soybean nucleus and that these proteins support the immune system through Rpp1.
**S07**

**Determining the Role of IDA (INFLORESCENCE DEFICIENT IN ABSCISSION)-like Genes in Root-Knot Nematode Infection of Roots**

Mark Tucker (mark.tucker@ars.usda.gov), USDA-ARS-BARC-SGIL
Joonyup Kim, USDA-ARS-BARC-SGIL, University of Maryland-College Park
Ronghui Yang, USDA-ARS-BARC-SGIL
Caren Chang, University of Maryland-College Park

The Arabidopsis gene INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) encodes a secreted signaling peptide that binds to two redundant receptor-like kinases (HAESA and HAESA-like 2), which signal a change in gene expression required for abscission (separation) of floral organs and the emergence of lateral roots through the root cortex. It was proposed that IDA might induce a signaling pathway common to many cell separation events. IDA-like (IDL) genes are conserved in every dicot genome examined and many monocot genomes. We also identified IDL genes in the genomic sequence of three root-knot nematodes (RKN), Meloidogyne sp. RKN are detrimental pathogens of many agriculturally important crops including soybean. The M. incognita IDL1 gene (MiIDL1) encodes a 47 aa open reading frame (ORF) with a 28 aa N-terminal signal peptide. MiIDL1 gene expression is low in eggs and pre-parasitic J2 but rapidly increases early after infection of roots. Treatment of Arabidopsis ida flowers and roots with the synthetic MiIDL1 peptide and expression of the full-length MiIDL1 ORF in transformed ida mutant plants rescued the delayed abscission and lateral rooting phenotypes of the knockout mutant. In addition, wild-type Arabidopsis plants were transformed with RNAi constructs to suppress MiIDL1 gene expression inside the nematode. Suppression of the MiIDL1 gene reduced the number and size of galls that formed on the roots. We propose that root-knot nematodes acquired an IDA-like gene by horizontal transfer or co-evolution that produces an effector that induces an IDA signaling pathway in the host.

**S08**

**A Molecular Tool to Increase Protein Content and Broad Disease Resistance in Soybeans**

Ling Li (liling@iastate.edu), Iowa State University
Mingsheng Qi, Iowa State University
Wenguang Zheng, Iowa State University
Jessica Hohenstein, Iowa State University
David Soh, Iowa State University
Xuefeng Zhao, Iowa State University
Chuanlong Du, Iowa State University
Dan Nettleton, Iowa State University
Gustavo Macintosh, Iowa State University
Greg Tylka, Iowa State University
Eve Syrkin Wurtele, Iowa State University
Steven A. Whitham, Iowa State University

Crop plants must integrate signals from the environment and prioritize responses to stresses that may occur individually or simultaneously throughout the growing season. Stress responses can adversely affect plant growth and quality traits such as protein and starch. The ability to optimize protein productivity of plant-based foods has far-ranging impact on world health and sustainability. Plant diseases each year cause major losses to crop production. The Arabidopsis thaliana QQS-orphan-gene modulates carbon allocation to protein and starch (1). Ectopic QQS expression increases protein content (2) in leaf and seed in soybean (3,4). QQS transcript levels are altered in plants under stresses and in mutants of genes involved in all sorts of stress responses, indicating that QQS may integrate primary metabolism with environmental perturbations, thus adjusting the plant’s adaption to abiotic and biotic stresses (5). The QQS protein binds to a transcriptional regulator in Arabidopsis and its soybean homologs: Nuclear Factor Y subunit C4 (NF-YC4). NF-YC4 overexpression in Arabidopsis and soybean mimics QQS-overexpression phenotype, increasing protein and decreasing carbohydrate (4). Mutants overexpressing genes related to QQS network have significantly increased resistance to plant pathogens and pests (6). Our data reveal indicate QQS exerts its effect via an interaction with a transcription factor conserved across eukaryotic species (4); these findings open a non-transgenic strategy to create high-protein soybeans and enhance broad-spectrum disease resistance (6).

S09

**A Developmental Genomics Analysis Identifies Expressed Genes Functioning in Defense to Root Pathogens**

**Vincent Klink** (heartwood27@hotmail.com), Mississippi State University
Gary W. Lawrence, Mississippi State University

Glycine max (soybean) has been used as a model to understand root defense process(es), focusing in on its interaction with parasitic nematodes. Toward this goal, candidate resistance genes have been identified and engineered into plasmid vectors that permit enhanced gene expression in a susceptible genotype. The results of those experiments are varying levels of suppressed pathogen success. In contrast, genetic engineering experiments that suppress candidate gene expression in a resistant genotype result in varying levels of increased pathogen success. The results demonstrate that it is possible to identify genes that are expressed in roots and determine if they function to suppress pathogen success. The results also demonstrate it is possible to determine whether the genes also function in other general aspects of root development.

S10

**Molecular Analysis of Microbial Defense Signaling Components in Soybean**

**Aardra Kachroo** (apkach2@uky.edu), University of Kentucky
MB Shine, University of Kentucky
Hexiang Luan, Nanjing Agriculture University
Pradeep Kachroo, University of Kentucky
Haiyan Zhi, Nanjing Agriculture University

Plants utilize multiple modes of defense signaling mechanisms to protect themselves from microbial pathogens including induced defenses in the infected tissues as well as systemic immunity in the distal uninfected tissues. We are studying the mechanisms by which plants perceive pathogens and the signaling pathways they utilize to activate such defense responses. Our studies related to the functional characterization of molecular components required for local and systemic defense signaling have highlighted some unique features of these pathways in soybean. For instance, we recently showed that both the phenylalanine ammonia lyase (PAL) and isochorismate synthase (ICS)-derived pathways contribute equally to pathogen-induced salicylic acid (SA) synthesis in soybean. This is unlike in many other plants, where pathogen-inducible SA is primarily derived from the ICS pathway. We have also identified several soybean proteins that directly bind pathogen effectors and regulate the outcome of pathogenesis by either modulating the virulence functions of pathogen effectors or directly contributing to host defense. For instance, we showed that enhanced disease susceptibility 1 (EDSI), not only regulates resistance (R)-protein derived signaling in soybean, but also interacts with the bacterial effector AvrA and is required for its virulence function in plants lacking the cognate R protein. In a recent study, we showed that eukaryotic elongation factor 1a (eEF1a) interacts with a viral effector and modulates the unfolded protein response associated with ER stress in soybean. This in turn promotes virus replication and thereby viral pathogenesis in soybean.

S11

**Toward Genetic Improvement of Soybean Drought Tolerance**

**Clinton Steketee** (steketee@uga.edu), University of Georgia
Mandeep K. Riar, North Carolina State University
Thomas R. Sinclair, North Carolina State University
Thomas E. Carter, Jr., USDA-ARS, North Carolina State University
William T. Schapaugh, Kansas State University
Ai-Ping Hu, Georgia Institute of Technology
Zenglu Li, University of Georgia

Drought stress is the most important abiotic limitation of soybean productivity. Combating this stress with irrigation is often not an option for farmers, making genetic improvement of drought tolerance in soybean necessary. However, accurate phenotyping for drought tolerance related traits is challenging and the genetic mechanisms governing these traits are poorly understood. Previous research in our program has identified QTLs for the slow canopy wilting trait by utilizing a RIL population derived from Benning and PI 416937 and differentially expressed genes under drought stress using a transcriptomic approach. To build on these findings, we have evaluated a panel of over 200 genetically diverse soybean lines genotyped with the SoySNP50K Infinium Chips for drought tolerance related traits in repeated growth chamber and field studies. Seven genotypes were identified in the top 15% for slow canopy wilting at two field locations (Athens, GA and Salina, KS) in 2015 that warrant further investigation. Difference in transpiration response of de-rooted soybean shoots to deionized water and an AgNO3 solution was measured in a walk-in growth chamber and several lines exhibit a response similar to the slow wilting genotype, PI 416937. Genome-wide association scans have identified putative regions responsible for slow canopy wilting and response to AgNO3 solution, and certain regions match previously identified QTL locations. Additional phenotyping of this panel and a RIL population will be conducted using the previously mentioned techniques and a novel field-based approach to visualize canopy architecture.
S12

Soybean Hydraulics and Water Use: FACE-ing the Future

Anna Locke (Anna.Locke@ars.usda.gov), USDA-ARS
Donald R. Ort, USDA-ARS, University of Illinois

SoyFACE is a free-air CO2 enrichment experiment in central Illinois where the impacts of climate change on soybean yield, physiology, and molecular biology have been investigated for 15 years. In the last several years, the experiment has been expanded to investigate multiple climate change conditions — ozone, increased temperature, drought, and heat waves — interactively with elevated CO2. A key question is how soybean water use will adjust to these conditions in the coming decades. Leaf hydraulic conductance links water supply with demand, describing the efficiency with which water can flow through the leaf to supply transpiration. Based on findings from SoyFACE about stomatal conductance, evapotranspiration, and productivity, we predicted that soybean leaf hydraulic conductance would have the plasticity to adjust to changing water supply and demand in different environmental conditions. Elevated CO2, elevated temperature (both short- and long-term), and drought were all investigated in field experiments. However, results from the field, backed up with chamber studies, showed the opposite: leaf hydraulic conductance remained unchanged across CO2 levels, temperatures, and soil moisture conditions. This apparently fixed capacity for leaf water transport has implications for high evaporative demand stress tolerance in soybean as well as improvement strategies.

S13

Genetic Improvement of Flooding Tolerance and Understanding the Underlying Mechanism in Soybean

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Climate change has made flooding more severe with a predicted 30% increase in heavy precipitation by 2030. Flooding is also confounded by an increase in irrigated acres that could result in excess water. This research aims to identify genetic resources and mechanisms for flooding tolerance and develop flooding-tolerant varieties in soybean. Initial screening of 450 diverse lines identified four most tolerant lines that were used to develop breeding and mapping populations. Recent screening of a core set of the USDA Germplasm Collection identified additional 24 tolerant lines including wild accessions that showed excellent tolerance. Four quantitative trait loci (QTL) were mapped using two mapping populations and the favorable alleles of two major QTL are from exotic parents. Three flooding-tolerant germplasm lines, which have similar yield potential as commercial checks under non-stress condition and outperform the commercial checks by 15-bushels/acre under flooding, were developed by marker-assisted selection. Fine-mapping of a major QTL delimited it into a genomic region containing two predicted genes. Naturally-occurring mutations in the 5’-UTR region of one candidate gene were identified to regulate the transcription and translation of the candidate gene and alter the flooding-induced auxin pathway to affect root growth during flooding. Pleiotropic effects of the candidate gene on partial resistance to Phytophthora sojae was observed, which provided the first evidence to support that flooding tolerance and Phytophthora sojae resistance shared auxin pathways and evolved together through natural and artificial selections. These results are expected to provide protection of yield against flooding and support sustainable soybean production.
S14
Genomics-Assisted Haplotype Analysis and Marker-Assisted Selection for Salinity Tolerance in Soybean
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Advances in next-generation sequencing generate large-scale genomic resources and enable the development of sustainable high-yield varieties that persist under biotic and abiotic stresses. Soil salinity is a major limiting factor affecting both crop development and yield. Soybean is sensitive to soil salinity causing a 20-40% reduction in seed yield. A dominant gene, Glyma03g32900 (GmCHX1) is primarily responsible for salt-tolerance. We utilized high quality (15x) whole-genome re-sequencing (WGRS) of 106 diverse soybean lines and identified three major haplotypes at GmCHX1 locus. The discovery of single nucleotide polymorphisms (SNPs) associated with structural variants facilitated the design of robust KASPar assays. Identified SNP markers were validated, and a strong correlation was observed between the genotype and phenotype (leaf scorch, chlorophyll content and Na+ accumulation) using a panel of 104 soybean lines and, an interspecific bi-parental population (F8) from PI483463 x Hutcheson. These markers precisely identified salt-tolerant/sensitive genotypes (>91%), and different structural-variants (>98%). Additionally, haplotype analysis and pedigree tracking of 93 U.S. ancestral lines were performed using publicly available WGRS datasets. This analysis showed that a majority of U.S. soybean cultivars are fixed for the salt-sensitive allele. The SNP assays, supported by accurate phenotyping, haplotype analyses and pedigree tracking information, will accelerate marker-assisted selection to enhance the development of high yielding, salt-tolerant soybean cultivars.

S15
Ethylene Signaling Negatively Impacts Cold Stress Responses in Soybean
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The growth season and crop yield of soybean is limited by temperature. The CBF cold responsive pathway has been well characterized in Arabidopsis, and soybean CBF homologous transcription factors have been described. Under cold stress, soybean CBF transcript levels increase similarly to reported responses in cold tolerant species; however, the expected CBF downstream targets appear unresponsive. In Arabidopsis, EIN3, a major component of the ethylene signaling pathway, is a known negative regulator of CBF cold regulation. Transcript analysis from cold treated unifoliate leaves of soybean seedlings revealed a 3.6 fold increase in EIN3 transcripts during early cold stress. As EIN3 transcripts do not significantly accumulate in cold treated Arabidopsis, we hypothesize that the observed cold induced EIN3 increase could be preventing effective CBF cold regulation in soybean. While silver nitrate is known to block the ethylene pathway in Arabidopsis and other species, the effect in young soybean seedlings is not presently reported. We have found that silver nitrate blocks the abscission of cotyledons and prevents yellowing of unifoliate leaves in soybean seedlings exposed to ethylene indicating silver blocks ethylene regulation in soybean. Initial experiments with a cold responsive reporter construct (AtRD29a::GFP/GUS) in soybean demonstrated that blockage of the ethylene pathway with silver nitrate prior to cold exposure led to significant increase in GUS activity level. This suggests that the ethylene pathway is interfering with the CBF cold regulation in soybean, likely through action of EIN3. These results provide potential targets for generating cold-hardy soybean crops.

S16
Genetic Control of Nodulation Specificity in Soybeans
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We will report the cloning of the soybean Rj4 gene that restricts nodulation by many Bradyrhizobium elkanii strains. The soybean-B. elkanii symbiosis has a low nitrogen-fixation efficiency, but B. elkanii strains are highly competitive for nodulation; thus cultivars harboring an Rj4 allele are considered favorable. Cloning the Rj4 gene is the first step in understanding the molecular basis of Rj4-mediated nodulation restriction and facilitates the development of molecular tools for genetic improvement of nitrogen fixation in soybeans. We finely mapped the Rj4 locus within a small genomic region on soybean chromosome 1, and validated one of the candidate genes as Rj4 using both complementation tests and CRISPR/Cas9-based gene knockout experiments. We demonstrated that Rj4 encodes a thaumatin-like protein, for which a corresponding allele is not present in the surveyed rj4 genotypes, including the reference genome Williams 82. Our conclusion disagrees with the previous report that Rj4 is the Glyma.01g165800 gene (previously annotated as Glyma01g37060). Instead, we provide convincing evidence that Rj4 is Glyma.01g165800-D, a duplicated and unique version of Glyma.01g165800, that has evolved the ability to control symbiotic specificity.
S17

Plant Immunity Plays an Important Role in Legume-Rhizobium 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. Our data describe novel and important findings that, for the first time, provide a mechanistic link between legume-rhizobia symbiosis and plant immunity. In order to better understand the biology of this mutualistic interaction, we conducted a phosphoproteomic study on soybean roots and root hairs in response to the symbiont Bradyrhizobium japonicum. Among the proteins phosphorylated rapidly upon inoculation was a protein previously characterized as playing a key, functional role in plant innate immunity. Silencing of the expression of this protein resulted in a significant reduction in nodule formation. In order to further study the specific role of phosphorylation, we introduced point mutations into the gene and then tested the resulting phenotype after constitutive expression in soybean transgenic roots. In the case of one, specific phosphorylation site, the expression of the mutated version resulted in significantly fewer nodules. Sequence comparison of an additional phosphorylation site suggested that it lies within a legume specific region, not found in the same protein from non-leguminous plants. Such a location suggests a legume-specific function, perhaps nodulation. Indeed, when a mutated version of this phosphorylation site was introduced into soybean, the transgenic roots produced significantly fewer nodules, suggesting that the site might be required for symbiotic signaling.

S18

Development of Next Generation Soybean Inoculants

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Maximum benefit of N2 fixation by soybean often requires the inclusion of selected strains of Bradyrhizobium japonicum as seed inoculants. The inoculant strain must be effective in its ability to fix N2 with the cultivar concerned and possess the ability to compete for nodulation with other strains of rhizobia that might be present in the soil. Strain competitiveness is influenced by the genetic diversity of both symbiotic partners (Triplett and Sadowsky 1992) as well as the soil environment in which nodulation occurs. Determination of strain competitiveness was previously seldom undertaken in inoculant selection programmes because of technical difficulties with the techniques used, mainly antibiotic-resistance and immunological methods. Here we report a PCR-based screening method that allows us to quickly screen thousands of strains in a short time period. This method allows us to differentiate the nodules infected from a commercial strain and an unknown new isolate when both of them were co-inoculated into soybean at equal ratio. More than two thousand nodule isolates have been screened for their nodulation ability and their nitrogen fixation ability against a commercial strain and a few strains with more effectiveness and competitiveness have been identified with 30% more nodulation capability than a commercial strain.

S19

Soybean-Associated Bacterial and Fungal Microbiota: Effects of Drought and Crop Diversification

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Soybean production is strongly influenced by biotic and abiotic stresses. Interrogation of microbial communities associated with plants grown under stressful conditions may provide insight into management strategies that better exploit microbial benefits. We first explored the changes in microbial communities during successive cycles of growth under dry and wet soil conditions. DNA samples from bulk soil, ectorrhizosphere, and endorrhizosphere were subjected to amplicon sequencing that targeted bacterial 16S rRNA gene and fungal ITS region. Consistent with improved water use efficiency, bacterial and fungal communities also shifted during the sequential plant growth cycles and across two plant genotypes. The drought treatment favored increases in the endorrhizosphere abundance of bacteria in the Glycomyces, Streptomyces and Dyadobacter genera, and fungi in the Fusarium genus, and decreases in the mycorrhizal fungi and some proteobacteria. We also explored the impact of cropping system on the bacterial and fungal communities associated with soybean and maize grown in a 2-year (corn/soybean) and a 4-year (corn/soybean/ oat +alfalfa/alfalfa) rotation. We observed a noticeably greater effect of the cropping system on fungal than bacterial communities. These cropping systems were associated with dramatic differences in soybean sudden death syndrome (SDS). We detected significantly higher populations of the SDS pathogen, Fusarium virguliforme, in soils from the high SDS 2-year rotation plots, and are using these data as a first step toward evaluating if protective microbial networks are present in the low SDS, 4-year diversified plots.
**S20**

**Efficient Genome Editing for Crop Improvement**

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The world faces tremendous challenges to feed the growing human population and the demand for plant-derived products such as food, feed, and biofuel demonstrates the importance of continuously improving crops with better yield and nutrition. The recent progress in genome engineering makes it feasible to precisely modify endogenous plant genes via two major repairing pathways: non-homologous end-joining (NHEJ) or homologous recombination (HR). Plant genome engineering requires the precise cleavage of plant chromosomal DNA to generate single or double-stranded DNA breaks using engineered nucleases. In this talk, the high-value traits under development at Calyxt, Inc using the TALEN® technology will be discussed. This will include a survey of the traits being developed in several crop species, such as soybean, potato and wheat.

**S21**

**Identifying Unique Phenotypes and Genotypes for Protein, Oil, and Carbohydrate Concentration in Soybean Seeds**

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Soybean seed composition is important in determining processed value, because the primary products are vegetable oil and protein meal for feed, food, and industrial applications. Concomitant increases in both total protein and total oil concentration in the seed have been difficult because the traits typically exhibit high negative phenotypic and genotypic correlations. Successful development of soybean lines with increased protein and oil in the seeds may be facilitated by use of different population structures and breeding and selection strategies. Results from over 50 biparental populations, reciprocal crosses, and two long-term recurrent selection populations will be presented, with identification of genomic regions important in determining the balance of proteins, lipids, and carbohydrates in the seed. Several hundred lines with seed protein and oil concentrations exceeding processor targets of 190 g kg-1 oil and 350 g kg-1 protein were recovered. Identification of unique QTL for oil, protein, and total (protein + oil) from different populations suggests further gains are possible.

**S22**

**Development and Characterization of Hypoallergenic Soybeans**

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The Food and Agriculture Organization of the United Nations has listed soybean as one of the eight most significant food allergens. Past research has led to the identification, characterization, and mitigation of some soybean allergens. In contrast to the role of soybean proteins as food allergens, only limited studies have been conducted on their role as allergens in animal feed. Soybean meal is extensively used in poultry, swine and fish feed since it contains higher protein levels with a superior amino acid profile and amino acid digestibility, increased metabolizable energy and lower fiber content. Pathological effects and immunological responses of soybean meal on animal performance have been previously examined, and these studies have led to the conclusion that soybean allergens could negatively affect animal performance. At a time when US soybean meal is facing increasing competition from alternative feed ingredients and other soybean producing countries, it would benefit US soybean farmers if hypoallergenic soybeans can be developed for both food and animal feed. Studies conducted in our laboratory and others have identified soybean β-conglycinin as a food and feed allergen. By employing RNAi technology, we have developed soybean lines that are devoid of the β-conglycinin, the major allergenic protein in swine and poultry feed. We have also developed soybean lines that lack this important allergen by traditional breeding. The development and characterization of hypoallergenic soybean lines will be highlighted in this presentation.
S23

**Genome Wide Association Study of Soybean Seed Composition Traits in Maturity Groups II to IV**

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Soybean [Glycine max (L.) Merr.] protein and oil are used worldwide in feed, food, and industrial raw materials. Many quantitative trait loci (QTL) controlling seed protein, oil, sulfur-containing amino acids, and fatty acids have been identified using family-based mapping populations. Soybean seed protein negatively correlates with seed oil and often with seed yield. Correlations also exist among the fatty acids. In addition, most seed composition traits are influenced by genotype × environment interactions. We evaluated seed compositions of 621 plant introductions (PIs) in maturity groups II to IV over multiple environments to identify useful alleles for several seed composition traits. The PIs with yellow seed coat, low seed shattering, low lodging, and low seed mottling were selected to avoid confounding effects on the traits of interest. Seed harvested from five environments in OH, IL, and NC in 2014 or 2015 were screened for protein, oil, amino acid, and fatty acid contents. Protein and oil content ranged between 29-44% and 12-21% on a 13% moisture basis, respectively; while cystein and methionine ranged between 0.39-0.60% and 0.40-0.56%, respectively. Distribution of fatty acid contents were between 8-15%, 2-8%, 14-53%, 29-63%, and 3-14% for palmitic, stearic, oleic, linoleic, and linolenic acid, respectively. All traits were highly heritable (H2 >0.7), but significant genotype × environment interactions were also observed. A genome wide association study was conducted with the phenotypic data and SoySNP50K data. More detailed results of the association analysis will be presented, which should be of interest to soybean scientists working on seed composition traits.

S24

**Development of a Soybean-Based Feedstock for Aquaculture**

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Soybean (Glycine max (L.) Merr.) is sought after for both its oil and protein components. Genetic approaches to add value to either component are ongoing efforts in soybean breeding and molecular biology programs. The former is the primary vegetable oil consumed in the world. Hence, its primary usage is in direct human consumption. As a means to increase its utility in feed applications, thereby expanding the market of soybean co-products, feeding trials were conducted designed to simultaneously displace of marine ingredients in aquafeeds with soybean-based protein and a high omega-3 fatty acid soybean oil, enriched with alpha-linolenic and stearidonic acids, in both steelhead trout (Onchorhyncus mykiss) and Kampachi (Seriola rivoliana). Aquafeed formulations with major reduction in marine ingredients, that incorporated high inclusion levels of soybean ingredients, resulted in more total omega-3 fatty acids in harvested flesh of the evaluated fish species. Building of these findings a genetic approach was pursued to design a prototype for a optimal identity preserved soybean-based feedstock for aquaculture, whereby a multi-gene stack strategy for the targeted synthesis of two value-added output traits, eicosapentaenoic acid (EPA) and the ketocarotenoid, astaxanthin, were introduced into the crop. To this end the systematic introduction of nine transgenic cassettes into soybean has been accomplished that translated to the production of these two high value aquafeed ingredients in seed.
S25
Engineering Altered Soybean Protein Composition and Content

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Soybean protein is a key component of industrial foods and nutritional source for animal feed. The soybean seed proteome is dominated by 11S and 7S storage proteins. Seed composition varies by genotype, environment, and lesions in gene expression of the major seed proteins. Soybean protein content is controlled by genotype and is a breeding trait. If either or both of the 11S or 7S storage proteins are mutated or silenced the soybean seed remodels its proteome to maintain the genotype-regulated protein content in a process termed proteome rebalancing. In proteome rebalancing the seeds accumulate alternate intrinsic seed proteins that results in a different proteome that maintains both genotype-controlled standard protein content and amino acid composition. The process of proteome rebalancing produces changes in the seed’s regulatory protein content that could provide a control mechanism. Several putative rebalancing regulatory proteins were identified in gene expression analysis of seeds that had both 11S and 7S storage proteins silenced. The regulatory proteins were screened using seed-specific over-expression and RNAi silencing in transgenic soybeans. One of these regulatory proteins appears to exert a direct control over protein content through the ABA regulon. This resulted in a metabolic nitrogen-push that increases in seed protein content without substantial alteration of the seed’s proteome. Together proteome rebalancing and protein content control provides enabling technology engineer novel seed protein traits in soybean and to exploit soybeans as protein biofactories useful to produce sector-specific functional food and feed.

S26
Soybean Kinome: Identification and Functional Classification

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The protein kinase gene family (kinome) is one of the most expanded and highly conserved plant gene families and constitutes the core components of numerous signaling pathways controlling almost all aspects of plant growth, development, and responses to environmental stimuli. Here we represent the identification, functional classification, and global analysis of gene expression profiles of the entire soybean kinome. The soybean kinome contains 2,166 putative kinases, categorized into 19 groups, 81 families, and 122 subfamilies. Collinearity analysis indicated that whole-genome segmental and tandem duplication events may have played the major roles in the expansion of the soybean kinome. Genome-wide gene expression analysis suggested regulatory functions over a wide range of biological processes and molecular functions. Gene co-expression network analysis of the 122 subfamilies suggested their implication in specific as well as synchronized developmental processes and stress signaling. Further analyses indicated that gene co-expression networks of soybean kinome offer such tremendous potential to identify highly connected genes “hub genes” that are trait-correlated in order to prioritize candidate genes for further functional genomics studies.
**S27**

**Dissection of Gene Networks that Govern Seed Development**

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The seed is a complex yet elegant structure that is an excellent model system for the study of plant development. It is comprised of three major regions; the diploid embryo and triploid endosperm arise from separate fertilization events, and the diploid seed coat is derived from maternal tissues. Each region is partitioned further into subregions, tissues, and cell types that each has a distinct morphology and physiological function. Another unique aspect of seed development is that it is temporally biphasic. During the morphogenesis phase early in seed development, both the embryo and endosperm undergo morphogenetic events that are required for formation of the seed subregions. Late in seed development during the maturation phase, the embryo and endosperm accumulate storage macromolecules, and the embryo becomes tolerant of desiccation. A comprehensive understanding of seed development requires knowledge of the gene regulatory networks that operate temporally and spatially within the seed.

We have profiled the mRNA transcriptomes of every subregion of soybean seeds at several stages of development using laser-capture microdissection coupled with RNA sequencing experiments. From this analysis, we have identified transcription factors (TFs) that accumulate in specific subregions and/or at specific stages of seed development. To define the gene regulatory networks that are governed by these TFs, we are identifying target genes directly regulated by these TFs in chromatin immunoprecipitation - RNA sequencing experiments. We are also characterizing the DNA methylome and modified histone occupancy of the target genes to obtain insight into the transcriptional mechanisms involved in regulating seed development.

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**S28**

**Functionally Characterizing Soybean Genes Involved in Defense by Using BPMV VIGS**

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We are interested in characterizing networks of soybean genes involved in immunity against pathogens. To facilitate identification of soybean defense genes, we have used virus-induced gene silencing (VIGS) mediated by Bean pod mottle virus (BPMV) to silence soybean genes at the single gene and gene family scales. We present an example of using VIGS to investigate the functions of soybean MAP kinases (MAPK) in regulating defense responses, and a second example of using VIGS to characterize the function of a gene targeted by a candidate effector produced by the soybean rust pathogen, Phakopsora pachyrhizi. In the MAPK study, we individually silenced 32 genes encoding MAPKs. GmMPK4 was identified as a negative regulator of defense and a positive regulator of plant growth, because GmMPK4-silenced plants were stunted and displayed constitutive defense responses including spontaneous cell death, increased salicylic acid, and enhanced resistance to pathogens. We have also identified a transcription factor, GmSPL12I, which negatively regulates defense. GmSPL12I is of interest, because it interacts with a small, cysteine-rich P. pachyrhizi effector candidate (PpEC) designated PpEC23. PpEC23 was identified in the haustorial secretome of P. pachyrhizi, and it was one of the PpECs that suppress effector- and/or pattern-triggered immunity in a screen using a bacterial type III secretion system-based delivery assay. The GmSPL12I-silenced plants had smaller leaves, increased defense gene expression, and enhanced resistance to pathogens. These results show that GmSPL12I is a negative regulator of defenses that may be targeted by P. pachyrhizi to enhance virulence.
S29

Use of Crispr/Cas Genome Editing Demonstrates a Critical Role for Uricase and Xanthine Dehydrogenase in Soybean Nitrogen Fixation and Nodule Development

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Previous biochemical studies suggested that de novo purine biosynthesis is required for the incorporation of fixed nitrogen in ureide exporting nodules, as formed on soybean roots. However, in many cases, the enzymes involved in this pathway have been deduced strictly from genome annotations with little direct genetic evidence, such as mutant studies, to confirm their biochemical function or importance to nodule development. While efforts to develop large mutant collections of soybean are underway, research on this plant is still hampered by the inability to obtain mutations in any specific gene of interest. However, this situation is now significantly changed through the ability to apply the methods of CRISPR/Cas9 genome editing using Agrobacterium rhizogenes-mediated hairy root transformation. Using this approach, we were able to generate homozygous mutant roots lacking either uricase (UOX) or xanthine dehydrogenase (XDH) activity. The uox knockout soybean mutants were unable to fix nitrogen, as exemplified by their internal greenish appearance reflecting a lack of leghemoglobin production. As added confirmation, a uox knock-out mutation generated through fast neutron mutagenesis displayed a similar phenotype. Similarly, a knock out XDH mutant, generated with the Crispr/Cas system, also displayed a fix− phenotype. These studies demonstrate the great utility of the Crispr/Cas system for studying root associated gene traits when coupled with hairy root transformation. Furthermore, these genetic studies confirm the critical role of the de novo purine biosynthetic pathway, not only in incorporation of fixed nitrogen but in the successful development of a functional, nitrogen fixing nodule.

S30

Movement of siRNAs into Arabidopsis Sperm Cells Directs Transposable Element Repression

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Plant small interfering RNAs (siRNAs) communicate from cell to cell and travel long distances through the vasculature. However, siRNA movement into germ cells has remained controversial. Movement of siRNAs into germ cells has gained interest because neighboring the sperm cells, the terminally differentiated pollen vegetative cell undergoes a programmed heterochromatin decondensation and transcriptional reactivation of transposable elements (TEs). Transcription of TEs leads to their post-transcriptional degradation into siRNAs, and it has been proposed that the purpose of this TE reactivation is to generate and load TE siRNAs into the sperm cells. I will present data demonstrating the molecular pathway of TE siRNA production in the Arabidopsis pollen grain and how we identified that siRNAs produced from pollen vegetative cell transcripts can silence TE transcripts in the sperm cells. Our data proves that TE siRNAs transit to the germ cells, providing a repressive force to inhibit TE activity in the germ cells and possibly in the next generation.

S31

Combinatorial Application of FANS with ATAC-seq to Identify Open Chromatin in Plant Genomes

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Chromatin structure plays a pivotal role in enabling proper control of gene expression. The abilities of transcription factors (TF) to bind cis-elements are often associated with accessible chromatin zones, in which chromatin is decondensed, exposing the DNA. Therefore, identification of these accessible regions throughout plant genomes is important to understanding the relationship between TF binding, chromatin status and thus the regulation of gene expression. Assay for Transposase Accessible Chromatin (ATAC-seq) is a recently developed and widely used technique to map open chromatin zones in animal and human genomes. Furthermore, this technique can be carried out on samples containing hundreds to thousands of cells. However, in plants, the existence of cell walls, robust cell types and numerous subcellular organelles have prevented the application of this technique. Here, we describe an assay combining ATAC-seq with Fluorescence-Activated Nuclei Sorting (FANS) to identify and map open chromatin, as a rapid and sensitive method for integrative chromatin status analysis in plant genomes. We are furthering development of this methodology by combining ATAC-seq with FANS and with samples collected from laser capture as this will enable cell-type specific analysis of chromatin states in any plant genome.
Inheritance Patterns of Transgenes and Targeted Mutations in a Soybean CRISPR-Based Mutagenesis System

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Junqi Liu, University of Minnesota
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Nicole Mihelich, University of Minnesota
Yer Xiong, University of Minnesota

There is considerable interest in using genome engineering technologies for gene function studies and genetic improvement of crop species. This talk will address our recent findings in soybean mutagenesis using a CRISPR/Cas9-based approach. To date, we have used CRISPR/Cas9 to induce targeted mutations in whole soybean plants for six different endogenous genes, including both in-frame and frameshift alleles. T0 plants exhibited a high rate of mutagenesis, including multi-allelic tissue samples, chimerism within individual plants, and simultaneous mutagenesis of paralogous genes. However, inheritance of both the CRISPR/Cas9 transgene and targeted mutations has been inconsistent among the different events. We have observed stable transmission of both transgene and mutations in some plants, while other events have displayed a complete loss of both transgene and mutations in the subsequent generation. Additionally, one event exhibited successful inheritance of two different mutations, but no plants were observed to inherit the transgene. These results provide insight into the opportunities and potential obstacles that exist in identifying and stabilizing heritable soybean mutations using our current CRISPR/Cas9 approach.

Insight into the Genomic Regions Under Breeding Selection and Genomic Selection for Yield in Soybean

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Increasing soybean yield potential is the ultimate goal for soybean breeding programs. Improved genetic yield potential is critical to the success of any new soybean cultivar and a main component that drives the profitability of soybean. Utilizing the genomic technology will help improve the rate of genetic gain in soybean yield. In this study, we attempt to identify the haplotype regions under breeding selection in soybean populations and build a reference population by utilizing elite breeding populations and advanced lines to conduct genomic selection (GS) for yield. Using SoySNP50k Infinium chips data, we performed genome-wide analyses of the North American soybean ancestral lines and modern cultivars and identified the signature of selection in these populations. Over 100 lines developed at eight public institutions were derived from exotic PI 416937 and have been entered into USDA Uniform Tests over past 15 years. We conducted the genome-wide analysis of these elite lines along with their parents derived from PI 416937 to identify the contributions of the haplotype alleles to these high yielding lines. Thirteen genomic regions from PI 416937 were selected for and 15 genomic regions that have been selected against. We have selected four elite soybean breeding populations with PI 416937 presenting in their pedigrees to form a reference population for genomic selection. Yield data from these populations have been collected over the past two years and the populations were genotyped with SoySNP6k Infinium chips. Preliminary results indicated the prediction accuracy of GS was 0.35-0.37. An integration of GS into soybean breeding programs will be discussed.

Nutrient Deficiencies: Beyond the QTL

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Iron and phosphorus are key mineral nutrients for plant growth and development. Biologically, these two minerals interact within the plant impacting storage, uptake, and transport of each other. As both nutrients are important factors for maintaining plant health, there have been a number of QTL identified for tolerance to deficient and toxic conditions. To complement these studies, our lab has grown soybean under both iron and phosphate deficient conditions. Leveraging genomic tools including RNASEq, the genome sequence, transcription factor databases, and clustering algorithms, we are identifying gene networks and expression profiles to characterize soybean’s response to iron and phosphate deficiency. These data will lead to an increased understanding of nutrient acquisition and use in legumes.
**Impact of Rhg1 Copy Number and Type on SCN Resistance**

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The major soybean cyst nematode (SCN) (Heterodera glycines Ichinohe) resistance locus Rhg1 is complex and contains tandem repeats of a 31.2 kb unit. Within these repeats, there are four genes and three have been implicated in contributing to resistance. Recent research has shown that across soybean germplasm the number of copies of the repeated unit ranged from one to ten and there are different repeat subtypes, defined by SNPs within the genes. The goal of our current project is to determine the phenotypic impact of variation for Rhg1 copy number and subtype. This objective was studied by developing F2 populations that segregate for this variation and testing these populations for SCN resistance and markers. These tests show that Rhg1 subtype impacts resistance as observed through subtype specific interactions with Rhg4. Within subtypes, an increase in copy number was associated with greater resistance. For example, within the Peking Rhg1 subtype, three copies conferred greater resistance than one copy and within the Fayette type, ten copies conferred greater resistance than six copies. In an evaluation of experimental lines from the SCN Northern Regional Tests that were developed with the PI 88788 source of resistance, there is evidence of variation in copy number with some lines showing fewer copies than Fayette, which has ten copies. The ability to estimate Rhg1 copy number and type combined with markers for Rhg4 improves our ability to predict SCN resistance responses of soybean experimental lines.

**Identifying Novel Alleles for Soybean Meal Composition Traits**

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Modified carbohydrate composition in soybean [Glycine max (L.) Merr.] improves metabolizable energy (ME) for monogastric animals. Incompletely removed during standard processing of soybean meal, raffinose family oligosaccharides (RFOs) reduce ME due to fermentation in the gut of monogastric animals, while sucrose increases ME. One of the goals of this project is to identify allelic variation for carbohydrate composition. Chemical mutagenesis via N-nitroso-N-methylurea (NMU) was used to create new alleles. NMU induces single-nucleotide polymorphism (SNP) mutations across the genome. Through the application of a reverse genetic technique, known as TILLING (Targeting Induced Local Lesions In Genomes), unique chemically induced mutations that have the potential to alter gene function can be identified within target genes. Our method makes this more affordable by surveying the available diversity in the population using next-generation sequencing, then isolating the desired mutant individuals by genotyping with a custom SNP marker. In this study we describe our method and report the successful identification of mutant alleles for genes that affect seed carbohydrate biosynthesis.

**Leveraging Genomic and Environmental Data for Predicting the Distribution of Yield of Candidate Varieties in Target Locations**

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Most agronomically relevant traits are affected by a large number of genetic (G) and environmental (E) factors. These factors can interact leading to genetic-by-environment interactions (G×E). Molecular markers (e.g., SNPs) and environmental covariates (ENV-COV) can be used to describe G and E factors. These two sources of information could be integrated into models that account for G, E and G×E. However, modeling interactions between predictors in two high-dimensional sets (e.g., SNPs and ENV-COV) can be extremely challenging. In 2014 we (Jarquin et al., TAG, 2014) proposed a methodology that allows integrating high SNP and ENV-COV in a model that accommodates G, E and G×E. In this presentation we will: (i) introduce the methodology, (ii) present results based on a large data set (n=28,554) generated by Arvalis-Institut-du-Végétal. This data originates on field trials conducted from 1997 to 2014 where commercial wheat varieties (601) were tested in (243) different locations covering all the relevant agronomic regions of France. Varieties were genotyped with two SNP arrays that combined gave (after QC) 213,339 SNPs. A total of 125 environmental covariates describing temperature, radiation and evaporative demand during five different phases of crop development were generated using climatic data and an ecophysiological model. Finally, (iii) we will discuss an extension of the methodology proposed by Jarquin et al. (2014) that will allow us to leverage trial data with historical wheatear information to derive predictions of the expected distribution of yield (over years) for candidate varieties on a set of target locations.
Drivers of Soybean Genome Size Change, and Other Insights from Multi-Species Legume Genome Comparisons

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Genome sequences have now been determined for more than a dozen legume species, and many more genome assemblies are in progress. Transcriptome and partial genomic sequences are also available for several wild Glycine species. Combining these resources adds power to various kinds of molecular-evolutionary analyses, enabling several kinds of new inferences: (1) evidence about how genomes have changed at various time scales, and after polyploidy; (2) evidence of shifts in selection pressure or copy number for particular genes or groups of genes; (3) evidence of gene loss or gain from particular genomic regions; (4) evidence from other species about gene function from mutants or association analyses. This talk will describe use of several on-line resources, including SoyBase and the Legume Information System (LegumeInfo), as research tools in these areas. One research conclusion is that genome fragmentation, such as occurred in soybean after polyploidy, systematically drives a decrease in genome size.

A Mutation in an Argonaute Protein Explains the Epistatic Interaction of the K and I Loci Controlling Seed Color Patterns

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Nearly all soybean varieties contain the dominant K1 allele. Interestingly, a recessive k1 mutation acts epistatically to overcome the dominant I and i-i alleles, which inhibit seed color, and extend the pigmented region over a larger surface of the seed coat. Thus, seed with the homozygous i-i, k1 genotype have a chimeric saddle pattern that mimics the i-k, K1 saddle phenotype. We have previously shown that the dominant i-i allele of the I (inhibitor) locus is composed of an inverted-repeat cluster of six chalcone synthase (CHS) genes on chromosome 8 whose unique arrangement generates CHS siRNAs that downregulate target CHS7 and CHS8 genes on non-linked chromosomes resulting in yellow seed coats. Using small RNA and RNA-Seq, we now demonstrate that CHS siRNAs are also the cause of chimeric seed coat patterns produced by the i-i and i-k alleles of the I locus which restrict pigment to only certain regions of the seed coats. In order to identify the unknown K locus, we used a combination of RNA-Seq data and whole genome resequencing and identified the k1 allele as a 129 bp deletion in a specific member of the Argonaute gene family (AGO5), which leads to premature termination of its translation product. The function of the AGO5 appears to be critical for maintaining sufficient levels of CHS siRNAs, thus explaining how the k1 allele reverses the phenotype of the seed coat regions from yellow to pigmented, even in the presence of the normally dominant i-i or I alleles.

Genetic Basis and Process of Soybean Domestication

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It is proposed that soybean has a single domestication origin. However, the genetic basis and selection process of soybean domestication remain to be deciphered. We have recently developed two recombinant inbred line (RIL) populations from two crosses between cultivated soybean Williams 82 (Glycine max) and two wild soybean (Glycine soja) accessions, and identified major genes underlying a few key soybean domestication transition traits, including soybean stem growth habit, hard-seededness and seed coat bloom etc., by quantitative trait locus (QTL) mapping. We have also identified genomic regions involved in introgression between G. max and G. soja, some of which seem to have undergone recurrent and/or reselection, as the against the targeted domestication traits. This study thus unveils the genetic control of several domestication traits and illustrates the dynamic process of soybean domestication.
S41
A Study of Molecular Evolution Patterns in the Genus Glycine

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The genus Glycine comprises two subgenera, the annual subg. Soja, which includes only the diploid (2n = 40) soybean and its wild progenitor, G. soja, and the perennial subg. Glycine, which includes over 30 diploid and tetraploid species belonging to numerous reproductively isolated genome groups. It thus presents a useful model to study selection on the expression of, and proteins encoded by, duplicated genes. We analyzed genic sequences and examined signatures of selection from de novo transcriptome assemblies of 12 accessions representing 5 wild perennial Glycine species. We found one gene, Glyma02g00320, that appears to be positively selected in the soybean genome lineage. Several variants within the sequence of Glyma02g00320 were soybean specific, including a prominent 24-base-pair deletion in the 5'-region containing the signal peptide. Glyma02g00320 encodes for a BolA4-like protein, with a possible role in mediating redox homeostasis in chloroplasts and mitochondria. We also investigated the expression levels of genes at two different light levels in the allotetraploid Glycine dolichocarpa AADD and its A and D genome diploid progenitors. Comparison of genes showing genome expression bias between two light conditions revealed several genes that change genome bias from A-bias to D-bias or vice versa between different light conditions. Promoter regions of the soybean orthologs of these genes were significantly enriched for light-responsive elements such as DPBF1&2, SORLIP2, Box II, and ARF1. These findings indicate that selection for redox balance has influenced soybean genome evolution and that in allotetraploid Glycine, expression partitioning of homeologs is mediated through transcriptional regulatory mechanisms.

S42
Genomic Approaches for Soybean Improvement

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Over the last ninety years, soybean yield has steadily improved. Yield increases are due in part to the modernization of farming practices, but also to genetic improvements made by breeders as they transformed ancestral land races to modern day elite cultivars. In addition to selecting for improved yield, breeders have selected for changes in maturity and greater tolerance to biotic and abiotic stress. We have used next generation sequencing technology to resequence the genomes of 80+ ancestral soybean land races and milestone cultivars, representing steady increases in yield, over 90 years of soybean improvement. Sequences have been aligned to the Williams82 reference genome allowing the use of bioinformatic techniques to identified more than 13 million single nucleotide polymorphisms (SNPs), 400 whole gene copy number variants (CNV) and 4,000 exon CNV. Genome wide association studies have identified a number of loci associated with yield increases. To facilitate the utility of these data, we have developed web resources for viewing and investigating these data.
The Long and the Short of Soybean Petioles: The Effect of a 3-bp Insertion on Plant Architecture and Harvest Index

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Dietary changes and increases in the world population have led to rising demands for sustainable sources of plant protein and oil. Modifications to plant canopy architecture could play an important role in meeting these global food demands, as was demonstrated during the Green Revolution in the 1960’s and 1970’s. Petioles are an important component of canopy architecture as they connect the leaflets to the stem in dicot species, but many of the mechanisms controlling petiole length are unknown. To our knowledge the effect of short petioles on seed yield has not been studied. Kilen (1983) identified a short petiole soybean mutant lps1 that segregated as a single, recessive locus. The mutation was first observed in 1976 segregating in an F3 row in a population of a cross between Forrest(2) x (PI 229358) and D71-6234. D71-6234 was derived from a cross between a high protein Lee type and PI 95960. None of the parents were observed to have the short petiole phenotype suggesting the mutation was spontaneous. This study was undertaken to identify the causal DNA polymorphism underlying the lps1 phenotype as well as to assess the utility of the lps1 mutation for improving soybean yield, agronomics, and physiology using Near-Isogenic Lines (NILs) generated during the trait mapping process. Whole-genome sequencing-based bulk segregant analysis was used to locate the chromosomal region harboring the lps1 mutation. Diversity analysis of sequences within this interval identified an in frame 3-bp insertion in an uncharacterized gene, and silencing of this gene by Virus Induced Gene Silencing successfully produced a short petiole plant. Preliminary yield trial testing of the NILs under narrow row spacing indicated that the short petiole trait does not negatively impact yield and may improve soybean agronomics through increased harvest index and decreased plant height.

Integrating Image Processing and Machine Learning to Decipher the Genetics of Iron Deficiency Chlorosis

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Advances in image-based phenotyping and machine learning (ML) is enabling accurate and precise trait collection. Digital imaging and ML enabled phenotyping were integrated to study the genetics of iron deficiency chlorosis (IDC) in soybean. Automated and fast phenotyping and subsequent decision support to generate IDC rating classification and severity from an association panel of 461 diverse plant introduction soybean accessions were used in genome wide association (GWA) studies. An end-to-end phenotyping workflow was utilized, which consisted of image capture, image processing to isolate canopy and removal of spurious features, IDC feature extraction, and use of supervised machine learning methods to design classifiers that link the automatically extracted features and visually rate IDC score. ML generated phenotypic data was subsequently utilized in GWA studies that validated previously reported loci on Gm03 and identified new loci controlling IDC. This study provides a framework for stress phenotyping in soybean and other crops for a more robust and quicker phenotyping through ground and aerial based systems, and integration with genome wide studies.
Syngenta, a leading developer of crop varieties (seeds) that provide food for human and livestock consumption, is committed to bringing greater food security to an increasingly populous world by creating a transformational shift in farm productivity. Syngenta Soybean Research & Development (R&D) is leading Syngenta’s corporate plant-breeding strategy by developing and implementing a new product development model that is enabling the creation of an efficient and effective soybean breeding strategy. Key to the new strategy is the combination of advanced analytics and plant-breeding knowledge to find opportunities to increase crop productivity and optimize plant-breeding processes. Syngenta uses discrete-event and Monte Carlo simulation models to codify Syngenta Soybean R&D best practices, and uses stochastic optimization to create the best soybean breeding plans and strategically align its research efforts.
POSTER ABSTRACTS
Use of Automated Image Analysis Tools to Quantify Soybean Composition: Tissues, Cells, and Cell Structures

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Microscopic histology is a tool providing colorimetric images of plant tissues for image analysis. Cellular events occurring during early seedling development are readily detected with fluorescent tags through light, transmission, or confocal microscopy and subsequent quantification. However, once soybeans initiate secondary growth through the production of active interfascicular cambia, techniques to process mature tissues for image analysis become limited. We are developing an automated image analysis method with ImageJ-Fiji software to obtain quantitative results from images acquired from soybean stems at peak vegetative growth. Histoslides of stem cross-sections are stained with fastgreen and safranin to differentiate primary and secondary tissues, and high resolution RGB images are acquired with light microscopy. Tailored algorithms facilitate accurate, fast, quantitative segmentation and quantification of complex tissues (cortex, pith, primary and secondary xylem, secondary phloem, and phloem fibers), cells (gelatinous fibers, vessels, parenchyma), and cell walls (primary and secondary). Our results illustrate a novel method of plant image analysis that we are using to identify drought-sensitive phenotypes during the susceptible seed filling stages of reproductive growth, R5 to R8.

Crosstalk of Transcriptomic Responses to Submergence and Drought in Leaves and Roots of Soybean

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The number of flooding and drought events is on the rise throughout the world due to global climate change, which has already resulted in agricultural and economic damage. These precipitation extremes can occur sequentially within a single crop growing season or independently in the same fields in different years. Therefore, understanding the regulatory mechanisms underlying tolerance to both of these stresses in major crops such as soybean can largely contribute to the development of climate-smart crop production systems. To this end, we conducted a genome-wide transcriptomic analysis in leaves and roots of soybean at the early vegetative stage under submergence, drought and recovery from these stresses. The plants were submergence for up to 3 d or withheld from water for up to 6 d followed by 24 h of recovery from both stresses. mRNA-Seq was conducted across 18 experimental conditions for both stresses and recovery in two tissue-types. The analysis identified 15,074 gene transcripts that responded to these stresses among which 73 were AP2/ERF transcription factors and 199 were MAP kinases. k-mean clustering and GO enrichment analysis of the identified differentially expressed genes displayed several stress-specific, tissue-specific and overlapped pathways associated with metabolism, development, response to stress, signal transduction, cellular homeostasis and redox regulation. Our study demonstrated the dynamic transcriptomic reconfiguration in soybean leaves and roots to acclimate to submergence and drought stresses.
P003
**Genome-Wide Analysis of Phosphoenolpyruvate Carboxylase Gene Family in Soybean**

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Phosphoenolpyruvate carboxylase (PEPC) plays an important role in assimilating atmospheric CO2 during C4 and crassulacean acid metabolism photosynthesis, and also participates in various non-photosynthetic processes including regulation of plant tolerance to stresses. However, a comprehensive analysis of PEPC family in soybean (Glycine max) has not been reported. A total of ten PEPC genes were identified in soybean and denominated as GmPEPC1-GmPEPC10. Based on the phylogenetic analysis of the PEPC genes from 13 higher plant species including soybean, PEPC family could be classified into two subfamilies, including plant-type PEPC (PTPC) and bacterial-type PEPC (BTPC). Members within each subfamily showed similar gene structures and protein motif combination patterns. Nineteen cis-regulatory elements (including ABRE, ARE, TGA-element, G-box, GARE-motif, ERE, GCC-box, HSE, LTR, MBS, S-box, TC-rich repeats, TCA-element, TGACG-motif, CGTCA-motif, WUN-motif, W-box) related to phytohormones, biotic and abiotic stresses were identified in the promoter regions of soybean PEPC genes, indicating their roles in soybean development and stress responses. The expression of GmPEPC genes in response to different abiotic stresses including aluminum toxicity (Al), cold, osmotic, and salt stress was evaluated by quantitative RT-PCR. GmPEPC6, GmPEPC8 and GmPEPC9 were significantly induced by Al, cold, osmotic and salt treatments. In addition, the enzyme activities of soybean PEPC were also up-regulated by Al, cold, osmotic and salt treatments, suggesting their potential roles in soybean response to these abiotic stresses.

P004
**Fine Mapping of an Iron Deficiency Chlorosis Tolerance QTL from Fiskeby III (PI 438471)**

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Soybean (Glycine max) is particularly susceptible to iron deficiency chlorosis (IDC) when grown in high alkaline soils. IDC tolerance historically has been difficult to achieve in the narrow U.S. breeding base. ‘Fiskeby III’ (PI 438471), a 00 maturity group Swedish edamame variety released in 1949, shows exceptional tolerance to a wide range of abiotic stresses, including IDC. To investigate the abiotic stress tolerance of ‘Fiskeby III’, the USDA-ARS developed a ‘Fiskeby III’ x ‘Mandarin (Ottawa)’ (PI 548379) bi-parental mapping population, which was previously used to map stress tolerance QTL. Major IDC tolerance QTL were discovered on chromosomes 5 and 6 in this population. Near isogenic lines (NILs) were developed from 20 heterogeneous inbred families (HIFs) from the ‘Fiskeby III’ x ‘Mandarin (Ottawa)’ bi-parental mapping population. F6 families that were heterozygous in the 1.5 mega base (Mb) chromosome 5 QTL were advanced. F7:8 plants that were homozygous for the ‘Fiskeby III’ or ‘Mandarin (Ottawa)’ genotype in the region were planted in an IDC nursery in 2015 to validate the QTL. Plants homozygous for the ‘Fiskeby III’ genotype were significantly more tolerant than plants homozygous for the ‘Mandarin (Ottawa)’ genotype (p < 0.001). Future directions include the development of analogous NIL pairs for the chromosome 6 QTL, and the identification of new recombinants for higher resolution of the chromosome 5 QTL, and backcrossing these traits into elite germplasm.
P005

Environmental Association and Evaluation of Abiotic Stress Tolerance Loci from Glycine soja

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The genetic diversity of soybean is relatively narrow due to the limited subset of wild progenitors from which it was domesticated, as well as selective sweeps during the production of elite lines. This narrow genetic base limits the identification of genetic loci responsible for traits of interest to breeders. While some effort has been made to exploit soybean landraces in modern breeding, the potentially rich reservoir of genes that reside in Glycine soja, soybean’s nearest wild relative, has been underutilized. Previous work explored the USDA G. soja collection, genotyped at 32,416 SNPs to identify population structure and test for associations with bioclimatic and biophysical variables (G3 6:835-843). Several candidate G. soja abiotic stress tolerance loci were identified in association with variables such as soil composition, soil pH, and monthly precipitation. Our current research efforts focus on identifying DNA sequence polymorphisms from G. soja candidate genes that may underlie these adaptations and functionally validating these alleles using isogenic or gene editing approaches. The identification of potentially adaptive variants in this collection may permit a more targeted use of this wild species for soybean improvement.

P006

Alternative Splicing Affects Target Gene Expression for miR398-CSD1 and miR172-AP2

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MicroRNA binding sites (MBSs) are frequently interrupted by introns and therefore require proper splicing to generate functional MBSs in the transcripts. Conversely, MBSs can be excluded from target transcripts during splicing of pre-messenger RNA, leading to different levels of regulation among different isoforms. Cu/Zn superoxide dismutase (CSD) plays a key role in detoxifying reactive oxygen species in response to stress conditions. APETALA2 (AP2) belongs to AP2/Ethylene Responsive Factor (ERF) family that has an important function as a transcription factor in regulating expression levels of downstream stress responsive genes. In this study, expression levels of miR398 and miR172 were analyzed in the legume Arachis hypogea (peanut) under drought conditions. Unexpectedly, the transcript levels of some AhCSD1 and AhAP2 isoforms were not correlated with miRNA levels under drought conditions. We report that the miR398 and miR172 binding sites in specific isoforms of AhCSD1 and AhAP2 are absent as a consequence of alternative splicing, which affects the levels of these transcripts under drought stress. Isoform AhCSD1-2.2, which lacks a binding site for miR398, contains an allelic polymorphism apparently derived from one of the diploid progenitors of allotetraploid cultivated peanut. In addition, we found isoforms of soybean CSD1 (GmCSD1) (Glyma.19G240400.1 and Glyma.03G242900.1) that lacked a MBS among multiple isoforms. We hypothesize that CSD1 transcript levels without a MBS are increased in legume crops to generate CSD1 protein for oxidative stress detoxification.

P007

Ethylene Signaling Negatively Impacts Cold Stress Responses in Soybean

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The growth season and crop yield of soybean is limited by temperature. The CBF cold responsive pathway has been well characterized in Arabidopsis, and soybean CBF homologous transcription factors have been described. Under cold stress, soybean CBF transcript levels increase similarly to reported responses in cold tolerant species; however, the expected CBF downstream targets appear unresponsive. In Arabidopsis, EIN3, a major component of the ethylene signaling pathway, is a known negative regulator of CBF cold regulation. Transcript analysis from cold treated unifoliate leaves of soybean seedlings revealed a 3.6 fold increase in EIN3 transcripts during early cold stress. As EIN3 transcripts do not significantly accumulate in cold treated Arabidopsis, we hypothesize that the observed cold induced EIN3 increase could be preventing effective CBF cold regulation in soybean. While silver nitrate is known to block the ethylene pathway in Arabidopsis and other species, the effect in young soybean seedlings is not presently reported. We have found that silver nitrate blocks the abscission of cotyledons and prevents yellowing of unifoliate leaves in soybean seedlings exposed to ethylene indicating silver blocks ethylene regulation in soybean. Initial experiments with a cold responsive reporter construct (AtRD29a::GFP/GUS) in soybean demonstrated that blockage of the ethylene pathway with silver nitrate prior to cold exposure led to significant increase in GUS activity level. This suggests that the ethylene pathway is interfering with the CBF cold regulation in soybean, likely through action of EIN3. These results provide potential targets for generating cold-hardy soybean crops.
P008  
**Prediction of Maturity Dates of Soybean via Remote Sensing**

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High-throughput phenotyping (HTP) using remote sensing is a fast developing technology which has the capacity to reduce the time it takes to measure phenotypic traits in the field. HTP shows particular promise as a method for predicting plant maturity. Maturity is the date where 95% of the pods reached mature color (R8 growth stage) and is commonly recorded on all yield plots in breeding programs by periodically walking through experiments and visually estimating maturity dates. Precise maturity dating is a time critical task; therefore, satellites and other previous methods of remote sensing would not be applicable to this research. To combat the limitations of other methods of remote sensing, we constructed a two-camera mounted Unmanned Aerial Vehicle (UAV) platform with the capacity to capture visible and near-infrared (NIR) images. This study was done in three broad steps: the acquisition of multi-spectral images using UAVs, constructing composite images of the visible and (NIR) images, and extracting digital values to build a model to predict maturity dates from images. Using these procedures, we were able to develop a binary prediction model from the multi-spectral image data to and achieved over 93% accuracy in classifying soybean maturity. The maturity model was validated in an independent breeding trial with a different plot type. These results show that remote sensing can be effectively used to estimate the maturity of plots but the analysis of images needs to be more efficient before it can be used routinely.

P009  
**A Genome-Wide Expression Profile Analysis Reveals Active Genes and Pathways Coping with Phosphate Starvation in Soybean**

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Phosphorus is one of the most important macronutrients that is required for plant growth and development. However, stress under low-P conditions has become a limiting factor that affects crop yields and qualities. Plants have developed strategies to cope with this, while few genes associated with low-P tolerance have been identified in soybean. Genome-wide analyses were performed on the roots and leaves of a low-P-tolerant accession and a low-P sensitive accession which were identified by hydroponic experiments under different P treatments. Through comparative analyses on the differently expressed genes, we explored 42 common genes that were highly correlated to low-P stress. The functional classification of these genes revealed 24 Gene Ontology (GO) terms of biological process including response to oxidation reduction, hormone stimuli, and biotic and abiotic stimuli. Additionally, three common pathways were identified. These results could not only promote the work on the molecular regulation mechanism under low-P stress in soybean, but also facilitate the cultivation of high-phosphorus-acquisition and high-phosphorus-utilization soybean varieties.

P010  
**A Genetic Strategy for the Synthesis of a High Saturated, Low Polyunsaturated Fatty Acid Soybean Oil**

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An effort designed to enhance carbon flux towards oil during soybean seed development targeted the introduction of a global regulator of lipid biosynthesis from Arabidopsis designated WRINKLED1 (AtWRI1) into soybean. Phenotyping of the derived transgenic soybean events led to the observation of elevated palmitic acid levels in the seed oil, up to approximately 20%, triggered by AtWRI1 expression. Subsequent stacking of AtWRI1 with the mangosteen steroyl-ACP thioesterase in soybean led to the synthesis of oil with over 30% saturated fatty acids. Co-expression of AtWRI1, with the mangosteen thioesterase, coupled with a concomitant down-regulation of the soybean FAD2-1 desaturase, led to the production of oil high in saturated fatty acids (app. 25%), elevated oleic acid (app. 60%), and a reduction in polyunsaturated fatty acids (< 10%). This novel soybean oil displays an oxidative stability index between 26 to 30 hrs and a melting point between 1.5°C to 3.8°C. These parameters ascertained on conventional soybean oil are approximately 6.5 hrs and -30°C, for oxidative stability index and melting point temperature, respectively.
P011
Development of a Soybean-Based Feedstock for Aquaculture

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Soybean (Glycine max (L.) Merr.) is sought after for both its oil and protein components. Genetic approaches to add value to either component are ongoing efforts in soybean breeding and molecular biology programs. The former is the primary vegetable oil consumed in the world. Hence, its primary usage is in direct human consumption. As a means to increase its utility in feed applications, thereby expanding the market of soybean co-products, feeding trials were conducted designed to simultaneously displace of marine ingredients in aquafeeds with soybean-based protein and a high omega-3 fatty acid soybean oil, enriched with alpha-linolenic and stearidonic acids, in both steelhead trout (Oncorhynchus mykiss) and Kampachi (Seriola rivoliana). Aquafeed formulations with major reduction in marine ingredients, that incorporated high inclusion levels of soybean ingredients, resulted in more total omega-3 fatty acids in harvested flesh of the evaluated fish species. Building off of these findings a genetic approach was pursued to design a prototype for a optimal identity preserved soybean-based feedstock for aquaculture, whereby a multi-gene stack strategy for the targeted synthesis of two value-added output traits, eicosapentaenoic acid (EPA) and the ketocarotenoid, astaxanthin, were introduced into the crop. To this end the systematic introduction of nine transgenic cassettes into soybean has been accomplished that translated to the production of these two high value aquafeed ingredients in seed.

P012
Impact of Growing Location in the Biomass Composition of Soybean (Glycine max L.) Seeds

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The market value and use of soybean seeds depends upon their biomass composition (content in proteins, oils, and carbohydrates). Factors including, but not limited to, genetics, fertilization, climatic conditions, and disease pressure, can modify biomass composition and consequently impact soybean price. Research challenges for improving soybean seeds currently aim at increasing the protein and oil content as well as modifying the composition in essential proteinogenic amino acid and fatty acids (FAs). The goal of this study was to quantify biochemical traits that are particularly important for the market value of soybean, specifically we determined: i) the levels of proteins and oils, ii) the content in proteinogenic amino acids by liquid chromatography tandem mass spectrometry, and iii) the FA composition by gas chromatography-mass spectrometry. These traits were measured in ten diverse soybean cultivars grown in four different locations in Ohio. The results highlighted that the growing site had an impact on the seed total dry weight and/or composition for the ten soybean cultivars under investigation, which can influence their market application and value. Specifically, we found that: i) the location had an influence on the levels of the valuable oleic acid (reaching up to 43.9% of total FAs instead of the common 23%); ii) there was an inverse correlation between the contents of proteins and essential amino acids; ii) the FA levels did not negatively correlated with protein content. Taken together, these results are expected to guide further breeding and/or metabolic engineering effort aiming to improve soybean composition in Ohio.
P013
An Induced Chromosomal Translocation in Soybean Disrupts a KAS 1 Ortholog and is Associated with a High Sucrose and Low Oil Seed Phenotype

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Mutagenesis is a useful tool in many crop species to create heritable genetic variability for trait improvement and gene discovery. In this study, a soybean Fast-Neutron (FN) mutant line was identified that has twice the amount of sucrose (≈8% on dry matter basis) and half the amount of oil (≈10% on dry matter basis) compared to wild-type. Bulked Segregant Analysis, comparative genomic hybridization, and genome resequencing were used to associate the seed composition phenotype with a reciprocal translocation between chromosome 8 and 13. This association was further validated in a backcross population through the development and use of translocation specific PCR primers. We hypothesize the translocation is responsible for the altered seed composition by disrupting a β-ketoacyl-[acyl carrier protein] synthase 1 (KAS 1) ortholog. KAS 1 is a core fatty acid synthesis enzyme which is involved in the conversion of sucrose into oil in developing seeds. To date, there have not been any characterized mutants of this gene in soybean. This novel finding may lead to new research directions for developing soybean cultivars with modified carbohydrate and oil seed composition.

P014
TILLING for Improved Soybean Feed for Use in Aquaculture

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The use of soy feeds in aquaculture remains limited due to the presence of anti-nutritional factors (ANF) including: undigested oligosaccharides raffinose and stachyose, lectins that damage the fish intestinal lining, and phytate that binds to and prevents the uptake of proteins and essential cations. This is particularly prevalent for high-value carnivorous species such as salmon, char and trout where feed rations can contain as little as 8% soy meal. Our final goal is to select and combine soybean mutant lines for sufficiently reduced ANFs to support increased feed conversion ratios (FCAs) and allow for greater soybean meal inclusion in feeding rations.

Arcadia possesses a robust soybean TILLING library, with mutation frequency of approximately 1/100Kb of DNA. This frequency creates enough genetic diversity to guarantee ~10 mutations/Kb of DNA, and thereby the potential to evaluate and select a trait from multiple mutants. We will present the status of our progress in the selecting of ANF mutants and their phenotype.

P015
A Profile of Amino Acid Root Exudates of Soybean

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An important part of the belowground portion of plants are the compounds that are exuded from plant roots. Together the roots and the area of the soil under the influence of these compounds make up the rhizosphere. Root exudates allow roots to interact with the soil as well as communicate with other plants and microorganisms. Plants are known to exude a number of different types of compounds including carbohydrates, lipids, phenylpropanoids, amino acids and many other compounds. Amino acids in root exudates represent an important fraction of plant root exudates, as they are a nitrogen source for microorganisms and can serve as chemotactic agents for others. Amino acid root exudates have been characterized in model systems under lab conditions, but remain largely unknown in important crops such as soybean (Glycine max). In this study we sought to identify and quantify the amino acids that are exuded by soybean roots in a soil environment during the first 14 days of growth. We grew sterile Glycine max cv. Lee seed for 14 days in sterile sand supplemented with 1/4 strength MS media. After 14 days the sand solution was washed and filter sterilized. Amino acid contents of were measured using LC MS/MS. Our preliminary data indicates that the amino acid root exudates of soybean varied with different soil conditions, light conditions, temperature conditions and stresses. Further testing is required to ensure optimal growing conditions for the soybean as well as the consistency of the results.
P016

Genetics of High-Stearic Acid Soybeans

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Stearic acid is a saturated fatty acid comprising about 4% of the fatty acid content of soybean (Glycine max) seeds. While some saturated fats have been shown to have a negative impact on human serum cholesterol levels, stearic acid does not. Soybeans with genetically elevated levels of stearic acid could be used to fill a need for solid fats without the need for hydrogenation. Mutations in the SACPD-C gene are known to increase stearic acid levels to ~14%, but these mutations must be combined with others to reduce linolenic acid levels for oil stability. We are also working to identify genes in addition to the SACPD genes that regulate stearic acid levels.

We have made genetic combinations of FAD2 x SACPD-C and FAD3 x SACPD-C. FAD3 - SACPD-C double mutants show both elevated stearic and decreased linolenic acid levels comparable to single mutant siblings, while FAD2 x SACPD-C show the expected elevated stearic levels, and mixed results for oleic acid levels. Other combinations that we have developed have shown mostly expected results. These promising results suggest that a goal of 20% stearic may be reached with further additive mutation combinations. Furthermore, it shows that the elevated stearic acid phenotype can be coupled with other economically useful oil phenotypes.

P017

Functional Validation of Soybean Seed-Related and Fatty-Acid Biosynthesis-Related Promoters through Transient and Stable GFP Expression

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Functional characterization of soybean promoters and their cis-regulatory elements is crucial for understanding gene regulation, as well as for the development and application of engineered soybean with specific, tunable gene expression. In this study, 15 promoter sequences were isolated from soybean seed-related genes (GmSeed), and 9 promoter sequences were isolated from fatty-acid biosynthesis pathway-related genes (GmFAB). Each promoter sequence was inserted upstream of the gfp reporter gene and the NOS terminator. The promoter constructs were assessed via transient expression in lima bean cotyledons, and stable expression in soybean hairy roots. In comparison to the CaMV35S promoter, most of the soybean promoters displayed weak but detectable activity, based on both transient and stable GFP expression. GmSEED10, GmFAB11, and GmFAB17 showed comparable or stronger expression than the CaMV35S promoter. In general, promoters that gave high transient expression with bombarded lima bean cotyledons also showed medium to high expression in stably-transformed soybean hairy roots, while those with low transient expression had relatively low expression in hairy roots. Based on the assessment using these rapid validation tools, constructs containing these same promoter::gfp::NOS terminator have been selected for production of transgenic soybean plants. Taken together, the present data confirmed functional promoter activity of these 24 promoters, expanding the toolbox of native soybean promoters.
P018  
**Growth Speed of Soybean Sprout and Expression Quantity of Genes Relating to Glyoxylate Cycle**

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Growth speed of soybean sprout is definitely different by genotypes. Oil seeds metabolize stored triacylglycerols by converting them into sucrose after germinating and these process contains β-oxidation and Glyoxylate cycle. Especially, Isocitrate lyase (ICL) and malate synthase (MS) are mainly required for those path ways. Thus, much expression of relating genes suggests much sucrose for growth energy. Four varieties of soybean seeds, ‘Pungsannamul’, ‘Wonheug’, ‘Neulchan’ and ‘Danbaeg’ were cultivated for five days in dark chamber with 23˚C watering (3min/4h). Each varieties in each day were estimated for whole length of sprouts and cotyledons were collected for quantifying gene expression of ICL, MS and Actin by Real-Time PCR. Sprout length of ‘Wonheug’ was longer than another varieties in each day and 22.9 cm in 5 day. Sprout length of ‘Pungsannamul’, ‘Neulchan’ and ‘Danbaeg’ were 13.3cm, 5.6cm and 14.8cm, respectively. Conversion value of expression quantity was from 0.96 to 1.21 in ICL gene and from 0.78 to 1.07 in MS gene. Expression of ICL increased from 1 day to 3 day but decreased from 4 day. Expression of MS was higher in 1 day and almost flat in from 2 day. ICL expression of ‘Wonheug’ from 1-5 day were 1.10, 1.20, 1.21, 1.15 and 1.00, respectively and significant higher in 3 day and 4 day compared to another varieties. Expression quantity of ICL gene was significantly and positively correlated with sprout length (r=0.86, p<0.01). This study showed that growth speed of soybean sprout was related to level of expression of ICL gene in seed.

P019  
**QTL Mapping for Glycinin and β-Conglycinin Contents in Soybean (Glycine max L. Merr.)**

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Compared to β-conglycinin, glycinin contains three to four times the methionine and cysteine (sulfur-containing amino acids), accounting for approximately 40% and 30%, respectively, of the total storage protein in soybean. Increasing the soybean storage protein content while improving the ratio of glycinin to β-conglycinin is of great significance for soybean breeding and soy food products. The objective of this study is to analyze the genetic mechanism regulating the glycinin and β-conglycinin contents of soybean by using a recombinant inbred line (RIL) population derived from a cross between Kefeng No.1 and Nannong 1138-2. 221 markers were used to map quantitative trait loci (QTLs) for glycinin (11S) and β-conglycinin (7S) contents, the ratio of glycinin to β-conglycinin (RGC) and the sum of glycinin and β-conglycinin (SGC). A total of 35 QTLs, 3 pairs of epistatic QTLs and 5 major regions encompassing multiple QTLs were detected. Genes encoding the subunits of β-conglycinin were localized to marker intervals sat_418-satt650 and sat_196-sat_303, which are linked to RGC and SGC; marker sat_318, associated with 11S, 7S and SGC, was located near Glyma10g04280 (Gy4), which encodes a subunit of glycinin. These results, which take epistatic interactions into account, will improve our understanding of the genetic basis of 11S and 7S contents and will lay a foundation for marker-assisted selection (MAS) breeding of soybean and improving the quality of soybean products.
**P020**

**Transcriptome Profiling of the Interaction Soybean-Phytophthora sojae as Mitigated by Silicon Amendments**

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Silicon (Si), as a soluble amendment, is reputed to offer protection to plants against many biotic and abiotic stresses, and in particular against biotrophic fungi. Soybean production is greatly affected by Phytophthora root and stem rot caused by Phytophthora sojae, a hemibiotrophic pathogen. The objectives of this study were 1) to augment soybean resistance to P. sojae by exploiting its natural ability to absorb Si; and 2) to investigate the mechanisms by which Si could confer protection. We tested the phenotypic response of soybean to P. sojae under Si+/Si- treatment. At 21 dpi, plants under Si+ treatment had a significantly higher survival rate and higher dry weight than control plants. In an attempt to understand the mechanisms inherent to Si prophylactic properties, samples of the soybean-P. sojae interactions in Si+/Si- treatment have been analyzed over different time points by RNA-Seq. Initial results indicate that defense responses in plants under Si treatment are elicited more rapidly and with greater intensity. Current analyses are also looking into the role of Si in the release of virulence factors by P. sojae.

**P021**

**Elevated Protein and Oleate Content in β-Carotene Enhanced Soybean Seeds**

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Transgenic soybean (Glycine max) plants overexpressing a seed-specific chloroplast targeted phytoene synthase gene from Pantoea ananatis accumulated 845 μg carotene g-1 dry seed weight with a 12:1 ratio of β to α. The β-carotene accumulating seeds exhibited a shift in oil composition increasing oleic acid with a concomitant decrease in linoleic acid. Specifically, the crtB transgenic seeds contained 45% oleic acid compared to 24% in the wildtype. Elevated oleic acid has been a target trait in soybean industry due to both its performance and health benefits. In addition to oil composition change, an increase in seed protein content by up 8% (w/w) was also observed in the crtB transgenics. The β-carotene accumulating seeds develop and germinate with the same timing and efficiency as nontransgenics. This is the first report of two desired collateral traits, high oleate and high protein, achieved by the expedient of increasing β-carotene content. Proteomic, transcriptomic and metabolomic analysis of the mid-maturation β-carotene cotyledons compared to the nontransgenic did not reveal any significant differences that would account for the altered phenotypes of high oleate and high protein content. Altered phytohoromone ABA levels were determined to be varied in the enhanced β-carotene seeds. The elevated β-carotene, oleic acid, and protein traits in these transgenic soybeans confer a substantial additive nutritional quality to soybeans with a single gene insertion.

**P022**

**Characterization of Seed Storage Proteins of Several Perennial Soybean Species**

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Perennial Glycine species, distant relatives of soybean, have been recognized as a potential source of new genetic diversity for soybean improvement. The subgenus Glycine includes around 30 perennial species, some of which are well adapted to drought conditions and possess resistance to a number of soybean pathogens. In spite of the potential of the various perennial Glycine species for soybean improvement, we know very little about their storage proteins and their relationship with cultivated soybean seed proteins. We have examined the seed protein composition of nine perennial Glycine species by SDS-PAGE and 2-D gel electrophoresis. The relationship between cultivated soybean and perennial soybean seed proteins were examined by immunoblot analyses using antibodies raised against several soybean seed proteins. Additionally, we have measured the trypsin and chymotrypsin inhibitor activities from cultivated soybean and perennial Glycine species, and have found marked differences between them. Our 2-D gel and immunoblot analyses demonstrate significant differences in the protein composition and size heterogeneities of the 7S and 11S seed storage proteins of soybean and perennial Glycine species.
P023  
**Identification and Characterization of Fast Neutron Mutants with Altered Seed Traits**

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The soybean genome is quite large with over 70% of the genes duplicated (1). However, the gene duplicates, in general, exhibit subfunctionalization suggesting that phenotypically altered mutants can be isolated (2). In order to develop a resource to investigate soybean gene function, we generated a mutant population using fast neutron radiation. Fast neutron irradiation induces genomic deletions which can be easily defined using comparative genome hybridization (CGH) (3,4). Phenotypic screens for seed traits identified a variety of seed composition mutants, including those affected in total oil, protein, sucrose, stacyose and phytate content, as well as variations in specific fatty acids. Thus far, eight of the 11 increased seed stearate mutants were found to have deletions in SACPD-C, which encodes a Δ9 - stearoyl - ACP desaturase involved in the desaturation of stearate precursors to oleate precursors. We also identified seed trait mutants with deletions in key genes involved in transcription regulation, fatty acid biosynthesis and storage protein production. Co-segregation analyses to identify causative deletions, and eventually causative genes, for the various seed trait phenotypes are underway.


P025  
**Use of a Multi-Trait Mixed Model for Genome-Wide Association Study of Protein and Oil Content in Soybean Seed**

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The fatty acid (FA) profile is important in determining the quality and uses of soybean oil. Understanding the genetic control of FAs will enhance our capacity to breed for improved soybean oil quality. Genome-wide association study (GWAS) is a powerful tool for investigating the genetic architecture of complex quantitative traits. In this study, 621 soybean accessions were selected based on maturity groups (II-IV), lodging score, seed color, pod shattering and other agronomic traits, and availability of the single nucleotide polymorphism (SNP) haplotype data. These soybean accessions were grown in four locations, in Columbus and Wooster Ohio, in Plymouth North Carolina, and in Urbana Illinois. Measurements of FA contents of all samples by gas chromatogram (GC) showed palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid ranged between 8 - 15%, 2 - 8%, 14 - 53%, 29 - 63%, and 3 - 14%, respectively. Over 30K polymorphic SNP markers from the SoySNP50K iSelect BeadChip (http://www.soybase.org) were utilized for quantitative trait loci (QTL) discovery. Best linear unbiased predictors (BLUP) values were calculated across environments and BLUP values were used for identification of QTL for these five FAs. Analyses on phenotypic variation in FAs and correlation among traits, and association analysis are in progress.
**P026**

**Identification of Quantitative Trait Loci Associated with Seed Isoflavone Concentration in Soybeans Grown in Soybean Cyst Nematode**

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Soybean (Glycine max [L.] Merr.) is one of the major isoflavone producing members of the Fabaceae family. Isoflavones function as phytoalexins in protecting plants against pathogens and as phytoestrogens in the human diet that have putative positive human health effects such as reduced risk of breast and prostate cancers, cardiovascular disease, and high blood cholesterol levels. Seed isoflavone concentration is a quantitative trait that is influenced by genetic and environmental factors. An increasing problem for soybean growers in most of the USA and in Ontario, Canada, is the presence of soybean cyst nematode (SCN; Heterodera glycines), the greatest yield-limiting pathogen in soybean, which is best managed by growing SCN resistant cultivars. The objective of this study is to identify quantitative trait loci (QTL) involved in controlling seed isoflavones in SCN infested and non-infested soil types. A segregating population of 111 F4:7 recombinant inbred lines (RILs), derived from a cross between DH4202 (moderately high isoflavone and SCN susceptible) and RCAT 1004 (high isoflavone and SCN resistant) was grown in two SCN infested and two non-infested fields in southern Ontario during the summer of 2015. Seed isoflavone levels were determined using near infrared spectroscopy. The RIL population was genotyped using genotyping by sequencing. The results of this study can lead to the identification of QTL associated with isoflavone accumulation as well as potentially SCN resistance, which will aid soybean breeders to expedite the development of SCN-resistant soybean cultivars with elevated seed isoflavones using marker-assisted selection.

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**P027**

**Analysis of Small RNA-Mediated Regulation of Soybean Cyst Nematode Resistance Genes in Soybeans**

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Small RNAs are key players in transcriptional and post-transcriptional regulation of gene expression. The roles of small RNAs are involved in plant development, stress response and disease resistance. A major soybean disease is the infection of soybean roots by soybean cyst nematode (SCN). SCN damages root systems and leads to a severe reduction in soybean yield. In terms of genomic architecture and structural variation, the copy number variation of three genes at Rhg1 locus is known to be associated with SCN resistance in soybeans. To explore the molecular mechanisms of SCN resistance with respect to functional genomics, this study performed the differential expression analysis of small RNAs in one SCN-susceptible soybean (Williams 82) and two SCN-resistant lines (Peking and PI 88788). Well-documented miRNA families such as miR390, miR395 and miR3508 were predicted to be differentially expressed among the three soybean lines. These miRNAs are related to root development, nutrient deprivation and stress responses. For small interfering RNA (siRNA) quantification in genic regions, GO term analysis revealed the set of genes involved in signal transduction was over-represented in differentially-expressed siRNA producing genes. At the Rhg1 locus, siRNAs in resistant soybeans were expressed at significantly higher levels than in susceptible soybean. Thus, small RNA regulation of gene expression may be affecting Rhg1-mediated SCN resistance mechanisms.
Enhancing Carbon Capture and Flux in Soybean

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A genetic approach targeting the development of a set of transgene stacks in both normal leaf and lanceolate leaf genetic backgrounds of soybean is being pursued. The desired outcome of this strategy is to alter carbon capture during vegetative stages and flux during reproduction stages in the crop, leading towards increase in total oil content. The rationale for pursuing creation of a multi-transgene stack designed to boost oil content in seed within a lanceolate leaf soybean, is that light penetrance through the canopy has an influence on protein/oil ratio of seed. Whereby, in conventional leaf morphology, pods towards the top of the canopy will possess seeds with higher protein to oil ratio, relative to those that develop in lower portion of the canopy. In a lanceolate leaf soybean genotype, the total protein/oil ratio in seed will be more uniform across canopy level. The selected transgenes designed to pull carbon towards oil during embryogenesis include a “master” regulator of lipid biosynthesis in Arabidopsis designated wrinkled-1 (Wri 1), two diacylglycerol acyltransferase genes, one from Arabidopsis (AtDGAT1) and the other from a diatom (Phaeodactylum tricornutum) (PtDGAT1), along with Arabidopsis ketoacyl-ACP synthase II (AtKAS II). While the transgenes designed to enhance carbon capture include a putative inorganic carbon transporter B (ictB) from Synechococcus sp and sedoheptolose-1,7-bisphosphatase (SBPase) from tomato. A two pronged approach to create these gene stacks is being pursued. First, crosses with the lead gene stack combination for carbon pull during reproduction stages (i.e. AtWri I, DGAT1(s), AtKasII), with transgenic events that carry the two-gene combination, shown to enhance photosynthesis, is ongoing. Secondly, depending on the gene stack combination, that translates to largest gains in carbon pull to oil and carbon capture via photosynthesis ascertained from the stacks created via crossing, a single T-DNA will be assembled and the optimal gene stack as a single introduced into soybean as a single transgenic allele.

Transcript Responses in Leaves to Very Early Infection by Sclerotinia sclerotiorum

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Transgenic soybean carrying an oxalate oxidase gene (OxO) that encodes for an enzyme catalyzing the degradation of oxalic acid (OA), shows enhanced resistance to Sclerotinia stem rot. This study applied RNA-Seq on soybean leaf tissue, 4 and 8 hours after a Sclerotinia sclerotiorum infected flower bud was placed on its surface, for both an OxO transgenic soybean line and its susceptible parental line AC-Calibri. By utilizing an OxO transgenic, we hoped to ascertain gene expression differences that might be related to the plant response to OA, from all other responses, as the OxO line, in theory, would remove the OA once it entered the apoplastic spaces (the OxO protein has an apoplastic secretion peptide signal). A 150nt paired-end RNA sequencing experiment was performed on 24 libraries, all treatments in triplicate. de novo assembly of the reads and a generalized linear model statistical analysis determined that 876 soybean genes were significantly differentially expressed in at least one pairwise comparison (FDR < 0.02). Gene expression patterns indicated that gene expression patterns did not differ much between the two genotypes. Genes induced commonly in both genotypes participated extensively in soybean basal defense, such as ethylene signaling pathways, WRKY-mediated transcription regulations and the phenylpropanoid pathways. Although most genes exhibited similar expression patterns between the two soybean genotypes, some groups were found to have higher fold changes at 4 hpi in OxO than in AC, including calcium signaling-related genes, ABC transporters and cell wall-based defense, suggesting potential roles in contributing to greater resistance of OxO.
P030

Isolation and Characterization of Four Soybean Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Promoters

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Promoters and contributing promoter elements are responsible for directing transcription according to tissue type, developmental stage, or various external stimuli. Identification of novel promoters and promoter elements expands the available toolbox for synthetic promoter design, and contributes to increasing our current understanding of transcriptional regulation mechanisms. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the primary enzyme involved in fixation of atmospheric carbon, consisting of eight large and eight small-subunits. The promoter regions of these nuclear encoded small-subunit genes are of interest because of the great importance of RuBisCO, and their induction by light. Using RNAseq analysis, four RuBisCO small-subunit promoters (rbcS 1-4) conferring high levels of expression in soybean tissues were identified. While rbcS1-3 showed high transcript levels in green tissues, rbcS4 expressed only in the root and nodule. Promoter regions were amplified and cloned into an expression vector directly upstream of the green fluorescent protein (gfp) coding region. Constructs were introduced into lima bean cotyledons via particle bombardment and GFP expression was monitored for 100 hours using an automated image collection system. Transient expression of all 4 rbcS promoters showed expression that was higher or comparable to the cauliflower mosaic virus 35S promoter. Stably transformed soybean hairy roots were generated for each promoter construct. These promoters seem to be light-inducible in soybean hairy roots, and light induction appears to be mediated through phytochrome. Light inducible promoters may be useful for mediating rhythmic transgene expression in the above ground portions of the soybean plant.

P031

The mPing Mutagenesis Project: Mutant Analysis and Activation Tagging

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The goal of our NSF funded soybean mutagenesis project is to develop resources for soybean gene discovery (soymutants.uga.edu). These resources are important for understanding basic plant biology, but may also reveal genes important for soybean yield and quality. Transposable elements are mobile DNA segments referred to as “jumping genes” because they can move from one location in the genome to another, and make up a large proportion of plant genomes. This movement can cause gene disruptions and phenotypic changes in plants. New mutagenesis systems have been developed for soybean including one utilizing mPing, a miniature inverted repeat transposable element from rice. In the summer of 2016, soybean mutants discovered in the mPing mutagenesis population were analyzed in the field (Athens, GA). This included genetic and phenotypic measurements of phenotypes with altered architecture, color, and developmental patterns. In addition to characterization of the mutants, mPing-based activation tagging is being developed to optimize this mutagenesis system. This involves insertion of promoter sequences into mPing, which can induce transcriptional activation of nearby genes, and reveal their function even if they are redundant or required. Previous research indicated that the addition of an enhancer sequence from a strong promoter reduced mPing’s transposition. To address this, a hyperactive version of mPing called mmPing20 was used to increase the transposition rate. Two activation tags using enhancer sequences from the promoter regions of the Figwort Mosaic virus and soybean β-conglycinin were constructed. To determine the efficacy of mmPing20-based activation tagging, the transposition of these two elements, mmPing20F and mmPing20B respectively were analyzed in yeast and prepared for analysis in plants.
P032
Identification of miRNAs and Their Targets in Cytoplasmic Male-Sterile Line NJCMS1A and its Maintainer NJCMS1B of Soybean

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Cytoplasmic male sterility (CMS) provides crucial breeding materials that facilitate hybrid seed production in various crops, and thus plays an important role in the study of hybrid vigor (heterosis), in plants. However, the CMS regulatory network in soybean remains unclear. MicroRNAs (miRNAs) play crucial roles in flower and pollen development by targeting genes that regulate their expression in plants. To identify the miRNAs and their targets that exist in the soybean CMS line NJCMS1A and its maintainer NJCMS1B, two small RNA libraries were constructed from the flower buds. A total of 105 new miRNAs present on the other arm of known pre-miRNAs, 23 new miRNA members, 158 novel miRNAs and 160 high-confidence soybean miRNAs were identified using high-throughput sequencing. Among the identified miRNAs, 101 differentially expressed miRNAs with greater than two-fold changes between NJCMS1A and NJCMS1B were discovered. The different expression levels of selected miRNAs were confirmed by stem-loop quantitative real-time PCR. A degradome analysis showed that 856 targets were predicted to be targeted by 296 miRNAs, including a squamosa promoter-binding proteinlike transcription factor family protein, a pentatricopeptide repeat-containing protein, and an auxin response factor, which were previously shown to be involved in floral organ or anther development in plants. Additionally, some targets, including a MADS-box transcription factor, NADP-dependent isocitrate dehydrogenase and NADH-ubiquinone oxidoreductase 24 kDa subunit, were identified, and they may have some relationship with the programmed cell death, reactive oxygen species accumulation and energy deficiencies, which might lead to soybean male sterility.

P033
Efficient Targeted Mutagenesis in Soybean by TALENs and CRISPR/Cas9

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Gene targeting (GT) is of great significance for advancing basic plant research and crop improvement. Both TALENs (transcription activator-like effectors nucleases) and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated 9) systems have been developed for genome editing in eukaryotes, including crop plants. In this work, we present the comparative analysis of these two technologies for two soybean genome editing targets, GmPDS11 and GmPDS18. We found GT in soybean hairy roots with a single targeting efficiency range of 17.5% to 21.1% by TALENs, 11.7% to 18.1% by CRISPR/Cas9 using the AtU6-26 promoter, and 43.4% to 48.1% by CRISPR/Cas9 using the GmU6-16g-1 promoter, suggesting that the CRISPR/Cas9 using the GmU6-16g-1 promoter is probably a much more efficient tool compared to the other technologies. Similarly, our double mutation GT efficiency experiment with these three technologies displayed a targeting efficiency of 6.25% by TALENs, 12.5% by CRISPR/Cas9 using the AtU6-26 promoter, and 43.4% to 48.1% by CRISPR/Cas9 using the GmU6-16g-1 promoter, suggesting that CRISPR/Cas9 is still a better choice for simultaneous editing of multiple homoeoalleles. Furthermore, we observed albino and dwarf buds (PDS knock-out) by soybean transformation in cotyledon nodes. Our results demonstrated that both TALENs and CRISPR/Cas9 systems are powerful tools for soybean genome editing.
Apple Latent Spherical Virus: An Emerging VIGS Construct for Functional Analysis in Soybean Genomics

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Virus induced gene silencing (VIGS) can be an efficient tool for functional analysis of candidate genes in plant genomic studies. Many VIGS constructs have been identified and successfully utilized in soybean functional gene analysis. Unfortunately, many of the current VIGS approaches for soybean have shown low efficiency of virus vector delivery, lack of gene silencing in certain tissues, poor stability of gene silencing, and the interference of phenotype determination due to meddling of viral symptoms. The objective of this study was to modify and evaluate a VIGS construct that can efficiently and stably silence genes throughout different tissues of the plant, specifically seed, without the interference of viral symptoms. Apple Latent Spherical Virus (ALSV) has previously been shown to silence genes in soybean at the seed level and appears to have great potential due to the efficiency of infection. We examined the efficiency of three different ALSV VIGS constructs, along with three different methods of delivery into soybean plant. We also observed the resistance or susceptibility of 20 different soybean cultivars to the virus and the ability of the virus to silence the phytoene desaturase (soyPDS) gene. We identified one construct that had 100% efficiency of VIGS using a rub inoculation method. Eight of the 20 cultivars had symptoms of PDS silencing at 20% or more efficiency. This approach has strong applications for the use of functional genomics approached for discovery of candidate resistance genes for Fusarium graminearum and other pathogens in soybean.

Pedigree-Based Seed Transcriptome Analysis of Landraces and Cultivars Representing 90 Years of North American Soybean Breeding

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Crop improvement through breeding has been shown to reshape crop genomes as the selection process in breeding results in the enrichment of beneficial alleles and their adjacent chromosomal regions. To identify those beneficial alleles, we characterized the genome and transcriptome-wide genetic and molecular changes that are associated with the enrichment process in soybean breeding populations. Using RNA-seq, we sequenced transcriptomes of mid-maturation seeds from 75 soybean genotypes that represent 90 years of public soybean breeding in North America. We identified approximately 90,000 SNPs and 31,000 expressed genes, which account for about 54% of all annotated genes. Our eSNP analysis revealed 4248 SNPs in 873 genes that are associated with and some possibly causative of expression changes. We integrated our sequence and expression data with a pedigree-based analysis of soybean lineages starting from ancestral landraces to commercially extremely successful varieties (milestone cultivars). We identified SNPs that are enriched and possibly selected for in all founder cultivar lineages. Our identity-by-decent analysis enabled us to trace the transmission of enriched SNPs through soybean lineages and to associate them with traits of interest. For example, we identified SNPs in the CNS lineage that are completely associated with resistance to the bacterial leaf pustule disease caused by Xanthomonas axonopodis pv. glycines. We also identified enriched SNPs that are correlated with expression variation. In conclusion, the pedigree-based analysis of enriched SNPs facilitates the association with gene expression changes and traits of interest that breeders have selected for in North American soybean breeding.
Expression of ictB and SBPase Transgenes to Enhance Photosynthetic Capacity in Soybean

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Enhancing photosynthetic capacity of crops is an avenue that may translate to improved yields. To this end we explored the expression of a putative inorganic carbon transporter B (ictB), from cyanobacteria, stacked with expression of the tomato sedoheptolose-1,7-bisphosphate (SBPase). We assembled two genetic constructs designated pPTN1284 and pPTN1285 carrying a soybean codon optimized versions of ictB and SBPase, under control of the 35S CaMV and pea Rbcs promoters, respectively. The former vector has the ictB transgene fused with the pea small subunit of Rubisco transit peptide (TP) to target protein to the plastid, while the latter ictB cassette in the latter plasmid is devoid of the transit peptide. In addition we assembled three additional vectors that harbor each of the respective transgenic cassettes separately. These are designated pPTN1274, pPTN1282, and pPTN1149, which carry the TP-ictB, ictB and SBPase cassettes, respectively. Data will be presented on the molecular and phenotypic analyses on the transgenic soybean events carrying these respective transgenic alleles characterized under greenhouse and field conditions.

Gene Expression Atlas: Co-Expression and Gene Regulatory Networks for the Identification of Key Soybean Traits

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Soybean is the 2nd largest crop in the USA and a very important food and feed source for human and animal health due to its high protein and oil content. In addition, soybean has the advantage that it does not require nitrogen fertilizer due to its symbiotic, nitrogen fixing relationship with rhizobial soil bacteria (Bradyrhizobium japonicum). The large size of the soybean genome, encoding for over 55,000 genes, create a formidable challenge for elucidation of gene function, especially those genes important for agronomic performance. In order to address this challenge, we developed an extended gene expression atlas for soybean, utilizing RNA-seq performed by the DOE Joint Genome Institute. Our effort was part of a larger project to develop similar transcriptome atlases for a number of so-called ‘flagship species’. We sequenced more than 100 libraries from various soybean tissues, treatment and conditions. Analysis of these data identified thousands of genes whose expression varied significantly between tissues and treatments. Further analysis of these data allowed us to identify genes that appear to be specifically expressed in different tissues. Co-expression, gene-regulatory network and metabolic pathway analysis of the data revealed a coordinated transcriptional control network related to such functions as seed development, nitrogen fixation, nutrient uptake and utilization. Furthermore, association of the fast-neutron mutants with these transcriptional regulators should allow us to analyze the functional role of these key genes with the ultimate goal of using this information for soybean improvement.
P038
A CRISPR Toolkit for Soybean

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With 6,500 publications last year alone, CRISPR technologies are rapidly being adopted across biology, changing the way questions of genetics are approached. CRISPR-mediated genome editing is the newest and cheapest way to create targeted gene knockouts or introductions that are valuable to gene-validation research and plant breeding. The technique utilizes endonuclease proteins like Cas9, guided by hair-pinned RNA sequences specific to targeted genomic regions. Four different endonuclease proteins are being adapted in an effort to expand CRISPR resources in dicots. These are the Cas9 genes from Streptococcus pyogenes, Staphylococcus aureus, and Streptococcus thermophilus, and the Cpf1 gene from Acidaminococcus spp. The S. pyogenes Cas9 has been the more widely used one. However, S. aureus Cas9 is the shortest Cas9 gene yet identified, and hence, is easier to use. Cpf1 from Acidaminococcus uses a significantly shorter RNA sequence. S. thermophilus is probiotic bacteria. Each of these endonucleases target different potential sequences in the genome, so having an assortment of endonucleases like Cas9 and Cpf1 provides flexibility when choosing targets for genome editing. This diversity of targeting is demonstrated in silico on a model of the phytoene desaturase gene in soy. The Cas9 and Cpf1 genes have been cloned into an expression cassette made of plant-derived sequences, which includes a nuclear localization signal from the E1 flowering gene from Glycine max. The editing efficiency for each endonuclease can be quickly assayed using soybean hairy roots.

P039
Progress on CRISPR/Cas9 Genome Editing in Soybean

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The utility of the CRISPR/Cas9 system for genome editing in both model and crop species has been widely explored in recent years. Optimization of different components in the CRISPR/Cas9 toolkit has proved to be essential for increasing mutagenesis efficiency, especially in crop species recalcitrant to genetic transformation such as soybean. Rapid screening of targets in transgenic soybean hairy roots can facilitate the optimization of CRISPR/Cas9 reagents used for genome editing. However, generating heritable mutations using whole plant transformation may require different backbone binary T-DNA vectors, promoters, and codon-optimized Cas9 nucleases. Our current mutagenesis efforts have achieved new mutations in a variety of endogenous genes for both hairy roots and whole plants derived from cotyledonary node transformation. In addition to gene knockouts, homologous recombination-mediated gene targeting and/or editing events were also detected in a high-throughput hairy root system. Finally, because editing of endogenous genes for subtle modifications such as SNP changes is preferred for some crop improvement strategies, we are also investigating how to further improve the efficacy of gene editing reagents for soybean.

P040
Identification of Quantitative Trait Loci Associated with Soyasaponin B Accumulation in Soybean Seeds

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Soybean (Glycine max (L.) Merr.) seed contains diverse forms of secondary plant metabolites called soyasaponoins. Soyasaponins are comprised of several groups, including the soyasaponin B forms. Soyasaponin B has putative health benefits and is of interest for its functional food properties. Soyasaponin I is the major soyasaponin B form derived from soybean seeds. Understanding the genetic control of soyasaponin accumulation is the key to developing Ontario-adapted soybean cultivars with improved soyasaponin profiles. The objective of this study was to determine the association of quantitative trait loci (QTL) with the concentration of soyasaponin I derived from soybean seed. A genetic mapping population consisting of 186 F3:7 recombinant inbred lines (RIL) of soyasaponin I was derived from the cross of OAC Wallace and OAC Glencoe was planted at two Southern Ontario locations in 2015. The concentration of soyasaponin I from seed produced in the 2015 field trials was determined using high-performance liquid chromatography (HPLC). The results demonstrated that the soyasaponin I concentration followed a normal distribution in the mapping population as would be expected for a quantitative trait. The minimum, maximum and average values of soyasaponin I were 2.92, 5.56, and 4.22 μmol g⁻¹, respectively. DNA was extracted from the mapping population and used in genotyping by sequencing (GBS). A linkage map and QTL analysis will be conducted in July, 2016. Identification of genetic markers associated with QTL for soyasaponin I can advance the development of Ontario-adapted soybean cultivars with desirable soyasaponin profiles for additional health benefits.
P041
Strategies for Generating Germinal mPing Insertions in Soybean
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A transposon-tagging resource has been developed to systematically mutate soybean genes allowing for the discovery of novel genes and mutant phenotypes. While the rice transposon, mPing, transposes when transformed into soybean along with the associated ORF1 and transposase genes, its germinal transposition rate is relatively low. Two complementary strategies are being used to increase the rate of germ line, and thus heritable, transposition. First, we are redesigning our vectors so that they use a native promoter that is strongly expressed in the meristem, such as the soybean ubiquitin promoter. The usage of an ubiquitin promoter to drive the expression of ORF1 and transposase ought to result in a high germinal transposition rate, which enhances our ability to generate novel mutants. The second strategy for increasing germinal transposition is tissue culture. Work in a number of species suggests that tissue culture is inducing transposon movement. As such, lines containing mPing, ORF1, and transposase have been placed in tissue culture and regenerated. The use of Illumina sequencing on pre- and post-tissue culture DNA makes it possible to assess changes in the location and copy number of mPing insertions. Regenerated plants are also being screened in the field for novel phenotypes.

P042
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 we assembled a T-DNA element that carries the Cassava Vein Mosaic virus promoter (CsMV), delineated by Ds. 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. We have identified 200 germinal transpositions to date of which 93 have been mapped via a TAIL PCR strategy to capture junction fragments. We are currently evaluating alternative, more throughput methods to identify and map Ds transpositions from field plantings of Ac/Ds stacked lineages.

P043
Identifying Genes Important for Determining Lateral Branch Angle in Soybean Using a Fast Neutron Mutagenized Population
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Soybean (Glycine max) genetic diversity has been reduced due to domestication and selective breeding. We used a fast neutron (FN) mutagenized population of soybean in background ‘M92-220’ and identified mutants that were altered in qualitative and quantitative traits. Changes in shoot architecture and particularly those that display differences in the angle of lateral branching are of interest since altering the shoot branching angle may influence light perception as well as harvest index, resulting in increased yield. We identified several unique mutant lines in the FN population that showed differences in the lateral branch angle. Our aim is to identify the loci responsible for the different phenotypes by mapping and eventually cloning the underlying gene(s). We are using a complementary approach of bulk segregant whole genome sequencing and array Comparative Genomics Hybridization to identify regions of the genome controlling these phenotypes. Using this approach we have identified candidate genes in the mapped regions that may be important for controlling lateral branch angle. Experiments are underway to functionally characterize the candidate genes as well as to determine the effect of lateral branch angle on overall yield of soybean.
P044
An RNAi Approach for Functional Analysis of Quantitative Resistance Genes Using Hairy Roots

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Fungal and oomycete diseases of soybean can cause significant yield losses for soybean growers. Development of soybean cultivars resistant to these pathogens has been shown to be an effective method in reducing their impact on yield. Identification of genes involved in disease resistance traits is critical to develop a better understanding of the factors that affect disease resistance and in guiding breeding programs that work to improve disease resistance in soybean. To accomplish this goal we have developed an RNA interference approach for the functional analysis of putative quantitative resistance genes using a soybean hairy root system. Genes identified as important to disease resistance using this system will be examined further to identify their specific role in protecting the plant from infection.

P045
Development and Application of a Novel Genome-Wide SNP Array

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Domestication of soybeans occurred under the intense human-directed selections aimed at developing high-yielding lines. Tracing the domestication history and identifying the genes underlying soybean domestication require further exploration. Here, we developed a high-throughput NJAU 355K SoySNP array and used this array to study the genetic variation patterns in 367 soybean accessions, including 105 wild soybeans and 262 cultivated soybeans. The population genetic analysis suggests that cultivated soybeans have tended to originate from northern and central China, from where they spread to other regions, accompanied with a gradual increase in seed weight. Genome-wide scanning for evidence of artificial selection revealed signs of selective sweeps involving genes controlling domestication-related agronomic traits including seed weight. To further identify genomic regions related to seed weight, a genome-wide association study (GWAS) was conducted across multiple environments in wild and cultivated soybeans. As a result, a strong linkage disequilibrium region on chromosome 20 was found to be significantly correlated with seed weight in cultivated soybeans. Collectively, these findings should provide an important basis for genomic-enabled breeding and advance the study of functional genomics in soybean.

P046
Use of Synthetic Promoters to Evaluate Regulatory Sequences in Soybean Promoters and Introns that Contribute to High Gene Expression

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Promoters can precisely regulate targeted gene expression in specific patterns mainly due to their contributing regulatory elements. Synthetic promoters, consisting of promoter core sequences with modifiers, have been developed as an efficient tool to evaluate potential regulatory elements that control specific gene regulation. The purpose of the present study was to identify and functionally characterize regulatory element sequences in promoters or introns. Element tetramers, core promoters and introns were assembled in different combinations in synthetic promoters and used to regulate a green fluorescent protein (gfp) gene. GFP expression was analyzed using both transient expression in lima bean cotyledons and stable expression in soybean hairy roots. Using our synthetic promoters, we were able to quickly validate potential cis-regulatory elements, investigate the effects of element flanking sequences, and evaluate intronic regulatory sequences contributing to intron-mediated enhancement (IME). With synthetic promoters, we identified G-box and GTAA elements responsible for high gene expression in rapidly growing tissues. We also identified 2-4 bp flanking sequences of the G-box element that significantly affected G-box activity. Manipulation of the proximal flanks of different G-box elements significantly enhanced promoter activity in both synthetic and native soybean promoters. Use of a synthetic promoter consisting of a G-box-containing tetramer, with a GmScreamM8 core promoter upstream of synthetic intron variants, we successfully identified a short intron repeated sequence significantly contributing to IME. Our research on the identification and characterization of soybean promoter and intronic regulatory elements may lead to more precise control of transgene expression and modulation of native gene expression via genome editing.
P047

Innovation of a Regulatory Mechanism Modulating Semi-Determinate Stem Growth through Artificial Selection in Soybean

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It has been demonstrated that Terminal Flowering 1 (TFL1) in Arabidopsis and its functional orthologs in other plants specify indeterminate stem growth through their specific expression that represses floral identity genes in shoot apical meristems (SAMs), and that the loss-of-function mutations at these functional counterparts result in the transition of SAMs from the vegetative to reproductive state that is essential for initiation of terminal flowering and thus formation of determinate stems. However, little is known regarding how semi-determinate stems, which produce terminal racemes similar to those observed in determinate plants, are specified in any flowering plants. Here we show that semi-determinate stem growth in soybean is modulated by transcriptional repression of Dt1, the functional ortholog of TFL1, in SAMs. Such repression is fulfilled by recently enabled spatiotemporal expression of Dt2, an ancestral form of the APETALA1/FRUITFULL orthologs, which encodes a MADS-box factor directly binding to the regulatory sequence of Dt1. In addition, Dt2 triggers co-expression of the putative SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (GmSOC1) in SAMs, where GmSOC1 interacts with Dt2, and also directly binds to the Dt1 regulatory sequence, as part of the repression complex. Heterologous expression of Dt2 and Dt1 in determinate (tfl1) Arabidopsis mutants enabled the creation of semi-determinacy, but the Dt2 expression in wild-type indeterminate (TFL1) Arabidopsis did not resulted in noticeable stem architectural change, further demonstrating the evolutionary novelty of the regulatory mechanism underlying soybean stem growth, which may pave new strategies for optimizing plant architecture for enhanced adaptability and yield potential in crops.

P048

Structural and Nucleotide Variation of Phytophthora sojae Isolates in Canada through Whole Genome Sequencing

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With the rapid expansion of soybean in Canada, Phytophthora sojae has found a new niche to establish its devastating presence. The best method to control this pathogen is through the use of varieties carrying resistance genes, following the gene-for-gene interaction where soybean resistance genes (Rps) provide immunity against P. sojae races carrying the corresponding Avr genes. However, the efficacy of Rps genes has been gradually lost as new races of P. sojae overcome this resistance. Breeders and growers are then confronted with the need to introgress Rps genes based on the P. sojae races found in the environment, without having clear information about it. This project aims to determine and characterize the presence and distribution of races/pathotypes of P. sojae in Canada. For this purpose, a collection of 31 P. sojae isolates representing the most common races found in Canadian fields was targeted for whole-genome-sequencing (WGS). Illumina sequencing yielded nearly 1 billion paired-end reads of 250 bp, leading to a total coverage of 75X per isolate. Following sequence alignment, structural variation and single nucleotide polymorphisms (SNPs) will be identified in order to develop PCR markers that could be used as diagnostic tools. These markers will be further used to specify races from plant and soil samples across soybean fields in Canada to develop a comprehensive map of the presence and distribution of P. sojae races.
P049
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 incorporated, including additions to the controlled vocabularies (ontologies) for soybean growth, development and phenotypic traits, soybean genes, 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 (http://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 and example data for entries in the Soybean Uniform Trials have recently been added. 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.

P050
Distinct Size Distributions of Small RNA Abundance in Soybean are Associated with a Missense Mutation in Dicer-like (DCL) 3

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Small non-coding RNAs are ubiquitous regulatory molecules that modulate gene expression at either transcriptional or post-transcriptional levels. In plants, small RNAs, which are 20 to 24 nucleotides (nt) in length, are generated from helical RNA precursors by an RNase III-type endonuclease known as Dicer. Generally, 24 nt small interfering RNAs (siRNAs) are the most abundant small RNAs in plant tissues, however, soybean (Glycine max) showed a distinct size distribution of small RNAs with 21 nt as most abundant. Here, we report that the reduced abundance of 24 nt siRNAs in soybean is associated with a missense mutation in the RNase III domain of GmDCL3. Small RNA sequencing of 102 soybean accessions showed divergent distributions of small RNA abundance, which have not yet been seen in any plant species. A genome-wide association analysis demonstrated that 24 nt siRNA abundance is tightly linked with an Arg-to-Cys change in GmDCL3. A multiple alignment of plant DCL3 proteins showed that only soybean DCL3 has the Cys mutation whereas DCL3 proteins in 17 other land plant species spanning ~500 million years of evolution have the Arg residue. Moreover, genotyping data of 302 soybean accessions revealed that 72.7% of soybean cultivars are homozygous for the Cys allele whereas none of the wild and 17.7% landraces are. This shift in allele frequency indicates that reduced abundance of 24 nt siRNAs might be involved with soybean improvement during the past century of modern breeding, or, alternatively, this allele may have hitchhiked with some other selected locus.
P051

**Genome-Wide Association Mapping of Sclerotinia sclerotiorum Resistance in Soybean Using Mainly Brazilian Genotypes**

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Sclerotinia stem rot (SSR) is one of the most significant diseases in soybean crop in Brazil. The use of molecular markers can facilitate the selection of genetic loci resistant to SSR. In this work we will use association mapping, identify chromosomal regions associated with reduced SSR severity. A collection of 430 genotypes of soybean from public sources, including many lines from private breeding groups in Brazil, were inoculated with Sclerotinia sclerotiorum isolate Jataí (Goiás State, Brazil) using the popular ‘straw test’ method. Four days post inoculation the length of necrotic lesion along the stem was measured. Inoculations were replicated 6-9 times, and the average lesion values used to serve as the phenotypic data values. The same plants used for inoculations had one developing shoot removed prior to inoculation to serve as tissue for DNA extraction using a CTAB method. The 384 samples with the most reproducible phenotypes and having at least six replications, are to be used for genotyping-by-sequencing (GBS) analysis using a protocol optimized for soybean. Molecular markers single-nucleotide polymorphisms (SNPs) will be identified covering all soybean chromosomes. After GBS library preparation, samples will be sequenced using the Illumina HiSeq4000 protocol. Processing of Illumina raw sequence read data, SNP calling and genome-wide association analysis will begin in the summer of 2016. The SNPs associated with enhanced resistance can be used to improve the ability to select new lines with more durable resistance.

P052

**Comprehensive Analysis of the Evolution of the Expression of Plant Genes: Insights at the Level of One Single Plant Cell Type**

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Our understanding of the conservation and divergence of gene expression patterns between plant species is limited by the resolution of the genomic and transcriptomic resources available. Specifically, the transcriptomes generated from plant organs and tissues are the reflection of the contribution of the different cell types composing the samples weighted by their relative abundances in the sample. These contributions vary between plant species affecting comparative transcriptomic analyses. To gain a deeper understanding of the evolution of gene transcription in and between plant species, single plant cell type models collected from different species should be used as models.

Applying this strategy, we performed a comprehensive analysis of the genomic and transcriptomic information collected at the level of one single cell type, the root hair cell, from two model plants: Arabidopsis thaliana and Glycine max. These two species were selected as models for this study based on the large amount of genomic and root hair transcriptomic information currently available and their relatively high percentage of gene orthology (i.e., 34.5%).

Upon comparative transcriptomic and genomic analyses, we identified 363 and 61 genes specifically expressed in soybean and Arabidopsis root hair cells, respectively. These data sets were used to analyze: (i) the distribution of the root hair genes on the different chromosomes, (ii) their genomic and transcriptomic evolutionary pathways, (iii) the conservation of the structure of their promoter sequences, (iv) the functional conservation of new root hair-specific cis-regulatory elements. The results show that root hair specific genes are enriched in the distal part of the soybean chromosomes. By using motifs discovery tools, we identify 2 specific cis-regulatory elements (not found in non-root hair genes) which are conserved in the 2 plant genomes. The details results will be present in addition to the validation of the regulatory elements.
P053

Association Mapping of Temperature Response in Agronomically Important Traits in Soybean

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Soybean [Glycine max (L.) Merr.] is one of the most globally important crops. Fluctuations in temperatures have a profound effect on soybean performance. However, soybean’s tolerance to temperature fluctuations has not been significantly examined. Our aim in this study is to identify genetic loci underlying temperature response of agronomically important traits in both wild (Glycine soja) and cultivated (Glycine max) soybeans. We phenotyped 192 G. soja and 457 G. max accessions grown under two temperature conditions (20°C and 30°C) at a constant 14-hour photoperiod. Measured traits include the temperature response of seedling emergence, R1 flowering, R5 flowering, R7 flowering, bud count, pod number, internode length, height, branching, lodging and vining. We observe that different temperatures significantly affected all of our traits with the exception of bud count. On average we observe that at higher temperatures plants reach R1 flowering, R5 seed-filling, and R7 pod maturity 10.0, 2.5, and 4.4 days earlier than at cooler temperatures, respectively. In G. soja a total of 17 temperature-related marker-trait associations were found for R1, R5, and R7 reproductive traits. In G. max 4 genetic loci were identified that exhibited significant marker-trait associations for temperature response in seedling emergence. Identified loci will help to elucidate the genetic diversity of temperature response in a wide range of soybean varieties and provide important genetic resources for breeding superior germplasm that are adaptive to fluctuating agricultural environments.

P054

Revitalizing Historical Resources: A Look at the USDA Soybean Isoline Collection

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The historical USDA Soybean Isoline Collection developed decades ago was created in order to isolate genetic intervals associated with contemporarily significant agronomic traits (Bernard et al., 1991). Previous studies have attempted to map intervals for these selected traits, however, RFLP markers did not provide high enough resolution to successfully identify all (Muehlbauer et al., 1991). Some success has been seen using RNA technologies to identify genes associated with some nutrient deficiencies (Peiffer, 2011), and some candidate genes have been identified. With the release of the SOY50KSNP genotyping array and the subsequent genotyping of the USDA germplasm, it is possible to continue the analysis of this historical collection (Song et al., 2013). This can be done by subsetting the 594 assayed isolines from Soybase and running comparative mapping procedures against their known genotyped parents. Here, we present preliminary results to the mapping of 48 intervals, and insights to the challenges associated with this most recent round of analysis.

P055

Development of a Wild Soybean Core Collection and Whole-Genome Analysis of Parental Stock and Random Lines of the G. max × G. soja

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Pair-wise genetic distances among all accessions within 1,168 G. soja accessions were calculated based on the SNPs in the SoySNP50K Illumina Beadchip and were used to classify the accessions into a pre-defined number of clusters, approximately 10% of the G. soja accessions which contain >90% of the diversity of the entire G. soja collections were included in the core collection and a number of wild soybean accessions were crossed with cultivated soybean in order to obtain lines with desirable performance. We sequenced the accessions in the core collections as well as parental stock and random lines of the G. max × G. soja populations created at USDA-ARS, NC and University of Missouri, and identified SNPs among core accessions, between wild soybean and cultivated soybean parents, as well as among the random lines, further analyses discovered SNPs with fixed or near fixed alleles in the G. max vs. G. soja parents and G. soja parents vs. random lines. This study will provide a baseline for the discovery of the genomic regions controlling desirable traits that affected by wild soybean and will facilitate genome-wide association analysis, parent selection, and efficient use of soybean germplasm diversity.
P056

Genomic Variation and DNA Repair Associated with Soybean Transgenesis: A Comparison to Cultivars and Mutagenized Plants

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Data gathered and communicated on the phenotypic and molecular variation induced by mutagenesis and genetic transformation will provide a scientific-based means to address concerns about the safety of these technologies. Recent work has assessed genomic structural variation (e.g., large deletions and duplications) and single nucleotide polymorphism rates among a sample of soybean cultivars, fast neutron-derived mutants, and five genetically transformed plants developed through Agrobacterium based transformation methods. On average, the number of genes affected by structural variations in transgenic plants was one order of magnitude less than that of fast neutron mutants and two orders of magnitude less than that observed between cultivars. Structural variants in transgenic plants, while rare, occurred adjacent to the transgenes and also at unlinked loci on different chromosomes. DNA repair junctions found adjacent to the inserted transgene and at unlinked sites were consistent with sequence microhomology across breakpoints. We are now developing experiments to more comprehensively assess the DNA sequence and transcriptional alterations observed following different mutagenesis (fast neutron and NMU), transformation (Agrobacterium and biolistic), and tissue culture (organogenic and embryogenic) treatments. These experiments will provide a fresh and comprehensive perspective on the genomic variation associated with these crop improvement technologies.

P057

A Systematic Approach to Routinely Define Haplotypes (and Alleles) on the Basis of Dense SNP Data

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Genes responsible for economic traits have been abundantly reported and still continue to be. For such known genes, breeding mostly aims to assess the allelic diversity captured in breeding collections and to identify individuals carrying favourable alleles at these genes. Genotyping-by-sequencing (GBS) can identify and genotype thousands of single nucleotide polymorphisms (SNPs) on a genomewide scale, thus providing sufficient coverage of SNPs to define major haplotypes. Ultimately, these haplotypes can be equated to specific alleles of a given gene. Therefore, the identification of haplotypes from large SNP data sets represents a promising approach to routinely assess allelic variation in large collections. The definition of useful haplotypes is nonetheless challenging and relies on: i) the ability to define a relevant interval around the gene, ii) the ability to discriminate between informative variants (co-transmitted with the gene) from others (independently transmitted), iii) the ability to classify and illustrate resulting haplotypes for further interpretation. Our work aims to develop a computational approach able to extract and process haplotypes of targeted genes from dense SNP data. We are developing and calibrating our method on four well-characterized maturity genes. In this work, we demonstrate how our approach can provide accurate and essential information for breeding purpose by i) delivering a quick and clear picture of a gene’s allelic diversity in a given collection and, ii) accurately discerning groups of individuals sharing the same allele, iii) allowing to estimate an allele’s respective genetic background.
P058  
**Structure and Diversity of the USDA Soybean Germplasm Collection**

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This study is the first report of quantitative and population genomic parameters along with phylogenetics based on the entire soybean collection of United State Department of Agriculture (USDA) germplasm, which provides novel insight of soybean germplasm structure. Germplasm studies provide plant breeders useful information to incorporate novel genetic resources into the breeding pipeline for the improvement of major agronomic traits based. We conducted comprehensive analyses on the all 19,652 USDA-ARS germplasm collection soybean accessions genotyped with the SoySNP50K Select BeadChip SNP array to elucidate structure and diversity in the collection. The majority of the accessions are from China, Korea, Japan, Russia, United States, Vietnam, India and Brazil. Hierarchical clustering was performed with Ward’s D method, using Nei’s genetic distance based on genomic information. The cladogram indicates the existence of eight major clusters (C1-C8). Each cluster has specific display particular properties with regard to major quantitative traits. C1 and C4 comprise the north-eastern Asian germplasm. C2 comprises the Korean germplasms. C3 represents the group with the largest number of entries, including the tropical and semi-tropical material. C5 display large seeds and may represent food-graded germplasm. C6 is abundant in germplasm with MGII. C7 comprises 99% of all wild relatives in the germplasm collection. C8 provided the highest yielding materials (assessed in the US). The classification and characterization of the germplasm collection into major clusters provides valuable information about to the genetic resources available to breeders.

P059  
**Whole-Genome Resequencing of a Collection of 102 Soybean Accessions from Canada**

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In this work, we report the resequencing of a collection of 102 Canadian soybean accessions. Selection of these lines was guided by a detailed genotypic analysis (>50,000 SNPs) to ensure an excellent coverage of the genetic diversity present in this germplasm. These accessions were sequenced to a mean depth of 11x. Using a new analytical pipeline that we have developed (Fast-WGS; unpublished), we identified 4.1M single (SNPs) and 284K multiple nucleotide polymorphisms (MNPs), as well as 642K InDels. To assess the quality of these genotype calls, we examined 47K SNP loci on the SoySNP50K array for which data was available for 19 accessions in common with our set of 102. A very high level of concordance (>99.98%) was obtained across this very large data set. Using haplotype and linkage disequilibrium analysis, we identified 1.7M tag SNPs which capture the haplotype diversity in this collection of short-season soybean. We then assessed how this exhaustive set of tag SNPs could be used as a reference panel to impute missing loci on accessions having undergone more shallow genotyping. A set of 530 soybean accessions were genotyped vis genotyping-by-sequencing (GBS) and this yielded a catalog of 156K SNPs. After imputation of all 1.7 M tag SNPs, the accuracy of the resulting data set was tested by comparing imputed genotypes to known genotypes. The very high accuracy of this imputation leads to a fine-scale genetic characterization of lines, almost equivalent to that achieved via resequencing, but at a fraction of the cost.

P060  
**The Evolution of FT Homologs and its Roles in Soybean Domestication**

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Gene duplication supplies raw material for mutation, drift and selection, and plays a major role in the evolution of functional novelty. Here, we investigate the evolutionary fate of a set of four recently duplicated genes: FT2a, FT2b, FT2c and FT2d that are homologs of the floral inducer FLOWERING LOCUS T (FT) in soybean. While FT2a maintained the flowering inducer function, the other genes went through contrasting evolutionary paths. Comparison of the genomic sequences and expression patterns between the wild (Glycine soja) and domesticated soybeans (Glycine max) revealed that FT2d obtained structure changes and became a pseudogene. Two of the homologs, FT2b and FT2c, evolved attenuated expression associated with a transposon insertion. In contrast to FT2b and FT2d whose mutational events occurred before the separation of G. max and G. soja, the evolution of FT2c is a G. max-lineage specific event. The domesticated FT2c allele carrying a transposon insertion is nearly fixed in domesticated soybeans, exhibits a signature of selection and affects photoperiodic flowering. These findings support that the domesticated FT2c allele was selected at the early stage of soybean domestication and show the important role of FT2c in soybean domestication. This work, together with previously observed evolutionary roles of FT in other crops, represents an example of convergent evolution in crop domestication genes.
P061
Methylation Affects Transposition and Splicing of a Large CACTA Transposon from a MYB Transcription Factor Regulating Anthocyanin Synthase Genes in Soybean Seed Coats

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We show that a transposon insertion in a MYB transcription factor at the R locus results in seed color variation in RM55-r-m, homozygous for mutable allele (r-m) specifying black and brown striped seeds, and also in RM30-R*, a stable black revertant isolate derived from the mutable line. Long range PCR, 454 sequencing of amplicons, and whole genome re-sequencing, determined that the variegated RM55-r-m line had a 13 kb CACTA transposon (TgmR*) in Intron2 of the R locus. The MYB encoded by R was expressed at very low levels in older seed coats of the black revertant RM30-R* line, but upregulated expression of anthocyanidin synthase genes (ANS2, ANS3) to promote the synthesis of anthocyanins. Surprisingly, the RM30-R* revertant also carried the 13 kb TgmR* insertion in Intron2. RNA-Seq analysis showed that intron splicing was accurate, albeit at lower levels, despite the presence of the 13 kb TgmR* element.

Whole genome methylation sequencing demonstrated that the TgmR* sequence was more methylated in RM30-R* than in the mutable RM55-r-m progenitor line. The stabilized and more methylated RM30-R* revertant line prevents effective binding of a transposase to its subterminal repeats and allows intron splicing to proceed, resulting in sufficient MYB protein to stimulate anthocyanin production and the black seed coat phenotype. This TgmR* element in soybean resembles McClintock’s Spm-suppressible and change-of-state alleles of maize and elucidates the opposite effects of the TgmR* element on intron splicing of the MYB gene depending on the methylation state of the element.

P062
Development and Validation of an Effective Greenhouse Assay for Evaluation of Stem Canker Resistance in Soybean

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Southern soybean stem canker inflicted large losses to growers in the southeastern United States in the 1980s. The most effective method of managing soybean stem canker is use of genetic resistance. An effective greenhouse screening method is important in continuing discovery of new sources of resistance. This study sought to develop an effective inoculation method that provided close to 100% disease incidence in susceptible plants and shortened the incubation time of the pathogen. Pre-soaking toothpicks in media broth, using wound sealant and humidity chambers, and inoculating seedlings at V2 growth stage all resulted in high disease incidence by three weeks post inoculation. Successful validation of this method was achieved with 18 southern stem canker susceptible soybean lines.

P063
Untying the Knot: Identifying a Genetic Mechanism for Southern Root-Knot Nematode Resistance

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Southern root-knot nematodes, or Meloidogyne incognita, are responsible for soybean yield losses of over one-hundred sixty thousand metric tons a year in the United States alone. With recent concerns about the use of nematicides on the environment, genetic mechanisms for resistance need to be identified and characterized to facilitate introgression into elite lines. A pair of duplicated candidate genes, Glyma10g016600 and Glyma10g016700, originally annotated as extensin proteins, are now recognized as containing Pollen Ole e-like domains. These genes are involved in drought tolerance but had not been identified as providing resistance to root-knot nematodes. Overexpression of these genes in hairy roots enhances the resistance of both susceptible and resistant genotypes, while CRISPR-mediated knockouts increase the susceptibility of both genotypes. Both extensin genes from the resistant type are upregulated in the presence of nematodes; the extensins from the susceptible type do not show such an increase in expression. The coding sequence of one of the extensins is identical in susceptible and resistant types, suggesting that resistance is associated with expression level. The promoter from one of the extensin genes from the resistant genotype has been sequenced and contains several differences relative to the reference genome that are not present in the promoter of the allele from the susceptible genotype.
P064
Mapping Host Resistance Genes to Soybean Sudden Death Syndrome

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Soybean Sudden Death Syndrome (SDS) is a disease caused by a soil-borne fungus, Fusarium virguliforme. The use of disease resistant varieties is the cornerstone of SDS management. Many genetic studies have attempted to identify genes responsible for the quantitative host resistance to SDS. Three recombinant inbred line (RIL) populations were evaluated for foliar SDS resistance at a naturally infested field site in Decatur, Michigan during the 2014 and 2015 growing seasons. Lines were evaluated for disease severity (DS) on a 1-9 scale, disease incidence (DI) as an estimate of the percentage of plants with symptoms per plot, and disease index (DI) as a metric which integrates DS and DI. A subset of lines from each population were genotyped with the SoySNP6K Illumina Infinium BeadChip. Linkage maps for each population were constructed using Joinmap. Composite interval mapping was done using WinQTLCartographer. Two quantitative trait loci (QTL) were identified across multiple years and/or populations. One QTL was on Chromosome 10, and appears to be in close proximity to the E2 maturity locus. Further data will be collected to determine if this QTL is truly responsible for SDS resistance, or if maturity conflated SDS phenotyping. The other QTL identified was on Chromosome 18, in a region which has been demonstrated to provide SCN and SDS resistance in many studies (Rg1/Rf52). Fine mapping of this QTL will seek to validate the identification of a receptor-like kinase responsible for SDS host resistance.

P065
Engineering Resistance to the Soybean Mosaic Virus (SMV) Using a Protease Detection System

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Genetically determined disease resistance is one of the most effective and environmentally sustainable approaches to control plant diseases. Traditionally, conventional breeding strategies have been used to introduce characterized disease resistance (R) genes into elite lines from germplasm collections. However, the durability of R genes introduced into crop species is often limited in field settings due to the low occurrence of available R genes with the desired recognition specificities. We previously reported a novel approach to expand the recognition specificity of an Arabidopsis R protein, RPS5. This strategy is based on our observation that modifying a proteolytic cleavage site sequence within a protease-targeted Arabidopsis host protein, PBS1, expands the recognition specificity of RPS5, thereby conferring resistance to a new suite of pathogens. We are now using this strategy developed in Arabidopsis to engineer durable, genetic-based resistance in soybean (Glycine max L.) to the Soybean Mosaic Virus (SMV) based on the recognition of the SMV protease, Nla. In the current study, we replaced the endogenous proteolytic cleavage site sequence in Arabidopsis PBS1 with the cleavage site sequence recognized by the SMV protease, generating AtPBS1SCS. Transient coexpression of AtPBS1SCS along with SMV protease and RPS5 in N. benthamiana resulted in an RPS5-dependent hypersensitive response. Consistent with the observed HR, immunoblot analysis confirmed the SMV protease cleaved the target AtPBS1SCS. Future work will test whether the modified Arabidopsis PBS1-RPS5 protease detection system can function in soybean to block symptom development and restrict systemic movement of SMV.

P066
Dissecting Plant-Mediated Pest Interactions in Soybean: Systemic Effects of Aphid Infestation

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Soybean aphids are specialized phloem-feeding insects that cause significant crop damage and yield reduction. Recent studies show that soybean aphid feeding systemically facilitates performance of both intra- and interspecific pests such as root-dwelling parasitic soybean cyst nematodes. To date, the few molecular studies of soybean aphid infestation have focused on locally infested tissues; no molecular data exist that explain soybean aphid-induced signaling between leaves and roots. We hypothesize that foliar aphid feeding activates plant-mediated systemic signaling to roots which in turn, facilitates soybean cyst nematode performance. We used RNA-seq to compare transcriptome changes in leaves and roots during an early (12h) and sustained (7d) foliar soybean aphid infestation in aphid-susceptible plants. Our results suggest that soybean aphid feeding triggers significant plant-mediated systemic signaling which may facilitate increased soybean cyst nematode performance.
P067

Elucidating Host Plant Resistance to Brown Marmorated Stink Bugs

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Halyomorpha halys, brown marmorated stink bug (BMSB), is a native insect of Asia. In 1996, BMSB was introduced to North America and has quickly become a noxious agricultural pest. In nearly 20 years, BMSB populations have steadily increased and are now high enough in the mid-Atlantic region to significantly reduced yield and create substantial economic losses to a wide range of plants and crops; including soybeans, corn, apples, grapes, vegetables, and several horticultural tree species. As of 2015, BMSB has been detected in 42 states and is a severe agricultural pest in nine states. On soybeans, damage from BMSB infestation ranges from puncture marks with seed discoloration and deformities to seed and pod abortion. Over the past three years, the USDA soybean germplasm collection (MGII-IV) has been screened for resistance to BMSB. Results from choice and no-choice tests indicate high levels of susceptibility in Ohio grown commercial varieties, however, several sources of strong resistance have been identified among the plant introduction collection. Resistance as measured by incidence and severity both appear to be quantitative. High broad-sense heritability estimates (H2 = 0.70) indicate that resistance characteristics are largely a result of genetic factors and can be introgressed into high yielding varieties. Genome wide association analysis of 202 individuals also revealed several significant SNPs in QTL regions linked to pod wall thickness, seed coat hardness, and aphid resistance. Moreover, detached pod assays indicate that BMSB resistant Pis are also resistance to Euschistus servus, brown stink bugs.

P068

Genetic Characterization of Rpp1 Mediated Resistance Using In-depth Transcriptomic and Genomic Analyses

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Asian Soybean Rust (ASR) is an important disease on soybean. Geneticists have found at least six different resistant alleles from soybean plant introductions (PI). This work focused on Rpp1 mediated resistance using PI561356 and PI594760. This study involved a time-course RNA-seq experiment to investigate the difference of transcriptome level regulation between two Rpp1-carrying resistant genotypes, and one susceptible genotype. De novo transcriptome assembly was generated using the Trinity software program. The N50 for Trinity assemblies was 2103 nucleotides, and the alignment rate is 92% in paired end mode, using Bowtie2 and RSEM for alignment and counting, respectively. Overall, 1277 soybean genes were selected as genes-of-interest based on edgeR and GFOLD results. Each individual gene was given category annotation according to the NCBI BLASTx results. We used 17 gene categories, and defense, DNA/RNA and signaling categories were the most represented. Heatmaps were made in the software program R to represent the overall expression profiles, and the two resistant soybean genotypes exhibited similar, as well as some different, expression patterns. Fifty-seven genes that were commonly differentially expressed in several previously conducted soybean-pathogen interaction microarray studies were selected from an in-house database for further exploration, and we will use the Fluidigm system to quantify their expression patterns using a mix of different soybean genotypes and rust isolates. Results of this research might contribute to better understanding of Rpp1 mediated soybean-rust interaction, and provide a list of Rpp1 candidate genes.

P069

Molecular Mapping of Quantitative Trait Loci from a Soybean Elite Line E05226-T Conferring Resistance to Pythium sylvaticum

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Pythium root rot is one of the most important diseases in soybean. The disease is caused by several species of the oomycete necrotrophic pathogen Pythium, such as P. sylvaticum and P. irregulare and P. ultimum. Of them, P. sylvaticum is the most prevalent species in Michigan soybean growing field. The objective of this study is to identify and validate resistant quantitative trait loci in MSU released soybean cultivars. An F4:7 RIL population with 226 lines was established by crossing a moderate susceptible cultivar E05226-T by a moderate resistant cultivar E09088. The disease resistance was measured by ratio of root weight change and ratio of plant weight change in greenhouse with temperature controlled at 20°. Linkage map was established using soy 6k beadchips with 1607 polymorphic SNP markers with 92 samples. Using QTl cartographer, two major putative QTLs were identified on soybean chromosome 2 (R2 = 21.3%, LOD = 4.31) and chromosome 5 (R2 = 17.3%, LOD = 4.85), and a minor putative QTL was identified on chromosome 15 (R2 = 9.30%, LOD = 2.94). All the QTLs were contributed from E05226-T. Further validation and fine mapping of the QTLs are in progress.
P070  
A Novel Source of Resistance to Defoliating Insects in Soybean  
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The study of defoliating insect resistance in soybean centers on the isolation and introgression of large-effect QTLs from two highly resistant plant introductions: PI 229358 and PI 227687. The line G00-3213 shows caterpillar resistance in the field. This resistance is not explained by previously identified QTLs, so it represents an additional source of resistance that can be pyramided with existing genes for enhanced resistance. G00-3213 and its progenitors were screened to identify the source and type of the resistance. Greenhouse bioassays were utilized to evaluate antixenosis (non-preference) and antibiosis (ability to harm insects) to caterpillars. In the antixenosis assay, percent defoliation was used as a measure of preference between all genotypes when given a choice. In the antibiosis assay, weight was used to quantify the effect of each genotype on insect health. One of the parents of G00-3213, Boggs, exhibits more antibiosis resistance and the same level of antixenosis resistance as G00-3213. Mapping is based on a RIL population to identify the QTLs responsible for resistance in Boggs.

P071  
The Genome of Soybean (Glycine max L.) has Resisted Diploidization  
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Nearly all plants show a whole-genome duplication (WGD) or polyploidy event somewhere in their evolutionary history. While many plants remain polyploid today, still many have become diploids over time due to the process of fractionation or diploidization. In this process, duplicated genes are deleted, paralogs gain new functions or lose certain functions, and duplicate segments are reorganized to form a condensed diploid genome. Soybean (Glycine max L.) is a prime example of this process at work, as although its genome is diploid, it has at least two major WGD events in its lineage: one shared with other papilionoid (Faboideae) legumes, which occurred approximately 55Mya, and one specific to Glycine from about 15Mya. Despite being a diploid plant, soybean still retains many genes in duplicate, with most genes being present in 2 or 4 copies. An analysis of synteny in Glycine max shows large contiguous blocks of synteny present in the genome covering about 91% of all genes therein, with most blocks belonging to the more recent Glycine duplication. Comparing the characteristics of these syntenic blocks in soybean to those in its fellow papilionoid Phaseolus vulgaris (common bean) shows that soybean blocks are longer, contain more genes, have slightly higher transposon insertion levels, and show less expression differentiation than those in P. vulgaris. Furthermore, these patterns of lower fractionation in G. max hold even when only considering syntenic blocks arising from the shared papilionoid duplication between the two species. As such, higher levels of transposon insertion and lower levels of expression differentiation within syntenic blocks in G. max are putative drivers of resistance to diploidization and fractionation in soybean.

P072  
Evaluation of the Putative Regulatory Element of the Arabidopsis Chloroquine-Resistance-Like Transporter (CLT3) Gene in Soybean  
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The homolog of the Plasmodium falciparum chloroquine-resistance like transporter in Arabidopsis thaliana, AtCLT3, is a plastid thiol transporter associated with stress responses in planta. AtCLT3 functions as a rheostat of cytosolic glutathione level, which in turn affects cellular redox potential. As a means to monitor the promoter activity of AtCLT3 in soybean, we fused the 1.5 kb up-upstream element of the gene call At5g12170 to the visual marker gene, GusPlus™. The derived binary plasmid harboring this cassette is designated pPTN1312. Among the ten independent transgenic soybean events obtained from transformations conducted with this binary vector two have been selected for down-stream characterizations. The selected two transgenic events, along with corresponding controls, are being monitored for GUS expression across developmental stages under non-stress conditions and upon challenge with soybean cyst nematode. Data will be presented on GusPlus™ expression patterns observed in the transgenic soybean events.
P073
Development and Implementation of SNP Genotyping Protocols for MAS of Soybean Cyst and Root-Knot Nematode Resistance

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Soybean cyst nematode (SCN, Heterodera glycines) is a devastating pathogen of soybean worldwide, while southern root-knot nematode (RKN) of the genus Meloidogyne is the most damaging nematodes in the southeastern United States. Control of these pathogens is difficult due to the life cycle of SCN and the wide range of host plants for RKN, so development of resistant cultivars is an effective way to combat these diseases. DNA markers have become powerful tools in performing marker-assisted selection against these diseases. Previously, our lab has developed functional SNP markers that can be used to effectively identify resistance against RKN and SCN, respectively. The RKN markers identify resistance against the most common root-knot nematode, M. incognita, while the SCN markers can detect the two loci involved in SCN race 3 resistance, and can also differentiate the two major sources of resistance (Peking or PI 88788) to SCN. Based on these results, we have developed and optimized the protocols of DNA extraction and KASP marker genotyping using either leaf or seed samples in a high-throughput setting. We have also developed strategies to implement the genotyping procedure in our breeding workflow to screen for plants with both RKN and SCN resistance. By using the genotype data obtained from leaf or seed samples, phenotypic resistance could be predicted and selections made for generation advancement in our breeding program. Using the procedure, over 15,000 samples were processed over the winter season for 2015-2016.

P074
Reactions of Soybean Plant Introductions with a Soybean Rust Resistance Allele at the Rpp3 Locus to a Pathogenically Diverse Set of Phakopsora pachyrhizi Isolates from the Southern United States

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A high percentage of soybean plant introductions (PIs) that have been resistant to soybean rust (SBR; Phakopsora pachyrhizi) populations and isolates from the United States carry a resistance (Rpp) allele at the Rpp3 locus, which was originally identified in PI 462312. The degree of similarity among these alleles is not known, though 37 of 52 PIs with an Rpp gene at this locus had a marker haplotype spanning the Rpp3 region that was identical to that of PI 462312. The objective of this study was to evaluate the reactions of 52 PIs with an allele at the Rpp3 locus to a pathogenically diverse set of monouredinial P. pachyrhizi isolates originating from Texas, Louisiana, Alabama, Florida, and Georgia. Detached leaflets were inoculated with a purified isolate and their reactions were rated after two weeks in culture. Leaflets from susceptible control plants were also inoculated and rated. Although many of the PIs had similar reaction patterns to the isolates, others had reaction profiles that were less similar to that of PI 462312, indicating that local mutations or other events affecting the ability of the host plant to recognize and react to SBR infection. These phenotypic reactions and more detailed genotype data can be analyzed together to determine which of the resistant PIs are most likely to carry unique Rpp3 alleles, and to guide decisions about which PIs are likely to be the most useful sources of alleles from the Rpp3 locus for breeding rust-resistant soybean.

P075
Soybean Derived miRNAs and Mungbean Yellow Mosaic India Virus Resistance

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Soybean is inflicted by many viruses causing serious diseases worldwide. In the tropical conditions, yellow mosaic disease (YMD) caused by Begomoviruses [Mungbean Yellow Mosaic virus (MYMV) and Mungbean Yellow Mosaic India virus (MYMIV)] is a hindrance to meet the demands of soy products. In order to understand host virus interactions at small RNA mediated RNA silencing pathways, soybean derived microRNAs (miRNAs) were studied. Expression profiling of conserved soybean miRNAs and antiviral miRNAs from soybean-that display propensity to bind and downregulate MYMIV genomes-were analyzed in two soybean genotypes [JS335 (susceptible) and UPSM534 (resistant)] with contrasting disease resistance trait against Mungbean Yellow Mosaic India virus (MYMIV). Expression profiling of conserved miRNAs and corresponding target miRNAs suggest the role of Argonaute (AGO) homeostasis in virus resistance along with the modulations in hormonal signaling pathways. Putative antiviral miRNAs showed over expression upon MYMIV infection in both the soybean genotypes. Furthermore, soybean derived miRNA potentially target and direct cleavage of viral mRNA encoding movement protein (BC1). Thus, this work provides molecular insights regarding changes in expression of miRNAs during MYMIV infection and improves deeper understanding of role of soybean miRNAs in MYMIV resistance.
P076

**A Meta-QTL Analysis of Root Structure, Quantitative Disease Resistance, and Abiotic Stress Resistance of Soybean Roots**

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Soil-borne pathogens, pest, and abiotic stress cause significant yield losses of soybean [Glycine max (L.) Merr.]. Soil-borne pathogens alone cause losses in excess of 300 million bushels or a cost greater than $3 billion. Losses could be limited by increased understanding of root resistance to soil-borne stresses. The soybean research community has completed numerous germplasm screening and genetic mapping studies to identify resistance against many yield-limiting soil-borne plant stressors. Over 400 QTLs have been identified representing 17 root structure and resistance traits. The objective of this work is to comprehensively extract relevant genetic information from independent studies for 14 root traits and consolidate the results to gain insight into root resistance. A meta-QTL analysis will be used to combine results for each individual trait in an iterative modeling process. The modeling process predicts the most likely number of QTL, the location of the QTL, and the effect of the QTL. To complete this objective we will (1) comprehensively identify mapping studies and QTLs associated with resistance to the soil-borne diseases, (2) collect information on the genetic location and effect of the QTL, and (3) use the collected information to perform a meta-QTL analysis in the program BioMercator. Meta-QTL results will be assessed for co-localization among loci controlling resistance against soil-borne biotrophs, necrotrophs, and abiotic stresses. The results of this study will be beneficial to soybean breeders by identifying QTL hotspots for disease resistance to each stress type and gaining a better understanding of the genetic architecture underlying these traits.

P077

**Screening Wild Relatives of Soybean for Resistance to White Mold**

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The fungal pathogen Sclerotinia sclerotiorum, white mold, is an important pathogen on soybean in New York State, where the climate is ideal for its growth. Yield losses due to white mold are rising at the same time that soybean cultivation is increasing in the state. The low genetic diversity of cultivated soybean and its quantitative trait response to infection with white mold have impeded breeding of durable resistance. As a means of identifying sources of qualitative resistance to white mold we are screening the perennial wild relatives for resistant genotypes. In addition to genetic bottlenecks associated with domestication of soybean (G. max), its annual progenitor, G. soja also experienced a major genetic bottleneck prior to soybean domestication. In contrast, although the wild perennial relatives of soybean are selfing, they have about a ten-fold higher level of genetic diversity than G. soja. We report the results of screening twelve diploid species of perennial Glycine for resistance to a virulent strain of S. sclerotiorum isolate found in New York. We have identified three diploid species on which we will concentrate further investigations, including metabolomics, transcriptomics and linkage mapping. Nine species of perennial Glycine are allopolyploids and we are investigating two of these species to determine if there is enhanced disease resistance responses when two genome groups are combined, as has been observed in other allopolyploid species.
P078

Defense Strategies from the Wild-Genome-Wide Association Analysis of Resistance to Soybean Cyst Nematode (Heterodera glycines) HG Type 2.5.7 in Wild Soybean (Glycine soja)

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Soybean cyst nematode (SCN) is the most destructive soybean pest [Glycine max (L.) Merrill] worldwide. Host plant resistance is the most environmentally friendly and cost-effective way of mitigating SCN damage to soybeans. Thus far, most of the commercial SCN-resistant soybean cultivars have been developed from very limited resistant germplasm resources. Overuse of these limited resistant resources has resulted in SCN race shifts in many soybean-growing areas. During the past decade, most studies used cultivated soybean (Glycine max) to select for resistant germplasm and to dissect the molecular basis of resistance. Moreover, the majority of the current resistant varieties are only resistant to HG Type 2 (race 1) and/or HG Type 0 (race 3); however, few studies have investigated HG Type 2.5.7, which is prevalent in the southeast USA. To broaden SCN resistance breeding resources and to mitigate nematode damage, we used Glycine soja, a wild soybean progenitor that shows much higher genetic diversity than cultivated soybean, to identify resistant accessions and to dissect the genetic basis of resistance to HG 2.5.7. A total of 235 G. soja accessions were evaluated; 43 were found to be resistant to SCN HG 2.5.7 (female index < 30) and could be considered exotic and novel SCN-resistant resources. We further conducted a genome-wide association study (GWAS) of HG 2.5.7 resistance with an association panel containing 235 wild soybean accessions using 41,087 single nucleotide polymorphisms (SNPs). A total of 10 SNPs distributed on chromosome 18 and chromosome 19 were found to be significantly associated with SCN HG 2.5.7 resistance. Three peak SNPs were located in the linked regions of the known quantitative trait locus (QTL) rhg1. Genes encoding disease resistance-related proteins with a leucine-rich region, mitogen-activated protein kinase (MAPKs), and MYB transcription factor were identified as promising candidate genes. The identified SNPs and candidate genes will benefit further marker assisted breeding and dissection of the molecular mechanisms underlying the soybean-SCN interaction.

P079

Expression of Soybean PIN Proteins During Infection with Phytophthora sojae

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The hormone auxin is involved in numerous plant processes, including responses to biotic and abiotic stresses. Plant pathogens are known to manipulate host auxin pathways to increase host susceptibility. The three major components of these pathways are metabolism, signaling, and transport. Genes involved in all three of these components are associated with quantitative trait loci (QTL) that confer resistance to Phytophthora sojae. To investigate the potential role of auxin transport in resistance to P. sojae, the expression of several PIN proteins was evaluated in Conrad (high partial resistance) and Sloan (moderately susceptible) at 0, 12, 24, 48, and 72 hours after inoculation (hai) using quantitative reverse transcription PCR. Glyma.19G128800, a putative PIN1-like gene, has increased transcription in both cultivars as the infection progresses. In contrast, Glyma.20G14300, a putative PIN3-like gene, has decreased transcription in Conrad as infection progresses. This same gene in Sloan increases in expression until 12 hai, but is also up regulated at the leading edge of the lesion margin at 72 hai. These data, in combination with biochemical and physiological approaches, will help contribute to an understanding of the role of auxin in response to biotic stress in soybean.

P080

QTL Mapping and Epistatic Analysis for Sudden Death Syndrome Resistance in Soybean

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Sudden death syndrome (SDS), caused by Fusarium virguliforme, has been a major disease of soybean [Glycine max (L.) Merr.]. Releasing of the SDS-resistant cultivar is the most cost-effective way to manage SDS damage. The objective of this study was to understand the genetic basis of resistance to SDS. A population of 129 F4-derived lines derived from GD2422 × LD01-5907 was evaluated for SDS resistance in the field from 2011 to 2012 and genotyped with 5361 polymorphic SNPs using SoySNP6K BeadChip. Five quantitative trait loci (QTL) were identified and mapped on linkage group C1, A2, H and G, separately. LD01-5907, a line with parentage of Hartwig, contributed the beneficial alleles for the QTL on LG A2 and G, while the beneficial alleles for the QTL on LG C1 and H were from GD2422. More significantly, the interaction between QTL on LG A2 and G was identified and confirmed on progeny line basis and single plant basis. Main effect of these two QTL together with the interaction effect in between explained 76% and 69% of phenotypic variation in 2011 and 2012 experiment, respectively. The preliminary results of fine mapping using residual heterozygous lines showed that QTL on LG A2 is in the vicinity of Rgh4, and QTL on LG G overlapped with Rgh1. Both of QTL showed recessive inheritance. Combination of genes on LG A2 and G conferred promising SDS resistance.
**P081**

**Untangling a Root-Knot Nematode Resistance QTL in Soybean: How Does One QTL Control Two Species of Nematodes?**

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Root-knot nematode (RKN) species are some of the most damaging nematodes, with estimated losses of $30 million/year in the Southeastern USA. While most soybean breeding efforts focus on Southern RKN, Peanut and Javanese RKN are emerging as serious pests due to the few resistant varieties available for these species. Previous research mapped a QTL for resistance to both Peanut and Javanese RKN to a similar region on Chromosome (Chr) 13. The aims of this study are to fine map the resistance QTL using SoySNP50K Infinium Chips data and KASPar assays, determine if one QTL is responsible for resistance to both nematode species, and identify the candidate gene(s) to develop SNP markers for marker-assisted selection (MAS). An F5-derived RIL population from CNS x PI200538 was used for fine mapping. The population was evaluated for Peanut and Javanese RKN resistance using a greenhouse bioassay. The target genomic regions were saturated with polymorphic SNP markers. Results indicate that two linked QTL located on Chr 13 control Javanese and Peanut RKN resistance, respectively. The QTL for Peanut RKN resistance is flanked by SNP markers GSM520 and GSM518 and the QTL for Javanese RKN resistance is within SNP markers GSM530 and GSM528, with the QTLs about 250 kbp apart. Based on the soybean reference genome, potential candidate genes are located within each QTL. The results of this research can lead to identification of functional markers associated with both RKN QTL, which will enable soybean breeders to conduct MAS of resistance to these two RKN species.

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**P082**

**Putatively Novel Sources of Resistance to Soybean Cyst Nematode**

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Soybean cyst nematode (SCN) is the most damaging pathogen in soybean in the USA. Two resistant loci, rhg1 and Rhg4 have been the main sources of resistance to SCN. Over 95% of SCN resistant cultivars used in the U.S. soybean production are derived their resistance from two genetic sources: Peking and PI 88788. This has become a major concern to soybean breeders. It is essential to identify new sources of resistance before the nematodes develop immunity to these two resistance sources. To discover new sources of resistance, we have genotyped 96 accessions, breeding lines and controls from various origins using the functional markers that were developed at rhg1 and Rhg4 loci as well as the functional marker for root-knot nematode resistance. These lines are also evaluated for SCN resistance using a greenhouse bioassay. The greenhouse phenotyping result was consistent with the maker prediction. Of 96 lines, 47 lines were classified as Peking type resistance through rhg1 and Rhg4 loci, while 27 lines classified as PI88788 type resistance with rhg1. Five lines rated as SCN resistance in greenhouse phenotyping do not carry either Peking or PI 88788 resistance alleles at rhg1 and Rhg4 loci. Based on haplotype analysis at the rhg1 and rhg4 loci assembled with Soy50KSNP Infinium Chip data, these lines were also grouped separately from PI 88788 and Peking. The results strongly indicated that these five lines might possess novel SCN resistance genes. Bi-parental populations have been developed and will be used to confirm these novel SCN resistance.

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**P083**

**Characterization of a Major QTL on Chromosome 18 Associated with Quantitative Resistance to Phytophthora Root and Stem Rot in Soybean**

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Phytophthora sojae is a soil-borne oomycete responsible for Phytophthora root and stem rot in soybean. The widespread deployment of race-specific Rps genes has led to a shift in physiological races of P. sojae. Partial resistance is quantitatively inherited and thus places less selection pressure on P. sojae populations. In a previous study, we identified a major quantitative trait locus (QTL) on chromosome 18 for quantitative resistance to P. sojae. As major QTL are uncommon in the soybean-P. sojae pathosystem, further investigation is warranted. Three RIL-F7 individuals heterozygous at the target QTL were selected and near isogenic lines (NILs) were developed to characterize and validate the QTL. The objectives of this study are to determine the allelic effect on partial resistance to P. sojae and test for pleiotropic effects against other soybean root pathogens and pests. Three sets of NILs were phenotyped for quantitative resistance to P. sojae using greenhouse (layer test) and growth chamber (tray test) based assays in conjunction with field evaluation. NILs were phenotyped for resistance to Fusarium graminearum and soybean cyst nematode to evaluate pleiotropic effects of the QTL on resistance to these pathogens. NILs with the resistant allele at the QTL, in general, were significantly more resistant to P. sojae in the tray test and layer test, whereas, no effects on resistance to either Fusarium graminearum or soybean cyst nematode were observed. This characterization of the QTL will facilitate cloning of the gene(s) controlling this trait and use of the resistance allele in breeding programs.
P084

Identification and Molecular Mapping of Rps11, a Novel Gene Conferring Resistance to Phytophthora sojae in Soybean

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Phytophthora root and stem rot (PRSR), caused by the soil-borne pathogen Phytophthora sojae, is a devastating disease of soybean [Glycine max (L.) Merr.] throughout the world. Deploying resistant soybean cultivars is the most effective and environmentally friendly approach to managing this disease. The soybean landrace PI 594527 was found to carry excellent resistance to all P. sojae isolates examined, some of which were capable of overcoming the major Rps genes, such as Rps1-k, Rps1-c, and Rps3-a, predominantly used for soybean protection in the past decades. A mapping population consisting of 58 F2 individuals and 209 F2:3 families derived from a cross between PI 594527 and the susceptible cultivar 'Williams' was used to characterize the inheritance pattern of the resistance to P. soja (Rps) in PI 594527. It was found that the resistance was conferred by a single Rps gene, designated Rps11, which was initially defined as an ~5 Mb genomic region at the beginning of chromosome 7 by bulked segregant analysis (BSA) with a nucleotide polymorphism (SNP) chip comprising 7039 SNP markers. Subsequently, simple sequence repeat (SSR) markers in the defined region were used to genotype the F2:3 mapping population to map Rps11 to a 225.3 kb genomic region flanked by SSR markers BARCSOYSSR_07_0286 and BARCSOYSSR_07_0300, according to the soybean reference genome sequence. Particularly, an SSR marker (i.e., BARCSOYSSR_07_0295) was found to tightly co-segregate with Rps11 in the mapping population and can be effectively used for marker-assisted selection of this gene for development of resistant soybean cultivars.

P085

Yield Drag Associated with the Aphid Resistance Gene Rag2 from PI 200538

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The soybean aphid (Aphis glycines Matsumura) is an exotic pest of soybean (Glycine max (L.) Merr.) that was first identified in North America during 2000. There are currently four known biotypes of soybean aphid and several genes that confer resistance to the pest. One such gene is Rag2, which was mapped from PI 200538 and has been introgressed into Midwestern adapted soybean lines. In previous studies conducted in aphid free environments, the Rag2 resistance allele was shown to be associated with a reduction in yield of approximately 120 kg ha-1 compared to lines without this allele. In our current study, we analyzed the introgressed genetic region of Rag2 in populations of near isogenic lines to localize QTL(s) causing yield drag associated with the Rag2 gene. The Rag2 genetic region was characterized with 23 genetic markers, and populations segregating for different intervals in the Rag2 region were tested in two Illinois environments in both 2014 and 2015 with no aphid infestation. Results from the tests indicate that QTL(s) causing yield drag are located within a ~5 Mb region approximately 2 Mb from Rag2. This information can be used to break the linkage between the yield reduction QTL(s) and Rag2, ultimately providing a higher yielding aphid resistant cultivar.
P086 Integrating GWAS and Expression Data for Functional Characterization of Resistance to Sclerotinia sclerotiorum in Soybean

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Sclerotinia is a ubiquitous phytopathogenic Ascomycete fungus capable of infecting a wide range of plants. On soybean plants, the disease is referred to as Sclerotinia stem rot (SSR). To dissect the genetic architecture of resistance to SSR, a high-density customized single nucleotide polymorphism (SNP) array (52,041 SNPs) was used to genotype two soybean diversity panels. Combined with resistance variation data measured in the field and greenhouse environments, genome-wide association studies (GWAS) were conducted to identify quantitative trait loci (QTL) controlling SSR. The mean level of LD, measured by $r^2$ declining to half its maximum value, was 370 kb and 240 kb in the two panels, respectively. The overall population structure was approximately coincident with geographic origin or maturity groups. The GWAS results identified 16 and 12 loci significantly associated with SSR resistance in field and greenhouse, respectively. Of these, 8 loci localized in previously mapped QTL intervals and 2 loci had a significant association with SSR across both environments. The expression level differences of genes that located in GWAS hit loci were compared between soybean resistance lines and susceptible lines through RNA-seq analysis. Diverse types of genes were identified involving in SSR resistance in term of up-regulating in the resistance lines and down-regulating in the counterparts. It demonstrates that GWAS integrated with gene expression data is a promising approach to dissect disease related traits. The loci and trait-associated SNPs identified in this study will aid efforts to improve SSR resistance in soybean and expedite positional cloning of the causal gene(s).

P087 Identifying Changes in Gene Expression that May Promote Virulence in the Soybean Aphid, Aphis glycines

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Insects have evolved mechanisms to evade plant defenses, facilitating their survival and proliferation. Plant cultivars that are resistant to insects (i.e. host-plant resistance) are better protected from herbivory and offer an environmentally safe tool for managing insect pests. Host-plant resistance is used against the most damaging insect pest of soybean: the soybean aphid, Aphis glycines. However, some populations of A. glycines can overcome resistance (i.e. virulent), threatening host-plant resistance durability. Our objective is to investigate genes that may play a role in A. glycines virulence. Previous research suggests that virulent aphids may avoid or suppress soybean defense. From these hypotheses, we predict that virulent aphids may increase or express novel genes coding for detoxification or effector proteins, which can modify plant cells to enable aphid feeding. These genes are known to be important for A. glycines interaction with resistant soybean. To identify potential mechanisms of aphid virulence, we performed RNA-sequencing of avirulent and virulent aphids fed on susceptible or resistant soybean varieties. We found 2546 and 2078 genes that were differentially expressed in virulent (relative to avirulent) aphids on susceptible and resistant soybean, respectively. Of these, 701 genes were expressed in virulent aphids when feeding on resistant soybean only. These 701 genes included a few genes that support predicted mechanisms of virulence, including suppression of defense and detoxification, in addition to other genes with unknown roles in aphid-plant interaction. Future analyses will focus on constitutive differences between virulent and avirulent aphids, and the role of effector proteins in virulence.
P088
Genetic Dissection of Aphid Resistance in Wild Soybean Glycine soja Accession 85-32
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The soybean aphid (SA) is a serious destructive insect pest for soybean production. Significant efforts have been put into identification of host plant resistance; several SA-resistance genes have been found in Glycine max. However, some of them have already been overcome by emerging new SA biotypes. Thus, identifying novel SA-resistance genes is critical to protect soybean yield. Two novel SA-resistant loci, Rag6 and Rag3c, from the G. soja accession 85-32 were previously detected in a 22Mb-interval on Chromosome 8 and a 1Mb-interval on Chromosome 16, respectively. Both loci conferred antibiosis resistance to aphids. No significant interaction was detected between Rag6 and Rag3c. The objective of this study was to fine map Rag6 and Rag3c. These two loci were validated with four F2 segregating populations. At the F2:3 generation, recombinant lines were selected to exclusively have either Rag6 or Rag3c with closely linked markers, and Rag6 and Rag3c were studied separately afterwards. With SNP markers developed from whole genome sequencing data, Rag6 was fine mapped to a 49Kb-interval on Chromosome 8 with the presence of three genes encoding leucine-rich repeat (LRR)-containing proteins. According to the exome capture sequencing data, one frame shift mutation occurred in one LRR gene, and six non-synonymous-coding mutations occurred in another LRR gene. qRT-PCR experiments have been conducted to determine which LRR gene is the candidate gene for Rag6. Rag3c was fine mapped to a 154Kb-interval on Chromosome 16 with the presence of nine genes, including one gene encoding leucine-rich repeat receptor-like protein kinase.

P089
Quantitative Amplification of Cleaved Ends (qACE) to Assay miRNA-Directed Target Cleavage
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microRNA (miRNA) regulation is crucial to achieve precise spatio-temporal expression patterns of their target genes. This makes it crucial to determine the levels of cleavage of a particular target mRNA in different tissues and under different conditions. We developed a quantitative PCR method “quantitative Amplification of Cleaved Ends (qACE)” to assay levels of specific cleavage products in order to determine the extent of miRNA regulation for a specific target gene. qACE uses cDNA generated from adapter-ligated RNA molecules and relies on a carefully designed fusion primer that spans the adapter-cleaved RNA junction in qPCR to specifically amplify and quantify cleaved products. The levels of full-length transcripts can also be assayed in the same cDNA preparation using primers that span across the miRNA cleavage site. We used qACE to demonstrate that soybean roots over-expressing miR164 had increased levels of target cleavage and that miRNA deficient Arabidopsis thaliana hen1-1 mutants had reduced levels of target cleavage. We used qACE to discover that differential cleavage by miR164 in nodule vs. adjacent root tissue contributed to nodule-specific expression of NAC1 transcription factors in soybean. These experiments show that qACE can be used to discover and demonstrate differential cleavage by miRNAs to achieve specific spatio-temporal expression of target genes in plants.

P090
The Role of microRNA 319 During Soybean (Glycine max) Nodulation
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Past work from our laboratory, as well as several others, has clearly documented an important role for miRNA in regulated legume nodulation. Therefore, miRNA represent another layer of regulatory complexity acting to either positively or negatively regulate nodule formation. As an additional example, we recently found that the relative levels of miRNA 319 were significantly reduced during nodule formation, while the potential targets of miRNA 319 were expressed significantly higher when measured at five different stages of soybean nodule development. The putative target genes of microRNA 319 include putative transcription factors, TCP4 (Teosinte branched 1, Cycloidea, PCF) and MYB (myeloblastosis) transcription factors (TFs). Over expression of two independent precursors of microRNA319 showed resulted in a significant increase in nodule number, suggesting that miR319 positively regulates soybean nodulation by targeting these transcription factors, which presumably normally act to suppress nodule numbers. Detailed characterization of miR319 and its target genes in soybean nodulation will be discussed.
P091
Use of Crispr/Cas Genome Editing Demonstrates a Critical Role for Uricase and Xanthine Dehydrogenase in Soybean Nitrogen Fixation and Nodule Development

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Previous biochemical studies suggested that de novo purine biosynthesis is required for the incorporation of fixed nitrogen in ureide exporting nodules, as formed on soybean roots. However, in many cases, the enzymes involved in this pathway have been deduced strictly from genome annotations with little direct genetic evidence, such as mutant studies, to confirm their biochemical function or importance to nodule development. While efforts to develop large mutant collections of soybean are underway, research on this plant is still hampered by the inability to obtain mutations in any specific gene of interest. However, this situation is now significantly changed through the ability to apply the methods of CRISPR/Cas9 genome editing using Agrobacterium rhizogenes-mediated hairy root transformation. Using this approach, we were able to generate homozygous mutant roots lacking either uricase (UOX) or xanthine dehydrogenase (XDH) activity. The uox knock out soybean mutants were unable to fix nitrogen, as exemplified by their internal greenish appearance reflecting a lack of leghemoglobin production. As added confirmation, a uox knock-out mutation generated through fast neutron mutagenesis displayed a similar phenotype. Similarly, a knock out XDH mutant, generated with the Crispr/Cas system, also displayed a fix- phenotype. These studies demonstrate the great utility of the Crispr/Cas system for studying root associated gene traits when coupled with hairy root transformation. Furthermore, these genetic studies confirm the critical role of the de novo purine biosynthetic pathway, not only in incorporation of fixed nitrogen but in the successful development of a functional, nitrogen fixing nodule.

P092
Differential Gene Expression in Two Nodule Zones of Soybean

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Leguminous plants like soybean develop a specialized organ called root nodule that harbors nitrogen fixing rhizobia bacteria. These bacteria fix atmospheric nitrogen fulfilling the nitrogen requirement of the plant and in return receive carbon for their survival creating a symbiotic relationship. During nodule development, root cortical cells undergo subsequent differentiation and de-differentiation processes. This process eventually leads to the formation of nodule primordia which later divides in two main nodule zones, nodule Parenchyma and Infection Zones. The mechanism by which cortical cells lead to the formation of two structurally and functionally different nodule zones is a major knowledge gap. We seek to evaluate the transcriptome profiles of these two nodule zones over a temporal scale in order to identify key determinants in the developmental identity of these zones. We adapted a cell type specific nuclear tagging method to isolate nuclei from nodule parenchyma (using the ENOD2 promoter) and nodule primordium /infection zone (using the ENOD40 promoter). Isolation of nuclear transcripts, subsequent validation by qPCR, and evaluation of transcriptome profiles by RNAseq will help achieve our goal of identifying key determinants of nodule zone identity. Ultimately, this finding will increase our knowledge in nodule developmental process which might help in optimizing nodule and nitrogen fixation in leguminous plants. Transfer of this trait to non-leguminous plants for a sustainable approach in fulfilling nitrogen requirements and reducing pollution caused by excessive use of chemical nitrogenous fertilizer will be beneficial and safe.

P093
Comprehensive Comparative Genomic and Transcriptomic Analyses of the Legume Genes Controlling the Nodulation Process

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Nitrogen is one of the most essential plant nutrients and, as a consequence, is one of the major limiting factors to crop productivity. Having the goal to perform a more sustainable agriculture, there is a need to maximize biological nitrogen fixation, a feature of legume plants. To enhance our understanding of the molecular mechanisms controlling the interaction between legumes and rhizobia, the symbiotic partner fixing and assimilating the atmospheric nitrogen for the plant, researchers developed genetic and genomic resources across different legume models (e.g. Medicago truncatula, Lotus japonicus, Glycine max and Phaseolus vulgaris). Using these resources, scientists identified key regulatory genes of the nodulation process from different legume species. Having the objective to maximize the transfer of knowledge between model legumes, we are taking advantage of the recent release of legume genomic and transcriptomic resources to highlight orthologous and functional relationships between legume genes controlling nodulation. Specifically, mining large transcriptomic datasets, we confirmed the induction of the expression of several orthologous genes in response to rhizobia across different legume plant species. This comprehensive study will not only help to transfer scientific knowledge but will also provide new insights into the evolution of the nodulation process in legume plants, especially in regard to the multiple duplication of legume genomes.
P094
Functional Characterization of GmFWL1, a Plasma Membrane Microdomain-Associated Protein Regulating Soybean Nodulation

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The soybean gene GmFWL1 (FW2.2-like1) belongs to a plant-specific family that includes the tomato FW2-2 and the maize CNR1 genes, two regulators of plant development. We previously demonstrated the specific expression of GmFWL1 in response to rhizobia. Silencing of GmFWL1 expression also significantly reduced soybean nodulation. While the biological role of GmFWL1 has been described, its molecular function and, more generally, the molecular function of plant FWL proteins is unknown. Here, we are presenting our work to functionally characterize GmFWL1. Specifically, co-immunoprecipitation and split luciferase assays show that GmFWL1 interacts with various proteins associated with membrane microdomains such as remorin, prohibitins and flotillins suggesting that GmFWL1 encodes a membrane microdomain-associated protein. Interestingly, applying comparative genomics, our biochemical analysis also revealed the interaction between GmFWL1 and GmFLOT2/4 (FLOTILLIN2/4), the soybean ortholog to Medicago truncatula FLOTILLIN4, a major regulator of the M. truncatula nodulation process. To complement our biochemical assays, we also conducted confocal and electron microscopic observations in homologous and heterologous plant systems to reveal the characteristic punctuate plasma membrane localization of microdomain-associated proteins in mock-inoculated root hair cells. Interestingly, upon rhizobia inoculation and similarly to MtFLOT4 and GmFLOT2/4, GmFWL1 protein was translocated at the tip of the soybean root hair cells, suggesting a role during the early events of nodulation and the strong cooperation between GmFWL1 and GmFLOT2/4.

P095
Identifying the Downstream Effectors of miRNA 160 in Soybean Root Nodule Development

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Soybean is an excellent candidate for sustainable agriculture due to its production of nutritious, multi-use beans and the ability to form symbiotic organs called root nodules that allow nitrogen fixation. Understanding how root nodules in soybean are formed may be one way of optimizing nitrogen fixation to sustainably enhance soybean yield and transferring the root nodule formation ability to other plants. microRNA 160 (miR160) has been shown to contribute to proper nodule formation by targeting repressor auxin response factor (ARF) transcription factors for proper auxin sensitivity, but the specific downstream effectors of this interaction remain unknown. This project seeks to resolve these downstream effectors by evaluating the cellular, spatiotemporal, and DNA-binding activity of a key target of miR160, ARF 16-2 by confocal microscopy, DamID-Seq, and protein-DNA binding assay. Confocal microscopy of 16-2 promoter:GUS or tdT fusions show that ARF 16-2 promoter is active in dividing and differentiating tissues such as primary root tips, lateral root primordium, and emerging nodules. In addition, 16-2 promoter:GUS or tdT fusions show that this promoter is active in the parenchyma and root stele of mature nodules. Interestingly, 16-2 promoter:16-2 CDS:tdT translational fusions show a reduction of tdT signal in mature nodule tissues, underscoring post-transcriptional regulation of 16-2, potentially by miR 160 during nodule maturation. When genomic binding profile of 16-2 is identified and evaluated, we expect to identify key downstream genes affected by its activity during nodule development.
P096
**Soybean Rhizosphere Bacterial Community Structure as Influenced by Root Isoflavonoids**

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Rhizodeposits play a key role in shaping rhizosphere microbial communities. In soybean, isoflavonoids are a key rhizodeposit component that aid in plant defense and enable symbiotic associations with rhizobia. However, it is uncertain if and how they influence rhizosphere microbial communities. Isoflavonoid biosynthesis was silenced via RNA interference in soybean hairy root composite plants and rhizosphere soil fractions tightly associated with roots were isolated using successive sonication. PCR amplicons from 16S rRNA gene variable regions V1-V3 and V3-V5 from these fractions were sequenced using 454. The resulting data was resolved using MOTHUR and vegan to identify bacterial taxa and evaluate changes in rhizosphere bacterial communities. The soybean rhizosphere was enriched in Proteobacteria and Bacteroidetes, and had relatively lower levels of Actinobacteria and Acidobacteria compared to bulk soil. Isoflavonoids had a small effect on bacterial community structure, and in particular on the abundance of Xanthomonads and Comamonads. The effect of hairy root transformation on rhizosphere bacterial communities was largely similar to untransformed plant roots with ~74% of the bacterial families displaying similar colonization underscoring the suitability of this technique to evaluate the influence of plant roots on rhizosphere bacterial communities. However, hairy root transformation had notable influence on Sphingomonads and Acidobacteria.

P097
**The Genetic Architecture Underlying the Complex Trait Controlling Soybean Seed Pigmentation**

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Soybean is a crop that produces high quality oil and protein. Yellow seed coats in soybeans account for most modern high yielding cultivars; however, a wide variety of seed coat and hilum pigmentations also exist, including diverse colors such as black, brown, imperfect black, redbrown, redbuff, gray, green, buff, as well as clear, which is the absence of pigmentation. Several mechanisms that make up the blueprint for the spectrum of pigmentation are multiple independent loci that interact such as the Inhibitor gene, the Tawny pubescence gene, the R gene, and the W1 gene for flower color. It is important to note that derivatives from a phenylpropanoid biochemical pathway are considered key factors in the pigmentation of soybeans. Our objective was to confirm the molecular basis and broaden our understanding of the components of this complex trait for seed coat pigmentation. Using genome-wide association, we were able to identify genes and the associated SNPs from unknown seed coloration lines. At the genomic locations, we were then able to test for accuracy of these alleles to predict their phenotypes. For instance, in gray seed coat lines, we were able to find a SNP that correlates to the Inhibitor gene, and for redbuff, a region containing SNPs on Chromosome 14 was identified that is linked to the w3/W3 allele that controls flower color pigmentation. In conclusion, we were able to determine the loci controlling the phenotypes for the pigmentation trait of several unknown soybean seed coats and hila.

P098
**Molecular Analysis of Sink Limited Soybeans**

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Senescence is the natural aging process during which nutrients are mobilized and exported to newer parts of the plant. During cotyledon senescence, nutrients are transported to the developing seedling, while during trifoliate leaf senescence nutrients are transported to the seed. Plants that do not produce a sink (depodded or sterile plants) have been shown to have altered physiological features including increased starch and nitrogen in leaves and a delayed senescence (or a “stay green” phenotype). RNAseq expression profiling was used to compare levels of gene expression in trifoliate leaves and cotyledons to identify transcriptional changes throughout development. A subsequent profiling experiment was performed to compare gene expression in sink-limited plants (depodded and ms6 male sterile) and controls.

Genes expressed during the later stages of leaf and cotyledon shared common functions, suggesting that mechanisms of senescence are conserved between tissues. Genes with predicted regulatory functions that may have a role in control of leaf or cotyledon senescence were identified. Transcription factor families such as the WRKY, NAC and GRAS were shown to be important regulators of plant senescence in both leaves and cotyledons, while bHLH and AUX/IAA families were important in early leaf development.

In sink-limited tissues, we observed continued high expression of genes associated with early leaf development. We identified genes that were not expressed during normal leaf development but highly expressed in sink-limited plants, including the D4 “non-yellowing” gene. These changes highlighted several metabolic pathways that were involved in distinct modes of resource portioning in the “stay green” leaves.
P099
A Pedigree-Based Germplasm Panel for Assessing Genomic Change in a Public Soybean Breeding Program

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Characterizing phenotypic and genotypic relationships is the foundation for future plant improvement efforts within a breeding program. Using historical pedigree records, the University of Guelph soybean breeding has created a Guelph Soybean Breeding Germplasm Panel. The varieties comprising the panel are historical founder, specialty trait and modern varieties representative of the breeding program over 80 years of soybean breeding. The Guelph Breeding Panel consists of 218 lines genotyped with genotyping-by-sequencing (GBS with >43k SNPs genome-wide), which were grown at two locations in Ontario, Canada (Woodstock and St. Pauls) in 2015. The panel was phenotyped for agronomic and seed traits including yield, canopy reflectance normalized difference red-edge index (NDRE), oil (range 12.6-22.4% dry basis), protein (range 37.6-50% dry basis), fatty acid profile and sugars. Field trials for 2016 are underway at the same locations. The goal of the panel is to determine genetic diversity and allelic changes through selection from founder to modern varieties, as well as assess dynamics of trait variation in the breeding program. Data on the genetic diversity of the University of Guelph’s soybean breeding program will be presented along with trait diversity and genome-wide trait associations within the Guelph Breeding Panel based on GBS results and 2015 field data. Yield gains throughout years of breeding will be described. Future use for the panel will include allele discovery and identification for targeting trait improvement in the breeding program.

P100
The Long and the Short of Soybean Petioles: The Effect of a 3-bp Insertion on Plant Architecture and Harvest Index

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Dietary changes and increases in the world population have led to rising demands for sustainable sources of plant protein and oil. Modifications to plant canopy architecture could play an important role in meeting these global food demands, as was demonstrated during the Green Revolution in the 1960’s and 1970’s. Petioles are an important component of canopy architecture as they connect the leaflets to the stem in dicot species, but many of the mechanisms controlling petiole length are unknown. To our knowledge the effect of short petioles on seed yield has not been studied. Kilen (1983) identified a short petiole soybean mutant lps1 that segregated as a single, recessive locus. The mutation was first observed in 1976 segregating in an F3 row in a population of a cross between Forrest(2) x (PI 229358) and D71-6234. D71-6234 was derived from a cross between a high protein Lee type and PI 95960. None of the parents were observed to have the short petiole phenotype suggesting the mutation was spontaneous. This study was undertaken to identify the causal DNA polymorphism underlying the lps1 phenotype as well as to assess the utility of the lps1 mutation for improving soybean yield, agronomics, and physiology using Near-Isogenic Lines (NILs) generated during the trait mapping process. Whole-genome sequencing-based bulk segregant analysis was used to locate the chromosomal region harboring the lps1 mutation. Diversity analysis of sequences within this interval identified an in frame 3-bp insertion in an uncharacterized gene, and silencing of this gene by Virus Induced Gene Silencing successfully produced a short petiole plant. Preliminary yield trial testing of the NILs under narrow row spacing indicated that the short petiole trait does not negatively impact yield and may improve soybean agronomics through increased harvest index and decreased plant height.
P101

Identifying Novel Resistance Genes against Phytophthora sojae Using an Effector-Directed Approach

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Phytophthora sojae (P. sojae), the causal agent of soybean root and stem rot disease, is an oomycete pathogen responsible for over $400 million dollars of soybean crop damage annually in the US, and over $1 billion dollars worldwide. For successful P. sojae infection, the pathogen must secrete effector proteins into the host, which function to repress natural defense systems. To date, P. sojae has been managed through the inclusion of P. sojae resistance (Rps) genes and quantitative trait loci (QTL) into commercial lines. Rps genes are able to recognize specific pathogen effectors inside the host and up regulate the defense response in turn. However, the effectiveness of current R-genes is decaying as certain strains of P. sojae evolve to overcome the resistance. The reduction of Rps gene effectiveness can be attributed to P. sojae's loss or manipulation of recognized effectors. This project seeks to identify novel genes conferring durable resistance. Resistance gene screening, targeting core P. sojae effectors would provide durable resistance, as a loss of these effectors by the pathogen would result in a loss of pathogenicity. An effector-based screening assay has been developed utilizing Pseudomonas fluorescens to rapidly screen soybean germplasm for resistance genes. Our strategy is to use the effector-based screening assay, along with a pathogen assay, to develop genetic maps of the novel genes conferring resistance to P. sojae.

P102

Population Genetic Study of High-Yielding Soybeans Derived from USDA Soybean Germplasm Collection Breeding Program

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Since 1979, we have been using exotic soybean germplasm in our breeding program to increase seed yield. Seventy-one high-yielding experimental lines that had seed yields equal to or greater than the best check in regional tests were selected for analysis. These experimental lines were developed from forty-two exotic accessions that had not previously been used in US soybean breeding. All experimental lines and exotic parents were characterized with the Soy50K SNP array. First, the population structure of the exotic founder lines of our breeding program and corresponding founders of the conventional Northern US elite cultivars was investigated, using Discriminant Analysis of Principal Components (DAPC), PCA and fastSTRUCTURE. Secondly, identification of candidate regions associated with selective pressure during the breeding process was performed using population genetic approaches including Fst, linkage disequilibrium (LD) and integrated haplotype score (iHS). These approaches were used to detect genomic regions selected during soybean improvement in each gene pool and to identify those selected regions that differed between gene pools. Tracing the selective signals to the donor accessions will enable marker assisted selection methods to be applied to combine haplotypes associated with increased yield from exotic germplasm and conventional lines within elite cultivar breeding programs.
P103

**Identification of New Sources of Resistance to Soybean Aphids (Aphis glycines Matsumura)**

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Soybean aphids are phloem feeding insect pests of soybean. Aphids divert plant assimilates for their nutrition and growth, causing yield losses of up to 50%. One of the management options for aphids is cultivation of resistant soybean varieties. Aphid resistance in soybean is conferred by Resistance to Aphis glycines (Rag) genes and in the United States five Rag genes (Rag1 to Rag5) have been identified to date. Although host plant resistance is an effective management strategy against aphids, aphid biotypes that can colonize resistant soybean have been discovered. The presence of aphid biotypes that can survive on aphid-resistant soybean indicates the need to identify more new and durable sources of aphid resistance. To identify new sources of aphid resistance, 308 USDA soybean accessions were screened for resistance to soybean aphids (biotype 1) using choice tests conducted in the greenhouse. Aphid numbers per plant and damage symptoms were used to assign scores to each plant and mean scores for each soybean line were used to categorize lines as resistant, moderately resistant or susceptible. From this panel, 16 lines were resistant or moderately resistant to aphids and their performance was similar to resistant checks. Genome-wide association analysis detected 15 significant SNPs on chromosomes 1, 10, 16 and 19, and 136 putative candidate genes were identified based on these SNPs. This study enhances the characterization of the underlying genetic architecture of aphid resistance and the resistant soybean lines identified can be utilized by plant breeding programs to develop varieties with durable aphid resistance.

P104

**A Molecular Tool to Increase Protein Content and Broad Disease Resistance in Soybeans**

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Crop plants must integrate signals from the environment and prioritize responses to stresses that may occur individually or simultaneously throughout the growing season. Stress responses can adversely affect plant growth and quality traits such as protein and starch. The ability to optimize protein productivity of plant-based foods has far-ranging impact on world health and sustainability. Plant diseases each year cause major losses to crop production. The Arabidopsis thaliana QQS-orphan-gene modulates carbon allocation to protein and starch (1). Ectopic QQS expression increases protein content (2) in leaf and seed in soybean (3,4). QQS transcript levels are altered in plants under stresses and in mutants of genes involved in all sorts of stress responses, indicating that QQS may integrate primary metabolism with environmental perturbations, thus adjusting the plant’s adaption to abiotic and biotic stresses (5). The QQS protein binds to a transcriptional regulator in Arabidopsis and its soybean homologs: Nuclear Factor Y subunit C4 (NF-YC4). NF-YC4 overexpression in Arabidopsis and soybean mimics QQS-overexpression phenotype, increasing protein and decreasing carbohydrate (4). Mutants overexpressing genes related to QQS network have significantly increased resistance to plant pathogens and pests (6). Our data reveal indicate QQS exerts its effect via an interaction with a transcription factor conserved across eukaryotic species (4); these findings open a non-transgenic strategy to create high-protein soybeans and enhance broad-spectrum disease resistance (6).

P105
Using Wild Soybean as a Resource to Increase Soybean Seed Yield

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It has been well documented that soybean has a narrow genetic base compared to many other crop species. To continue to make improvements in yield, it is important to selectively increase the genetic base of the commercially used gene pool to add diversity. In previous studies, the domestication of soybean from wild soybean (Glycine soja) was identified as the most influential genetic bottleneck. Wild soybean has consistently been shown to have higher diversity, making it a potentially valuable resource for breeding. Selection of key traits related to domestication caused selective sweeps around the genes controlling those traits, potentially resulting in a loss of genetic diversity in those regions. In an attempt to recover some of the diversity in the regions of the selective sweeps as well as other chromosomal regions, we assembled 300 lines derived from 23 different G. max x G. soja crosses involving 12 G. soja accessions. These sets of lines were developed through both backcrossing and single crosses and all lines were selected for agronomic appearance. Yield testing was conducted at two locations in 2015. Out of 253 lines that were directly compared to the G. max parent, 43 lines were not significantly different from the soybean parent. The set of 43 lines are derived from five different crosses involving five G. soja accessions. The lines will also be genotyped using GBS, producing SNPs to identify introgressed regions from G. soja. Introgressions found in chromosomal regions subjected to selective sweeps during domestication will be of special interest.

P106
Initiating a Genomic Selection Pipeline for Public Soybean Breeders Using Uniform Regional Test Data

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The USDA coordinated Uniform Soybean Tests are a rich source of publicly available phenotypic data. Performed annually, since 1939, these tests are replicated, region-wide field trials of elite lines submitted from breeding programs across the US and Canada. In addition to yield measures, the phenotypic data includes a range of agronomic, seed quality, and disease scores that are integral to the cultivar release decisions made by public soybean breeders. Despite the extensive effort that went into collecting this data, relatively little comprehensive exploration of the dataset has been performed, largely due to the inaccessible nature of the records, which currently exists in books, pdfs, and discordant spreadsheets. To take advantage of this resource, we are developing a searchable database to house the phenotypic data of the Northern States (maturity groups 00-IV), along with the relevant environmental and pedigree data. Additionally, soybean entries from the past 10 years of tests will be genotyped using Genotyping by Sequencing (GBS) technology. This will provide a framework for analyzing many aspects of the performance and genetic composition of elite public soybean varieties, both overtime and across various growing environments. Ultimately, with the addition of genotypic data, we hope to develop robust genomic prediction models that will serve as a community resource for breeders across the northern soybean growing region. Currently, phenotypic records from years 1989-2015 have been processed, and entries from the 2015 regional trials have been genotyped. Over these 27 years, 7,642 genotypes have been assayed in over 199 locations.

P107
Insight into the Genomic Regions Under Breeding Selection and Genomic Selection for Yield in Soybean

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Increasing soybean yield potential is the ultimate goal for soybean breeding programs. Improved genetic yield potential is critical to the success of any new soybean cultivar and a main component that drives the profitability of soybean. Utilizing the genomic technology will help improve the rate of genetic gain in soybean yield. In this study, we attempt to identify the haplotype regions under breeding selection in soybean populations and build a reference population by utilizing elite breeding populations and advanced lines to conduct genomic selection (GS) for yield. Using SoySNP50k Infinium chips data, we performed genome-wide analyses of the North American soybean ancestral lines and modern cultivars and identified the signature of selection in these populations. Over 100 lines developed at eight public institutions were derived from exotic PI 416937 and have been entered into USDA Uniform Tests over past 15 years. We conducted the genome-wide analysis of these elite lines along with their parents derived from PI 416937 to identify the contributions of the haplotype alleles to these high yielding lines. Thirteen genomic regions from PI 416937 were selected for and 15 genomic regions that have been selected against. We have selected four elite soybean breeding populations with PI 416937 presenting in their pedigrees to form a reference population for genomic selection. Yield data from these populations have been collected over the past two years and the populations were genotyped with SoySNP6k Infinium chips. Preliminary results indicated the prediction accuracy of GS was 0.35-0.37. An integration of GS into soybean breeding programs will be discussed.
P108
Mapping Traits Related to the Domestication of Soybean from Glycine soja

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Soybean (Glycine max) was domesticated in East Asia from the wild progenitor Glycine soja. The domestication process led to many morphological changes that are essential for cultivation, including larger seed size and upright growth. The objective of this research is to map the genes or QTLs that underlie traits differentiating the two species to help understand the genetic basis of soybean domestication. Through genetic dissection of the key domestication-related traits, we also hope to identify targets for introgressing novel alleles or genes into Glycine max which were eliminated by selective sweeps during domestication. To accomplish our objectives, we performed field evaluations of two RIL populations consisting of a total of 800 lines from interspecific crosses between Williams 82 and the wild accessions PI468916 and PI479752. These lines were selected from a total population of over 3,000 lines to represent all combinations of extreme phenotypes for each pair of traits. The experimental lines were grown at two locations for three years. Eleven traits were evaluated, including days to R1 and R8, height, lodging, stem diameter, leaflet size and shape, pod shattering, and seed weight. QTL mapping was done using SNP markers obtained through genotyping-by-sequencing. This mapping study detected both previously identified and novel QTL for traits relating to soybean domestication.

P109
Detection of Transmission-Disequilibrium in Elite Pedigrees Can Improve Selection Inference and Genomic Prediction

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The soybean community possesses extensive pedigree information spanning the last seventy years of modern breeding. Lines from these pedigrees have often been preserved and can now be genotyped using a range of genome-wide platforms. Previous researchers have used pedigree information and modified transmission-disequilibrium tests to identify alleles under selection. Though informative, these studies suffered from low marker density or gapped pedigrees that made rigorous statistical inference problematic. Even more problematic is the question of how the strength of selection in prior improvement can be used to enhance contemporary breeding efforts. Using 131 genotyped individuals comprising 53 breeding trios, we identified regions that have been selected during the development of elite Southeastern cultivars. We demonstrate how, after normalizing by linkage disequilibrium in these populations, a reasonably accurate and general genomic prediction model can be formulated. Potentially, such a model can be integrated with program-specific yield data to enable prediction accuracies that make genomic selection a more effective breeding strategy.

P110
Fine Mapping and Candidate Gene Analysis of Root Nodules in Soybean

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Biological nitrogen fixation (BNF) is a complex process involving the interaction between the plant and the symbiotic rhizobia in soybean. Higher rate of soybean nitrogen fixation is important to achieve high yield and good quality. Root nodule number (RNN) is significantly correlated with the rate of nitrogen fixation, but few studies about quantitative trait loci (QTL) associated with RNN are available in soybean. In this study, an F5:7 recombinant inbred line (RIL) mapping population derived from the cross between soybean local cultivar, Yiqianli, and wild soybean accession, Changling was developed. The 200 RILs were evaluated for BNN in 2014 and 2015. Genotypes were determined for 200 RILs using 4564 specific length amplified fragment (SLAFs) markers. The high-density genetic map obtained has 3403.90 cM with an average interval of 0.94 cM. Composite interval mapping (CIM) analysis was used to identify QTL for BNN. A major QTL, qBNN7, was located on chromosome 7 with the flanking markers M33856 and M33594, which explained 31.7% of the phenotypic variation. Within this interval, 6 candidate genes for BNN were annotated. Three candidate genes in that major QTLs were obtained. It has been confirmed that two of the candidate genes encoded receptor kinases, and the remaining candidate gene encoded C2H2 zinc finger protein through the verification and functional annotation of all predicted genes and their protein products. In addition, all these three candidate genes were expressed in soybean root nodules. The candidate gene encoded C2H2 zinc finger protein has the highest expression level. However, there is a need to further study and exploring the modulating action and mechanism of these three candidate genes on the formation of soybean root nodules.
P111

A Brassica napus Bi-directional Promoter Exhibits Differential Leaf-expression Patterns in Soybean Transgenic Lines

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Many plant species are capable of being transformed with multiple transgenes from other species to introduce agronomically desirable traits or characteristics. However, these genes are frequently controlled by identical or homologous promoters. Homology-based gene silencing is likely to arise when multiple introduced transgenes are driven by the homologous promoters. Here, we report the expression of a bi-directional promoter derived from Brassica napus (BBP) driving two reporter genes GUS and GFP. Agrobacterium-mediated transformation of soybean (Glycine max) was carried out with two experimental binary vectors consisting of BBP regulating GUS in the forward, or the native orientation, and GFP in the reverse orientation, or BBP regulating the reporter genes in the opposite orientation. Transgenic plants generated displayed the expression of GUS and GFP confirming that BBP drives expression in both directions at reasonable levels. Depending on the orientation of the genes there are differences in the expression patterns within the leaf tissues. Thus, bi-directional promoters can be used to effectively drive multiple transgenes for desired traits.

P112

Positional Gene Cloning and Sequencing of Two Soybean Cyst Nematode Resistance Loci

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Glycine soja, the wild annual ancestor of soybean, can be used as a source of genetic diversity for the crop. PI 468916, a Glycine soja line, contains two resistance QTL (cq-SCN006 and cq-SCN007) to soybean cyst nematode (SCN) Heterodera glycines, a major pest of soybean. These loci have been fine mapped to intervals of 300 kilobase pairs and 147 kilobase pairs, respectively. Through screening a fosmid library and de novo Illumina sequencing, the sequences of the two intervals are being elucidated in detail. The alignment of the sequence of the DNA from the resistance gene interval in PI 468916 to Williams 82 allows for the discovery of differences between the two sequences, of which there are many. Changes in the sequences include single nucleotide variants as well as insertion or deletions. In some areas of these loci we have observed a very high frequency of variants (both SNVs and indels) which are similar to a kataegis or mutational storm. These regions of localized hypermutation have been observed in some cancer genomes. We analyzed these differences in the sequences of PI 468916 and Williams 82 for their potential to alter encoded proteins, and affect splice sites or promoters. Very many of these differences could be the source of the SCN resistance of these loci. Precise identification of these novel resistance loci could be helpful in precision breeding approaches or using transgenic methods to introduce new resistance into soybean.
CONNECT TO THE INTERNET

1. Connect to the following wireless network: HyattMR
2. Open your web browser. This will automatically take you to the login page. If not, please type www.psav.com into your address bar and the login page should come up.
3. Enter the following case-sensitive password: SoyRules
NEARBY RESTAURANTS

(See next page for listings)
NEARBY RESTAURANTS

Market Stand Café
350 North High Street
0.0 miles from Hyatt Regency Columbus

Gourmet Grill
400 North High Street, Greater Columbus Convention Center
0.0 miles from Hyatt Regency Columbus

Subway
400 North High Street, Greater Columbus Convention Center
0.0 miles from Hyatt Regency Columbus

Mykonos Gyros
400 North High Street, Greater Columbus Convention Center
0.0 miles from Hyatt Regency Columbus

Gallerie Bar and Bistro
401 North High Street, Hilton Columbus Downtown
0.0 miles from Hyatt Regency Columbus

Martini Modern Italian
445 North High Street
0.1 miles from Hyatt Regency Columbus

Gordon Biersch Brewery Restaurant
401 North High Street
0.1 miles from Hyatt Regency Columbus

Buca di Beppo
343 North Front Street Downtown, Arena District
0.1 miles from Hyatt Regency Columbus

Barley’s Brewing Company
467 North High Street
0.2 miles from Hyatt Regency Columbus

Bareburger
463 North High Street
0.2 miles from Hyatt Regency Columbus

Kooma Sushi Restaurant
37 Vine Street
0.2 miles from Hyatt Regency Columbus

Japanese Steak House
479 North High Street
0.2 miles from Hyatt Regency Columbus

Rodizio Grill
125 West Nationwide Blvd.
0.2 miles from Hyatt Regency Columbus

Double Comfort Restaurant
505 North High Street
0.2 miles from Hyatt Regency Columbus

Bd’s Mongolian Grill
295 Marconi Blvd.
0.2 miles from Hyatt Regency Columbus

The North Market
59 Spruce Street
0.2 miles from Hyatt Regency Columbus

Hyde Park Prime Steakhouse
569 North High Street, The Cap at Union Station
0.3 miles from Hyatt Regency Columbus

Elevator Brewery & Draught Haus
161 North High Street
0.3 miles from Hyatt Regency Columbus

Nada
220 West Nationwide Blvd.
0.3 miles from Hyatt Regency Columbus

Sunny Street Café
277 West Nationwide Blvd.
0.4 miles from Hyatt Regency Columbus

Marcella’s Ristorante
615 North High Street
0.4 miles from Hyatt Regency Columbus

The Pearl
641 North High Street
0.4 miles from Hyatt Regency Columbus

Restaurants include, but not limited to: Dos hermanos, Flavors of India, Hubert’s Polish Kitchen, Little Eater, Nida’s Sushi
COLUMBUS ENTERTAINMENT

Local Shopping:
• North Market Farmers Market, 59 Spruce Street
• Short North “Art and Soul of Columbus,” shortnorth.org/shopping/
• The Shops on Lane Avenue, theshopsonlaneavenue.com
• Easton Town Center, 160 Easton Town Center
• Polaris Fashion Place, 1500 Polaris Parkway

Local Movie Theaters:
• AMC Lennox Town Center 24, 777 Kinnear Rd.
• Grandview Theater and Drafthouse, 1247 Grandview Ave.
• Gateway Film Center, 1550 N High Street
• Studio Movie Grill, 175 W Nationwide Blvd.

Local Parks:
• Goodale Park, 120 W. Goodale Street
• North Bank Park, 311 W. Long Street
• Scioto Audubon, 400 W. Whittier Street
• Chadwick Arboretum & Learning Gardens, 2001 Fyffe Rd.

Local Transportation:

Bus Service:
• COTA, One-way fares as low as $2; day passes as low as $4.50, COTA.com, (614) 228-1776
• CBUS, FREE downtown Columbus circulator running every 10-15 minutes from the Short North Arts District to the Brewery District, Cota.com/ CBUS, (614) 228-1776
• COTA AirConnect, Cost $2.75 each way, runs to/from John Glenn International Airport and downtown Columbus

Taxi Service:
• Yellow Cab, (614) 444-4444
• Orange Cab, (614) 414-0000

Other Options:
• Car2go, Columbus.car2go.com, 1-877-488-4224
• CoGo, cogobikeshare.com, 1-855-877-COGO

Local Attractions:

Center of Science and Industry (COSI)
333 W. Broad Street, www.cosi.org
COSI is one of the premier public education centers in the U.S.
Over 33 million visitors have learned about science, industry, health, and history through interactive exhibits and activities.

Columbus Museum of Art
480 E. Broad Street, www.columbusmuseum.org
Formed in 1878, the Columbus Museum of Art is the oldest art museum in Ohio. Notable paintings by Picasso, Juan Gris, and Monet can be observed in the permanent collection.

Franklin Park Conservatory and Botanical Gardens
1777 E. Broad Street, www.fpconservatory.org
Franklin Park Conservatory is a beautiful facility, featuring both indoor and outdoor gardens. The diverse plant collections and nature-based exhibits make this one of the most enjoyable destinations in central Ohio.

Columbus Zoo and Aquarium
Take a trip around the world by visiting the Columbus Zoo and Aquarium. There you can observe more than 9,000 animals, representing 650 species of interesting critters.

Thurber House
77 Jefferson Ave., www.thurberhouse.org
The Thurber House is located at the former home of author and cartoonist James Thurber. Literary fans will enjoy this tribute to the literary and artistic achievements of Thurber.

Topiary Garden
480 E. Town Street, www.topiarypark.org
Consider visiting this unique public art exhibit. Located on a seven acre park, the Topiary Garden is a ‘real-life’ interpretation and model of the painting “A Sunday Afternoon of the Isle of La Grande Jatte.”

Billy Ireland Cartoon Library & Museum
1858 Neil Avenue Mall, www.cartoons.osu.edu
The Billy Ireland Cartoon Library & Museum is home to the largest collection of cartoons and comics. Over 300,000 original cartoons, 45,000 books and 2.5 million comic strips are in the current collection.

Wexner Center for the Arts
1877 N. High Street, www.wexarts.org
Located on the Ohio State University campus, the Wexner Center for the arts is OSU’s multidisciplinary laboratory of contemporary arts. Visit the Center to observe original art exhibits, films, and performances.

Ohio State Fair
717 E. 17th Ave., www.ohiostatefair.com
Enjoy the final day of the Ohio State Fair on August 7th. The fair will be host to diverse exhibits representing what makes Ohio great! Enjoy the daily activities, food vendors, and agricultural education booths.

*For more information on Columbus attractions and events, please visit Experience Columbus at www.experiencecolumbus.com