Biennial Cellular and Molecular Biology of the Soybean Conference

Come together, right now, over the soybean: with apologies to John & Paul

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#SOY2023

UNIVERSITY OF NEBRASKA–LINCOLN

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WELCOME TO SOY2023

The inaugural biennial conference on the Cellular and Molecular Biology of the Soybean was held 36 years ago (Soy1986) at Iowa State University, with the stellar USDA soybean geneticist, Randy Shoemaker, serving as the host.

The 11th biennial, Soy2006, was held in Lincoln, Nebraska, hosted by the University of Nebraska-Lincoln soybean geneticist, James Specht. Seventeen years later, the conference, Soy2023, returns to Lincoln, Nebraska.

Soy2023 brings together a diversity of STEM professionals and learners to exchange ideas and research outcomes, and to discuss challenges to enhance our understanding of soybean biology. Through the sharing of multipronged experimental studies with Glycine max and complementary organisms, the 19th Cellular and Molecular Biology of the Soybean Conference will span the scale from the single cell to the fields to collectively advance our knowledge of this wonderful legume feedstock that is globally valued for its quality oil and protein.

The Program Committee is grateful to the Session Chairs for their willingness to serve and make Soy2023 a success. For those of you have visited the capital city of the "Cornhusker" State, welcome back. For those who are visiting for the first time, have fun.

Sincerely,

Soy2023 Program Committee

Marc Libault

Toshihiro Obata

Katarzyna Glowacka

Support Staff

Connie Hansen

Lana Johnson

Fran Benne

Kool-Aid the fruit-flavored drink mix that powered the baby boomer generation was created by Edwin Perkins from Hastings, Nebraska.

The Reuben sandwich is made of corned beef, Swiss cheese, sauerkraut & Russian dressing on rye bread. The popular sandwich was first created by Reuben Kulakofsky, a Lithuanian born restaurateur, who lived in Omaha, Nebraska.

SOYBEAN COMMUNITY AWARDS

Mary Coker Joslin Early Career Award

As a student as Vasser College in 1942, Mary Coker Joslin crossed lines Tokyo (from Japan) and Nanda (from Korea), to eventually generate the line Majos. Majos was used to develop Hampton and Stuart, which were released in 1962, both lines reported high yields, shatter resistance, high oil, and disease resistance. These lines were considered valuable contributions to soybean growers in the Southeastern US. Mary Coker Joslin may be the first recorded female plant breeder.

Richard (Dick) Bernard Mid-Career Award

Dr. Bernard was a USDA-ARS Research Geneticist at the University of Illinois who made immense contributions to soybean genetics and breeding including developing the Clark and Harosoy near-isogenic line collections, and the development of Williams, a soybean line that occupied a substantial portion of USA acreage in the 1970s. These contributions continue to be utilized by soybean geneticists, breeders, and growers today.

2022- Kristin Bilyeu, USDA-ARS, Columbia, Missouri

2022- Jianxin Ma, Purdue University

William J. Morse Career Achievement Award

In 1907, William Morse was hired by the USDA as a breeder working with grasses, legumes, and new forage plants, making him the first USDA soybean breeder. Morse was one of the founders of the American Soybean Association and oversaw the expansion of soybean from 1,629 acres in 1909 to 15 million acres in 1943. One of his greatest contributions was participating in the Dorsett and Morse expedition in 1929, a 2-year excursion to China to collect soybean varieties and learn about soybean growing and processing. This expedition returned with 4,451 varieties, 22% of which are still in the USDA germplasm collection, including over 100 varieties of edamame, which was not previously known in the US.

2022- Brian Diers, University of Illinois

2022- Lila Vodkin, University of Illinois

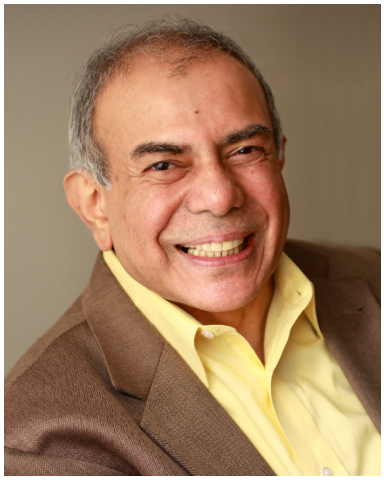
PLENARY SPEAKERS

JULIA BAILEY-SERRES

Director, Center for Plant Cell Biology (CEPCEB) | Director, NSF Plants3D NRT | Institute of Integrative Genome Biology | UC MacArthur Foundation Chair | Distinguished Professor of Genetics | Bailey-Serres Group & Plasticity Project

Bailey-Serres' program conducts translational plant biology research activities, from gene to field scale, to gain insight on genetic underpinnings that impart protection of yield in crops when challenged with abiotic stresses including drought, flooding and nutrient limitation.

Botany and Plant Sciences, 4119 Genomics Building, University of California, Riverside, CA 92521; 951-827-3738 or serres@ucr.edu



GANESH KISHORE

Co-founder and Co-Managing Partner at Spruce Capital Partners and MLS Capital Fund II.

Kishore's career has provided leadership in biotechnology research, development and business. His leadership accomplishments have led to innovations that have had significant impact on the global bioeconomy.

kishore@sprucecp.com and https://www.sprucecp.com/kish

MARÍA EUGENIA ZANETTI

Professor of Molecular Biology and Biotechnology, Universidad Nacional de La Plata, Buenos Aires, Argentina

Eugenia Zanetti's program is broadening our understanding of the mechanisms underlying the relationship between members of the Fabaceae and the nitrogen fixing symbionts within the Rhizobiaceae.

Instituto de Biotecnología y Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CCT La Plata CONICET, Calle 115 e/ 49 y50 C.P.1900 La Plata, Argentina. ezanetti@biol.unlp.edu.ar

AGENDA

THURSDAY, AUG. 10

TIME	EVENT
12 pm	Poster set-ups can proceed
3:30 pm	Workshop (Science Communications)
5:30 pm	Welcome/Opening Remarks
5:45 pm	**Ganesh Kishore** – Plenary Speaker
6:50 pm	Awards (SoyGec Rep)
7 pm	Soybase Workshop
7 pm	Dinner on your own

FRIDAY, AUG. 11

TIME	EVENT
7:30 am	Registration Desk Opens
8 am	**BIOTIC INTERACTION SESSION 1** Session Chair: **Melissa Mitchum**, Department of Plant Pathology, University of Georgia
8:10 am	**Adam Steinbrenner**, Assistant Professor, Department of Biology, University of Washington – *Soybean bites back: Interspecies transfer of a receptor gene to resist lepidopteran pests*
8:40 am	**Andrew Scaboo**, Assistant Professor, Division of Plant Science and Technology, University of Missouri – *Beyond the norm: New strategies using native genes for combating virulent soybean cyst nematode populations*
9:10 am	**Jen Jaqueth**, Research Scientist, Corteva Agriscience – *Deploying disease resistance genes in a CRISPR edited Disease Super Locus*
9:40 am	*__Sutton Tennant__, Graduate Student Researcher, Department of Agronomy and Horticulture, University of Nebraska–Lincoln – *Nuclear retention of transcripts as regulatory mechanism of protein translation in soybean root and nodule cells* *Poster selected for oral presentation.
10:10 am	Coffee Break

AGENDA

FRIDAY, AUG. 11

TIME	EVENT
10:30 am	**GENETIC/BREEDING FOR OUTPUT TRAITS** Session Chair: **Felix Fritschi**, Division of Plant Science and Technology, University of Missouri
10:35 am	**Carrie Miranda,** Assistant Professor, Department of Plant Sciences, Soybean Breeding Program North Dakota State University – *Determining genetic mechanisms of maturity in North Dakota: expanding the molecular model for MG 00 and 0*
11:05 am	**Siva K. Chamarthi**, Postdoctoral Fellow, University of Missouri – *Association mapping confirms known loci and identifies new loci which control canopy temperature in Soybean*
11:35 am	**Benjamin Fallen**, Research Agronomist, USDA-ARS Soybean and Nitrogen Fixation Research Unit, Raleigh, NC – *Developing Genetically Diverse Soybean Germplasm with an Improved Seed Composition*
12:05 pm	*__Hyojin Kim__, Postdoctoral Researcher, Center for Plant Science Innovation, University of Nebraska–Lincoln – *Development of EPA- and Astaxanthin-Enriched Soybean Germplasm for Aquaculture Feedstocks* *Poster selected for oral presentation.
12:35 pm	Lunch Provided
1:35 pm	**PROTEOMICS/METABOLOMICS** Session Chairs: **Doug Allen**, USDA-ARS and Donald Danforth Plant Science Center, and **Michaela McGinn**, Smithbucklin
1:40 pm	**Ruthie Angelovici**, Associate Professor, University of Missouri – *Can multi-omic integration lead to a better understanding of proteomic reprogramming and rebalancing? Lessons learned from Arabidopsis*
2:10 pm	**Kristin Haug Collet**, Research Scientist, Corteva Agriscience – *A single amino acid mutation in a transcriptional repressor increases oil and protein content in soybean*
2:40 pm	**Timothy P. Durrett**, Associate Professor, Kansas State University – *Orchestrating seed metabolism to enhance synthesis of novel oils*
3:10 pm	*__Trish Tully__, Postdoctoral Associate, Donald Danforth Plant Science Center – I*ncreasing Sulfur Content in Soybean Seed Protein* *Poster selected for oral presentation.
3:40 pm	Coffee break

AGENDA

FRIDAY, AUG. 11

TIME	EVENT
3:50 pm	**GENOMICS/TRANSCRIPTOMICS** Session Chair: **Jason Nichols**, Principal Scientist, Syngenta Crop Protection, LLC
3:55 pm	**Aamir W. Khan**, Postdoctoral Researcher, Division of Plant Science and Technology, University of Missouri – *Advancing Soybean Genomics for Enhanced Haplotype-Based Trait Mapping*
4:25 pm	**Gary Stacey**, Curator's Distinguished Professor, Divisions of Plant Science and Biochemistry, University of Missouri – *Soybean has a lot to Offer*
4:55 pm	***Sergio Alan Cervantes-Perez**, Postdoctoral Research Associate, Department of Agronomy and Horticulture, University of Nebraska–Lincoln – *Tabula Glycine: The Glycine max single-cell resolution transcriptome atlas* *Poster selected for oral presentation.
5:25 pm	Break
5:40 pm	**María Eugenia Zanetti** – Plenary Speaker
6:35 pm	Dinner on your own/Poster/Social
7:05 pm	Saltdogs vs Kansas City - game starts at 7:05 pm Haymarket Park - 403 Line Drive Circle

AGENDA

SATURDAY, AUG. 12

TIME	EVENT
7:45 am	Registration Desk Opens
8 am	**BREEDING/GENETICS FOR YIELD/PROTECTION OF YIELD** Session Chairs: **Aaron Lorenz**, University of Minnesota, and **Carrie Miranda**, Assistant Professor, North Dakota State University
8:05 am	**Leah McHale**, Professor of Breeding & Genetics, The Ohio State University – *Breeding Soybean for Quantitative Disease Resistance to Phytophthora sojae*
8:35 am	**Natalia de Leon**, Professor, Department of Agronomy, University of Wisconsin, Madison – *Ten Years of the Genomes-to-Fields Maize GXE Project: Lessons and Opportunities*
9:05 am	**Kyle Kocak**, Research Scientist, Corteva Agriscience – *Breeding for Yield in Industry*
9:35 am	***Vishnu Ramasubramanian**, Postdoctoral Associate, University of Minnesota – *A Genomic Selection Pipeline for Public Soybean Breeding Programs* *Poster selected for oral presentation.
10:05 am	Coffee break
10:35 am	**BIOTIC INTERACTION SESSION 2** Session Chair: **Andrew Bent**, Department of Plant Pathology, College of Agricultural & Life Sciences, University of Wisconsin–Madison
10:40 am	**Naoufal Lakhssassi**, Associate Scientist, Adjunct Assistant Professor, Department of Plant Soil and Agricultural Systems, Southern Illinois University at Carbondale – *An update about SCN resistance: Peking-type and other new SCN resistant sources*
11:10 am	**Shin-Yi Marzano**, Research Molecular Biologist, U. S. Department of Agriculture ARS – *Developing alternative viro-control and RNAi-based approaches to reduce white mold infection*
11:40 am	**Francois Belzile**, Plant Science Department and Institute for Integrative and Systems Biology (IBIS), Université Laval – *Using genomics to unravel the complexities of the interaction between soybean and Phythophthora sojae*
12:10 pm	***Md Sabbir Hossain**, Doctoral Student, Department of Agronomy and Horticulture, University of Nebraska–Lincoln – *An integrated single-cell comparative transcriptomic and evolutionary analysis of the legume membrane microdomain-associated protein-coding genes during the nodulation process* *Poster selected for oral presentation.
12:35 pm	Lunch Provided

SATURDAY, AUG. 12

TIME	EVENT
1:35 pm	**GENETIC ENGINEERING** Session Chairs: **Robert Stupar**, Department of Agronomy and Plant Genetics, University of Minnesota, and **Wayne Parrott**, Department of Crop and Soil Sciences, University of Georgia
1:40 pm	**Margaret Frank**, Assistant Professor, School of Integrative Plant Science, Plant Biology Section, Cornell University – *Engineering approaches to develop hybrid soybean*
2:10 pm	**Gunvant Patil**, Assistant Professor, Institute of Genomics for Crop Abiotic Stress Tolerance, Texas Tech University – *Editing for abiotic stress outcomes in soybean*
2:40 pm	**Nathan Hancock**, Associate Professor, University of South Carolina Aiken – *Harnessing the mPing Transposable Element for Gene Discovery and Precision Genome Engineering*
3:10 pm	***Vikranth K Chandrasekaran**, Postdoctoral Fellow, Division of Plant Sciences, University of Missouri – *Facilitating gene discovery in soybean through mutagenesis: Identification of novel genes controlling the production of four-seeded pods* *Poster selected for oral presentation.
3:40 pm	Coffee break (20 minute break)
4:10 – 6:10 pm	**PANEL DISCUSSION** *Opportunities in advancing genetic and genomic translations in soybean biology* PANEL DISCUSSION LEAD **Brad Zamft** – Project Lead, x.company PANELIST **Shveta Bagga** – Protein Core Technologies Lead, Corteva Agriscience **Selma Davis** – Head of Soybean Product Design, Bayer Crop Science **Tengfang Huang** – VP & Head of Research, Traitology **Ian Miller** – Chief Development Officer, Pairwise The panel will be an open dialog to discuss issues/challenges that are associated with getting agricultural technologies, with a little soybean centric to it, from the lab to the marketplace. Topics will include STEM workforce needs, logistics of identity preservation of output traits, along with discussion around future investments to help foster getting technologies onto the market, including outreach activities to better inform the consumer about the challenges facing the global food supply chain and safety of traits developed through the tools of biotechnology.

SATURDAY, AUG. 12

TIME	EVENT
6:30 pm	**Julia Bailey-Serres** – Plenary Speaker
7:15 pm	Social/poster viewing
7:45 pm	Conference dinner
8:45 pm	Social/on your own/poster awards

SUNDAY, AUG. 13

TIME	EVENT
9 am	SoyGEC Report
10 am	Closing remarks

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SPEAKER ABSTRACTS

BIOTIC INTERACTION SESSION 1

SESSION CHAIR: **Melissa Mitchum**, Department of Plant Pathology, University of Georgia

Soybean bites back: Interspecies transfer of a receptor gene to resist lepidopteran pests

Adam Steinbrenner, Assistant Professor, Department of Biology, University of Washington

Plant defense responses to pests and pathogens require immune receptor genes to activate resistance. We recently identified Inceptin Receptor (INR), a receptor which detects In11, a ubiquitous 11-amino acid peptide in the oral secretions of Lepidopteran larvae (caterpillars). In11 elicits strong anti-herbivore direct and indirect defenses on cowpea, common bean, mung bean, and other legumes, but is inactive on all tested soybean varieties. Comparative genomic analysis revealed that the INR gene is specific to the ~28 my old tribe of Phaseoloid legumes, but is absent in the subtribe Glycininae which includes wild, perennial, and cultivated soy (Glycine sp.), suggesting recent secondary of In11 recognition in critical crop lineages. We hypothesized that restoration the missing INR immune receptor would enhance soybean responses to herbivory. Transgenic lines of Williams 82 (WT) soybean expressing INR from common bean (Phaseolus vulgaris) restored In11 responsiveness measured by rapid In11-induced release of the defense hormone ethylene. Beet armyworm (Spodoptera exigua) larvae reared on a T1 line (segregating for INR) gained 15% less weight than on a separate line lacking the transgene. We will report on ongoing experiments to measure both direct and indirect defenses to herbivory in INR-transgenic soybean lines, including analysis of In11-induced specialized metabolites and plant volatiles. INR provides a potential defense trait for recruitment of natural enemies and induction of endogenous inducible defenses, to augment Bt-based transgenic resistance to Lepidopteran pests.

Beyond the norm: New strategies using native genes for combating virulent soybean cyst nematode populations

Andrew Scaboo, Assistant Professor, Division of Plant Science and Technology, University of Missouri

TBD

Deploying disease resistance genes in a CRISPR edited Disease Super Locus

Jen Jaqueth, Research Scientist, Corteva Agriscience

Disease resistance is one of the top priority traits for farmers. In 2021, US farmers lost 318 million bushels of corn yield to four top fungal diseases: northern leaf blight, southern rust, gray leaf spot and anthracnose stalk rot. This need is expected to increase in the coming years, due to the changing climate that can affect disease patterns and severity of infection. Earlier this year, Corteva announced that we are using a CRISPR gene editing approach to reposition native resistance genes into a single location in the genome. This gene-edited product will harness corn's native resistance to these four diseases giving farmers a sustainable alternative to fungicides. Most of these disease resistance genes originate from non-US maize varieties, therefore we will be offering US farmers novel sources of disease resistance. While this gene-edited plant breeding approach is initially being applied to corn diseases that most concern North American farmers, it has the potential to be scaled to other crops, incorporate other diseases or be otherwise tailored to specific geographies.

*Nuclear retention of transcripts as regulatory mechanism of protein translation in soybean root and nodule cells

Sutton Tennant, Graduate Student Researcher, Department of Agronomy and Horticulture, University of Nebraska–Lincoln, sutennant@huskers.unl.edu

The central dogma of molecular biology follows a simple path, DNA is transcribed into transcripts in the nucleus, and transcripts are then translated into proteins in the cytosol. However, many studies reported that protein production is not solely impacted by the level of expression of genes, but by many other regulatory processes. The number of studies exploring these post-transcriptional regulatory processes in plants is sparse. Here, combining the use of single-nucleus transcriptomic and high-resolution fluorescent in situ hybridization technologies, we provide a new perspective on the role of the nuclear retention of transcripts as a central mechanism to control RNA biology and the biology of plant cells.

SPEAKER ABSTRACTS

The analysis of confocal microscopic images of transcripts at the sub-cellular resolution combined with the use of a specifically designed Image J software package, clearly revealed the differential nuclear retention of transcripts between genes, cell types, and organs of the soybean root and nodule. This work reveals the influence of the sub-compartmentalization of transcripts as another regulatory mechanisms of protein translation and a new understanding of the central dogma of molecular biology.

*Poster selected for oral presentation.

GENETIC/BREEDING FOR OUTPUT TRAITS

SESSION CHAIR: **Felix Fritschi**, Division of Plant Science and Technology, University of Missouri

Determining genetic mechanisms of maturity in North Dakota: expanding the molecular model for MG 00 and 0

Carrie Miranda, Assistant Professor, Department of Plant Sciences, Soybean Breeding Program, North Dakota State University

Production areas of soybean have grown in North Dakota to make it the number one crop in the state however state yield averages are among the lowest in the Midwest. Maturity is one of the most important agronomic traits impacting yield potential. North Dakota is characterized by having a short season length due to frost and is necessary to have early maturing soybean. The predominate maturity groups grown in North Dakota are MG 00 and 0. It is possible to "fine tune" maturity to a region/environment to maximize yield potential. The major genetic mechanisms of soybean maturity are well characterized The genes E1, E2, and E3 have the largest effect on soybean maturity, where the functional allele of these genes condition for late maturity and the null or semi-functional alleles condition for early maturity. It has been determined that variations of the non-functional or semi-functional alleles of these three genes create the MG 00 or MG 0 phenotype. However, it is not understood which combination of alleles is most favored for breeding purposes which can potentially affect yield. In addition, since these major genes are not fully functional it can be hypothesized that there are other genes that play a major effect in this environment which may be only minor in other maturity groups with functional E genes. The goal of this research is to determine the major effect maturity alleles present in North Dakota MG 00 and 0 germplasm. This will be accomplished by exploring known maturity gene alleles in the North Dakota State University breeding program to determine which alleles are favored. In addition, these alleles will be determined over time in historical cultivars to determine if preference changed over time. These results will enrich knowledge of the maturity molecular model necessary to create an MG 00 and 0 cultivar and could possibly identify new genetic mechanisms for yield gains.

Association mapping confirms known loci and identifies new loci which control canopy temperature in Soybean

Siva K. Chamarthi, Postdoctoral Fellow, University of Missouri

Siva K. Chamarthi1,[2,5], Avjinder S. Kaler[2], Hussein Abdel-Haleem[3], Felix B. Fritschi[1], Jeffery D. Ray[4], James R. Smith[4], C. Andy King[2], Larry C. Purcell[2], Jason D. Gillman[5*]

[1]University of Missouri, Columbia, MO; [2]University of Arkansas, Fayetteville, AR; [3]USDA-ARS, Maricopa, AZ; [4]USDA-ARS, Stoneville, MS; [5]USDA-ARS, Columbia, MO

Drought is a major global constraint for crop productivity in rain-fed areas and Improving crop tolerance to drought is of critical importance. Less than 10% of soybean acreage is irrigated and 20-70% of soybean growing regions experience significant drought in any given year. Drought incidence and severity are projected to increase due to the ongoing effects of climate change. Active transpiration under drought indicates a genotype that still has access to soil moisture. Thus, canopy temperature (CT) can be a scalable and indirect measure of transpiration and stomatal conductance with value in distinguishing differences among genotypes in response to under-water replete and water-limited conditions. The objectives of the present study were to confirm the loci associated with CT identified previously by genome-wide association mapping and to identify novel loci using a panel of 205 diverse, maturity group (MG) IV soybean accessions.

SPEAKER ABSTRACTS

These 205 accessions were planted in seven locations across two years under six irrigated and six drought environments. Within each location, CT was normalized (nCT) on a scale from 0 to 1. By conducting genome-wide association mapping (GWAM), we identified 163 significant SNPs represented by 117 loci associated with nCT. Of these loci, 69 were consistent with earlier studies and 48 were novel loci. Remarkably, two SNPs found in our new study matched previously discovered regions for nCT and slow canopy wilting, located near the Glyma04g36870 and Glyma04g37330 genes, which have annotations involved in the regulation of water transport, stomata, and temperature responses. We also identified extreme genotypes with cooler canopies and slow wilting, as well as warmer canopies with fast wilting, compared to previous studies. We identified 156 candidate genes out of 163 significant SNPs, with 15 SNPs located within genes related to plant stress responses and other drought-related traits. These confirmed genomic loci provide a valuable resource to enhance soybean drought tolerance by pyramiding favorable alleles for nCT.

Developing Genetically Diverse Soybean Germplasm with an Improved Seed Composition

Benjamin Fallen, Research Agronomist, USDA-ARS Soybean and Nitrogen Fixation Research Unit, Raleigh, NC

Soybean is a major source of protein and oil in animal and human diets. Almost 75% of the total soybean meal produced in the U.S. is used for feeding livestock, with poultry and swine being the two largest consumers. To meet the growing demand for soybean meal previous efforts were focused on quantity over quality, which inadvertently led to a decrease in protein content due to an inverse relationship between protein and yield. The narrow genetic base of the U.S. soybean crop limits the ability to enhance seed composition, specifically increasing the protein content. The genetic and phenotypic diversity in wild soybean makes it an excellent source for soybean improvement. The challenge when breeding with wild soybean is the undesirable agronomic traits inherited from the wild parent. We accessed the impact of wild soybean on protein content and subsequent selection for yield. Our research led to the development of more than a dozen breeding lines with a 12.5-50% soja genome shown to yield 91-104% and exhibit 103-106% seed protein compared to elite checks based on >25 environments of data. This highlights the progress being made to develop breeding lines from wild soybean, that are genetically diverse with high yields and improved seed composition. Efforts are currently underway to determine the impacts of wild soybean on protein functionality and dietary nutrition.

*Development of EPA- and Astaxanthin-Enriched Soybean Germplasm for Aquaculture Feedstocks

Hyojin Kim, Postdoctoral Researcher, Center for Plant Science Innovation, University of Nebraska–Lincoln, hkim20@unl.edu

The omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and carotenoids such as astaxanthin are recognized for their health-promoting qualities. Marine fish and fish oil currently provide the main sources of EPA/DHA and astaxanthin for human consumption, but require this lipophilic compounds in their feed for aquaculture production. To provide a land-based source of these high-value feed components, we introduced the EPA and astaxanthin biosynthetic pathways in soybean by gene stacking. Our first version of aqua-soybean stacking EPA and astaxanthin biosynthetic genes showed poor seed quality such as reduced seed oil (<20% of seed weight), abnormal seed shape, low germination rate, decreased ABA level, and less EPA level (<2% of total fatty acids). From the design–build–test–learn (DBTL) cycle, the first version of aqua-soybean was crossed with high-alpha-linolenic acid soybean to improve EPA level. These crosses showed the enhanced seed quality such as high EPA level (~10% of total fatty acids) and normal seed shape, and the unexpected results such as improved germination rate. We generated only EPA soybean line or astaxanthin soybean line, separately. Our EPA soybean line accumulated up to 15% EPA of total fatty acids in seed with normal seed quality and germination rate. In addition, we developed astaxanthin-producing lines by introduction of biosynthetic genes (AaCBFD2 and AaHBFD1) with/without phytoene synthase gene (ZmPSY). Overall, our work represents a step toward viable soybean-based sources of astaxanthin-enriched fish oil for aquaculture production.

*Poster selected for oral presentation.

SPEAKER ABSTRACTS

PROTEOMICS/METABOLOMICS

SESSION CHAIRS: **Doug Allen**, USDA-ARS and Donald Danforth Plant Science Center, and **Michaela McGinn**, Smithbucklin

Can multi-omic integration lead to a better understanding of proteomic reprogramming and rebalancing? Lessons learned from Arabidopsis

Ruthie Angelovici, Associate Professor, University of Missouri

The ability of seeds to 'reset' the seed's amino acid content and composition back to their original states despite large proteomic elimination is termed "proteomic re-balancing, but its molecular mechanism remains elusive. Understanding the cellular responses underlying this phenomenon is a first step to understanding how seeds exert can such high resilience to large perturbations in their proteome. Toward this goal, we performed comparative metabolic and proteomic analyses of seven "re-balanced" mutants of the three main seed storage proteins (SSPs) in Arabidopsis (cruciferins). This analysis uncovered two conserved cellular responses in the seeds: an elevation in the reactive oxygen species (ROS) scavenging system, and an adjustment of the translational machinery composition, especially ribosomal proteins. The former suggests that storage proteins are involved in regulating seed redox homeostasis, and the latter suggests that the composition of the translational apparatus is key to the plastic rebalancing response of seeds. Further in-depth analysis of the rebalanced cruciferin triple mutant during seed maturation supported the initial finding and highlighted the early onset of proteomic rebalancing. Overall, our study uncovered two unexpected players in seed protein content and composition and opened new venues for crop seed biofortification.

A single amino acid mutation in a transcriptional repressor increases oil and protein content in soybean

Kristin Haug Collet, Research Scientist, Janel Bettis, Clay Bettis, Gina Zastrow-Hayes, Shreedharan Sriram, Yang Wang, Andrew Foudree, Merideth Hay, Zhan-Bin Liu, John Everard, Bo Shen, Corteva Agriscience, Johnston, Iowa, kristin.haugcollet@corteva.com

Climate change is a growing concern, especially the increase of CO2 release from use of fossil fuels. To help reduce carbon emission, demand for renewable biofuels is increasing. This is driving an increased demand for plant oil production especially for soybean oil, which is the second largest oil source for renewable biofuel production. To discover new genes for increasing oil in soybean, we have identified high oil mutants by high throughput single seed screening. One of these high oil mutants has been fully characterized and the mutant gene has been mapped, cloned, and validated by CRISPR gene editing. The mechanism of this novel transcriptional repressor, which regulates oil accumulation in soybean, and its application in high oil soybean development will be presented.

Orchestrating seed metabolism to enhance synthesis of novel oils

Linah Alkotami[1], Dexter White[1], Maliheh Esfahanian[2], Brice Jarvis[2], Andrew Paulson[3], Somnath Koley[4], Kathleen M. Schuler[1], Jianhui Zhang[5], Chaofu Lu[5], Doug K. Allen[4,6], Young-Jin Lee[3], John Sedbrook[2], **Timothy P. Durrett**[1]

[1] Department of Biochemistry and Molecular Biophysics, Kansas State University; [2] School of Biological Sciences, Illinois State University; [3] Department of Chemistry, Iowa State University; [4] Donald Danforth Plant Science Center; [5] Plant Sciences & Plant Pathology Department, Montana State University; [6] Agricultural Research Service, U.S. Department of Agriculture

Acetyl-triacylglycerols (acetyl-TAG) are TAG that possess an sn-3 acetate group instead of a long chain fatty acid. This unusual structure confers useful properties to acetyl-TAG, including reduced kinematic viscosity and improved cold temperature performance. Acetyl-TAG are synthesized in nature in the endosperm of many Euonymus seeds by unique diacylglycerol acetyltransferases (DAcTs). Isolation and expression of DAcT enzymes from different Euonymus species has resulted in the synthesis of acetyl-TAG in a number of oil seed crops, including soybean. This talk will discuss different approaches that have been used to increase the amount of acetyl-TAG synthesized in transgenic seeds. For example, the isolation of a higher activity DAcT enzyme from E. fortunei enabled increased acetyl-TAG levels. In addition, targeting of competing endogenous enzymes, using RNA interference or CRISPR-based genome editing, further enhanced the levels

of acetyl-TAG synthesized. The combined effect of these different strategies has resulted in oil seeds that accumulate over 95 mol% acetyl-TAG, higher than the levels naturally found in Euonymus seeds. Despite the almost complete replacement of the type of TAG synthesized, properties of the transgenic seeds such as seed fatty acid content or seed weight were comparable to those of wild-type seeds, though a slight delay in germination was observed. Imaging of lipid accumulation in these ultra-high acetyl-TAG seeds has revealed the location of residual endogenous TAG, offering future strategies for the development of seeds that synthesize pure acetyl-TAG.

*Increasing Sulfur Content in Soybean Seed Protein

Trish Tully, Postdoctoral Associate, Donald Danforth Plant Science Center
TLA Tully[1], D Duressa[3], V Veena[1], TP Durrett[3], DK Allen[1,2]
[1.] Donald Danforth Plant Science Center, St. Louis, MO, 63132; [2.] United States Department of Agriculture, Agricultural Research Science, St. Louis, MO, 63132; [3.] Kanas State University, Manhattan, KS, 66506

Protein is one of the most valuable biomass components of soybean seeds and accounts for ~40% of seed biomass. However, soy protein is not optimal for animal meal-based diets as it is deficient in sulfur containing amino acids (cys + met). In the past, attempts to increase sulfur content in soy protein have been focused on the protein level, including heterologous protein expression and overexpression of endogenous storage proteins with high sulfur content. Unfortunately, these approaches have had limited success. Here we illustrate the first steps in increasing sulfur content of soy protein at the metabolic level. In wildtype soybean, low molecular weight thiols downstream of cysteine (γEC and hGSH) accumulate over the course of development. This sequesters sulfur in non-proteogenic compounds rather than in the protein found in meal. To increase cysteine availability for protein synthesis, we have generated RNAi-knockdown lines targeting CGL; the enzyme responsible for diverting cysteine towards γEC and hGSH biosynthesis. RNAi-knockdown lines show decreased expression of all CGL homologues with seeds that are morphologically similar to wildtype. Observed levels of free amino acids and sulfur intermediates show an increase in free cysteine and a decrease in both γEC and hGSH in CGL-knockdown lines relative to wildtype. CGL-knockdown lines also show an increase in total protein as well as an increase in protein-bound cysteine relative to wildtype. Further work will combine the CGL-knockdown lines with protease-knockdown lines; combining a push and a protect to result in a hypothesized greater increase in both total protein and protein-bound cysteine.

*Poster selected for oral presentation.

GENOMICS/TRANSCRIPTOMICS

SESSION CHAIR: **Jason Nichols**, Principal Scientist, Syngenta Crop Protection, LLC, jason.nichols@syngenta.com

Advancing Soybean Genomics for Enhanced Haplotype-Based Trait Mapping

Aamir W. Khan[1], Heng Ye[1], Henry T. Nguyen[1],*

[1]Division of Plant Science and Technology, University of Missouri, Columbia, MO, USA.
nguyenhenry@missouri.edu

Soybean is a major source of protein and oil worldwide. Understanding soybean domestication, genome architecture and identifying major trait linked loci accelerates genomics-assisted trait mapping efforts. Complete, gapless genomes assemblies are a prerequisite for investigating the full architecture of complex regions like centromeres or telomeres and for covering the complete gene-space of the genome for a species. Here, we report long reads enabled near-gapless genome assemblies for Williams 82 and Lee soybean cultivars. A total of ten chromosomes in Williams 82 and eight in Lee were entirely reconstructed in single contigs without any gap. Comparison of improved genome assemblies for both Wm82 and Lee with their previous versions show improvements in current genomes by filling gaps and correcting the inversions and complex rearrangements on different chromosomes. Gene annotations of these genome assemblies resulted in identification of genes in gap filled regions. We used the near gapless Williams 82 genome as a reference to map the sequence data of 514 soybean accessions from USDA genebank including 268 landraces, 216 cultivars, 30 breeders' lines representing different maturity groups to develop a global variation map of 6.6 million single-nucleotide polymorphisms (SNPs) and 1.35 million insertions-deletions (InDels). This variation map serves as a resource for identifying haplotypes linked with

distinct phenotypes and for genome wide association studies utilizing higher marker density. The genome-wide deleterious mutations limiting fitness were identified. Resources from this study will aid haplotype-based trait mapping in soybean and the identified deleterious mutations could be potential targets for gene-editing.

Soybean has a lot to offer

Gary Stacy – Curator's Distinguished Professor, Divisions of Plant Science and Biochemistry, University of Missouri, Columbia, staceyg@missouri.edu

As I travel around and meet various scientists, I often hear comments about how soybean is so difficult to work with, cannot be transformed, lacks important resources for study, etc. I find that even folks in the soybean community share some of these same complaints. However, in my experience, there are very few things that cannot be done with soybean and the practical use of soybean rivals and, in some cases, surpasses that of the 'so-called models'. Genetics/ molecular biology had its start with bacteriophage, progressing through use of *E. coli*, yeast, *C. elegans*, mice, and now we see a great deal of research conducted directly with humans. Likewise, plant molecular biology began with algae, progressing via Arabidopsis, other models (e.g., Medicago) and now can be broadly applied to crop plants. In my seminar, I will illustrate how we use soybean in much the same way one would use Arabidopsis to address both discovery and application. I will also outline the various resources that exist now for the study of soybean, which may not be fully apparent to the soybean community writ large. It is important that the community be aware of these resources and that they communicate broadly the utility of soybean for research. For example, one important goal is to recruit and expand the soybean community by informing young scientists that soybean, indeed, has a lot to offer.

*Tabula Glycine: The Glycine max single-cell resolution transcriptome atlas

Sergio Alan Cervantes-Perez, Postdoctoral Research Associate, Department of Agronomy and Horticulture, University of Nebraska–Lincoln, alan.cervantes@unl.edu

Soybean (*Glycine max*), one of the most important crops in the world, is an essential source of protein and oil with high nutritional value for human and animal consumption. It also has the capability to establish symbiotic interactions with nitrogen-fixing soil bacteria. Synthetic biology offers an opportunity to improve important soybean agronomic traits. However, the development of well-sounded synthetic biology strategies requires a deep understanding of the gene expression and their associated regulatory mechanisms in each cell/cell type composing the plant. Here, we present "Tabula Glycine" the *Glycine max* single-cell resolution transcriptome atlas. This atlas is composed of ~133,000 nuclei isolated from 11 organs using the single-nuclei RNA-sequencing approach. Our analysis revealed 172 different groups of nuclei clustered based on their transcriptomic profile. Using spatial transcriptomic technology and comparative genomic approaches, we functionally annotated ~80% of the clusters composing the Tabula Glycine. Focusing on the transcriptional patterns of the soybean transcription factor (TFs) genes, we observed that their activity is sufficient to define a cell type, supporting the idea that TFs genes are key regulators of cell identity. Among the soybean epidermal cells, the "root hair cell" cluster is characterized by its unique transcriptome, likely a reflection of its biological specialization and polar elongation. Notably, we found 93 TFs co-expressed in the soybean root hair including orthologs to Arabidopsis TFs controlling root hair cell development and to Medicago TFs controlling the early stages of infection by rhizobia. The Tabula Glycine is a high-resolution functional genomic resource for the soybean community.

*Poster selected for oral presentation.

BREEDING/GENETICS FOR YIELD/PROTECTION OF YIELD

SESSION CHAIRS: **Aaron Lorenz**, University of Minnesota, and **Carrie Miranda**, Assistant Professor, North Dakota State University

Breeding Soybean for Quantitative Disease Resistance to *Phytophthora sojae*

Leah McHale, Professor of Breeding & Genetics, The Ohio State University
Anne Dorrance, William Rolling, Stephanie Karhoff, Cassidy Million, Christian Vargas-Garcia, Sungwoo Lee, Rouf Mian

Phytophthora root and stem rot, caused by the oomycete *Phytophthora sojae*, is an economically significant disease of soybean. More than 30 race-specific Resistance to *P. sojae* (Rps) genes have been identified and characterized. Though

surveys in the North Central US have shown that, with the complexity of *P. sojae* field populations, none of the deployed Rps-genes confer resistance to all *P. sojae* isolates. In contrast to Rps genes, quantitative disease resistance (QDR) toward *P. sojae* is a complex trait and generally considered to be non-race specific. QDR is usually controlled by many small-effect loci throughout the genome, though there are a few notable exceptions. While the space, time, expertise, and isolate maintenance required for disease assays in phenotypic selection makes breeding for QDR towards *P. sojae* challenging, there is potential to improve breeding progress with traditional marker assisted selection (MAS) targeting the limited large effect QDR loci and/or genomic selection to combine small-effect QDR loci. Integrated genomic methods have implicated potential molecular mechanisms, including glutathione metabolism, auxin and jasmonic acid signaling pathways, and root architecture, in QDR to *P. sojae*. As we build our knowledge of the molecular basis of individual QDR loci, traditional MAS can be applied to purposely combine multiple mechanisms of QDR for optimal, durable defense.

Ten Years of the Genomes-to-Fields Maize GXE Project: Lessons and Opportunities

Natalia de Leon, Professor, Department of Agronomy, University of Wisconsin, Madison

Population growth and climate concerns demand that we increase the sustainability and efficiency of crop production. The Maize GXE project, part of the Genomes to Fields (G2F) initiative, is a multi-institutional effort focused on assessing the differential effect of environmental components on the performance of diverse maize genotypes with the goal of optimizing crop performance for specific environments. Since 2014, a research network of more than 30 principal investigators across almost 20 universities and research institutions has collected phenotypic, genotypic and environmental data for thousands of diverse maize varieties across more than 280 agriculturally relevant locations in North America. The collected information and network of collaborators have contributed to the advancement of technologies and tools that address the goal of the project and have supported scientific progress of the maize research community as a whole. This presentation will provide a description of the framework of the project and current activities and share opportunities under consideration moving forward.

Breeding for Yield in Industry

Kyle Kocak – Research Scientist, Corteva Agriscience

Modern soybean breeding programs rely on sophisticated data collection and analytical methods to drive genetic gain. These methods have changed and adapted alongside the technology that enables their use in applied settings. Ultimately plant breeders have to make decisions into which germplasm is released commercially as well as recycled for improving the genetic base. The amount of data underlying and augmenting these decisions has been growing rapidly in the past decades. This talk will highlight how data and models have helped applied plant breeders deliver genetic gain for key traits such as yield and yield stability for soybean farmers in North America.

*A Genomic Selection Pipeline for Public Soybean Breeding Programs

Vishnu Ramasubramanian, Postdoctoral Associate, University of Minnesota, vramasub@umn.edu

Genomic selection has become an important part of plant and animal breeding programs to accelerate genetic gain. We've implemented a GS pipeline designed for soybean public breeding programs in the US Midwest using open source tools that are currently available. Herein, we describe the steps and tools in the pipeline and discuss results for the Northern Uniform Soy Trial Population. Soybeanbase, an instance of breedbase hosted by BreedingInsight is used for the storage of both genotypic and phenotypic data. Filtering of markers and lines are done in the R environment using rTASSEL and custom R scripts. Imputation using LD – K-nearest neighbors imputation (LDKNNi) implemented in rTASSEL often showed the highest imputation accuracy in our test data. An optimized training subset selected using the STPGA package demonstrated higher accuracies in 5-fold cross-validations compared to a randomly selected subset for certain combinations of candidate and training set sizes. In this version, we've implemented genomic prediction models using the rrBLUP, BWGR, BGLR and SOMMER packages for single trait and multiple traits across single or multiple environments. We've also implemented models that include GxE interactions and environmental covariates using the SOMMER and envRtype packages. Our preliminary results indicate that modeling GxE interactions significantly improved prediction accuracies for a subset of the NUST population and including environmental covariates improved prediction accuracies only for some conditions. In the future, we plan to refine these methods and optimize the GS pipeline.

*Poster selected for oral presentation.

SPEAKER ABSTRACTS

BIOTIC INTERACTION SESSION 2

SESSION CHAIR: **Andrew Bent**, Department of Plant Pathology, College of Agricultural & Life Sciences, University of Wisconsin–Madison

An update about SCN resistance: Peking-type and other new SCN resistant sources

Naoufal Lakhssassi, Associate Scientist, Adjunct Assistant Professor, Department of Plant Soil and Agricultural Systems, Southern Illinois University at Carbondale

Naoufal Lakhssassi[1*], Sushil S. Chhapekar[2*], Vikas Devkar[3], Dounya Knizia1, Heng Ye[2], Abdelhalim ElBaze[1], Tri Vuong[2], Gunvant Patil[3], Henry Nguyen[2], Khalid Meksem[1]

[1]Department of Plant, Soil and Agricultural Systems, Southern Illinois University, Carbondale, IL, USA; [2]Division of Plant Sciences, University of Missouri, Columbia, MO, USA. [3]Institute of Genomics for Crop Abiotic Stress Tolerance, Texas Tech University, Lubbock, TX, USA.

Soybean cyst nematode (SCN) is the first devastating pathogen in soybeans and causes more than $1.5 billion in damages annually. Genetic variability within SCN populations plays a crucial role in the ability of the pest to adapt and overcome management practices, including planting resistant soybean varieties. Since 1960, several QTL for resistance to SCN have been identified. Advancements in molecular biology and genomic technologies have significantly facilitated the process of gene cloning and functional characterization of two key SCN resistance genes, the Rhg4 (GmSHMT08 gene) and rhg1-a (GmSNAP18 gene). PI 88788-type derived resistance cultivars were commonly and widely used due to their effectiveness against specific SCN populations. However, continuously planting of soybean cultivars that are derived from the same SCN resistance genetics, or relying on a limited set of resistant cultivars, creates selection pressure on the SCN population that shifted over time to become more virulent and better adapted to the resistant cultivars that are being used. Therefore, it is necessary to identify and characterize additional sources of SCN resistance to combat this ever-changing pest. Soybean exotic line PI 567516C carries two novel genes for SCN resistance that seem to display different resistance mechanisms other than the already known Rhg4 and rhg1 genes. We recently fine-mapped a novel SCN-resistant loci qSCN10 (O) (to a 142-kb) region containing 15 genes on Chr. 10) from soybean accession PI 567516C. Functional characterization of this novel region identified two novel genes that reduce cyst number in fully susceptible soybean line Williams-82 by 52.64% and 57.9%. Understanding the molecular basis of SCN resistance allows breeders to develop soybean cultivars with more effective and durable resistance.

Developing alternative viro-control and RNAi-based approaches to reduce white mold infection

Shin-Yi Marzano, Research Molecular Biologist, U. S. Department of Agriculture ARS, shinyi.marzano@usda.gov

Growers lack effective genetic tools to manage losses caused by *Sclerotinia sclerotiorum* because of a lack of resistance to the pathogen in germplasms. This necessitates the identification of alternative sources of resistance or methods for the disease control. In this talk, I will explain our efforts in developing mycoviruses as well as identifying strong candidate genes from *S. sclerotiorum* as the targets for the development of alternative pesticides. I will also talk about the effects of endophytic colonization of avirulent strain of *S. sclerotiorum* on soybean plants, which may shed light on ways to manipulate pathogen-plant interactions by involving mycoviruses capable of extracellular transmission.

Using genomics to unravel the complexities of the interaction between soybean and *Phythophthora sojae*

François Belzile, Yanick Asselin and Richard Bélanger, Département de phytologie, Centre de recherche et d'innovation sur les végétaux, Université Laval, Québec, Qc, G1V 0A6, Canada,
Francois.Belzile@fsaa.ulaval.ca

Phytophthora sojae is arguably one of the most important pathogens of soybean worldwide. A common method of control resides in the introgression of specific resistance genes (*Rps*) in elite material. These *Rps* genes have a gene-for-gene relationship with avirulence (*Avr*) genes of *P. sojae*, which triggers defense reactions. Over the years, some *Rps* genes have lost their efficacy as a result of adaptation by the pathogen leading to new alleles of *Avr* genes whose product is no longer recognized by the product of the *Rps* gene. This situation compromises, for breeders and growers, the lasting reliance on

genetic control. This problem is compounded by the rapid evolution of *Avr* genes and the fact that very few *Rps* genes have actually been precisely identified and cloned. Following extensive surveys of *P. sojae* isolates throughout soybean growing areas in Canada, we were able to define SNP haplotypes for the alleles of *Avr* genes associated with the most commonly deployed *Rps* genes, *Rps1a, Rps1b, Rps1c, Rps1k, Rps3a,* and *Rps6*. Based on this information, we have developed molecular tools that can specifically identify each haplotype of these six *Avr* genes found in the isolates of *P. sojae* recovered in soybean fields. We have further been able to identify markers that will discriminate isolates on the basis of their virulence toward the newly identified *Rps11*. These tools can be easily exploited by breeders and farmers to introgress and use cultivars carrying the proper *Rps* genes in a given environment. In parallel, we have used RenSeq to precisely define *Rps* regions and genes, an approach that has allowed a much clearer understanding of the 35 or so reported *Rps* genes along with a finer resolution of their position in the soybean genome. Once optimized, this approach should facilitate introgression of those genes by breeders.

*An integrated single-cell comparative transcriptomic and evolutionary analysis of the legume membrane microdomain-associated protein-coding genes during the nodulation process

Md Sabbir Hossain, Doctoral Student, Department of Agronomy and Horticulture, University of Nebraska–Lincoln, mhossain8@huskers.unl.edu

Legumes have the unique ability to fix atmospheric dinitrogen through a mutualistic symbiosis with rhizobia. This symbiosis starts with the infection of the legume root hair cell and ultimately leads to the development of a new root organ, the nodule. The rearrangement of the plasma membrane is a pre-requirement for rhizobial infection. For instance, membrane microdomain-associated proteins (MMAPs), including FW2.2-LIKEs (FWLs), flotillins (FLOTs), prohibitins (PHBs), and remorins (REMs), play a crucial role in the initiation and development of the infection threads, a tube-like structure that allow rhizobia to infect the plant cells. In this poster, using state-of-the-art single-cell transcriptomics technology, we evaluate the co-expression pattern of the MMAPs in each cell type composing the *Glycine max* and *Medicago truncatula* nodules. As a result, we identified two GmFWLs, one GmFLOT, two GmPHBs, and three GmREMs, and, and two MtFWLs, two MtFLOTs, three MtPHBs, and one MtREM genes preferentially expressed in the infected cells of the soybean and Medicago, respectively. Expanding our analysis to the entire soybean single-cell transcriptomic atlas, we confirmed the specific expression of these soybean MMAPs in the infected cells of the nodule. The phylogenetic analysis of the legume MMAPs revealed that most of the nodule-infected cell-specific MMAPs co-cluster together suggesting their early functional allocation to regulate the nodulation process.

*Poster selected for oral presentation.

GENETIC ENGINEERING

SESSION CHAIRS: **Robert Stupar**, Department of Agronomy and Plant Genetics, University of Minnesota, and **Wayne Parrott**, Department of Crop and Soil Sciences, University of Georgia

Engineering approaches to develop hybrid soybean

Margaret Frank, Assistant Professor, School of Integrative Plant Science, Plant Biology Section, Cornell University Cornell University

TBD

Understanding nutrient uptake and its potential role in water deficit conditions

Gunvant Patil, Assistant Professor, Institute of Genomics for Crop Abiotic Stress Tolerance, Texas Tech University, https://patil-lab-ttu.com/

Understanding the molecular basis of differential mineral element uptake, translocation, and accumulation in soybean is vital for developing improved cultivars. In this study, we profiled diverse soybean germplasm and identified novel QTLs associated with several mineral nutrients including Silicon (Si). Silicon (Si) is considered a beneficial element due to its ability to enhance plant growth and mitigate a variety of abiotic and biotic stresses. Outside of monocot plants, less is

known about the transport and localization of Si in different crops, especially soybean. In this study, we combine association mapping, electron microscopy, and single nuclei transcriptomics and gene editing to quantify Si uptake, deposition, and characterize candidate genes for Si transport and accumulation in the soybean leaf. The snRNAseq analysis identified a potential Si efflux gene which is localized in the epidermal cells of Si-treated plants. Functional characterization using CRISPR/Cas9 of Si transport gene identified its role in water stress conditions.

Harnessing the mPing Transposable Element for Gene Discovery and Precision Genome Engineering

C. Nathan Hancock[1], Peng Liu[2], Zara Lacera[1], Megan Collins[1], and R. Keith Slotkin[2]

[1] University of South Carolina Aiken, Aiken, SC, USA; [2] Donald Danforth Plant Science Center, St. Louis, MO, USA

Efficient identification and manipulation of genes influencing agronomically important traits is crucial for crop improvement. Leveraging the mPing transposable element from rice, we have developed mutagenesis resources capable of generating both knockdown and overexpression phenotypes. Experiments in soybean show that mPing-based activation tags incorporating enhancer sequences can induce upregulation of nearby genes. To enhance mutagenesis efficiency, hyperactive versions of mPing and the Pong transposase proteins responsible for mobilizing these elements are being engineered. This technology has been advanced in collaboration with Keith Slotkin's Laboratory at the Donald Danforth Center by establishing a reliable method for sequence-specific targeting of mPing insertion in plant genomes. Linking Pong Transposase to CRISPR/Cas nucleases allows for the excised mPing elements to be inserted into the Cas targeted double stranded breaks. They have successfully used this technology to deliver enhancer elements, open reading frames, and gene expression cassettes into targeted locations in Arabidopsis and soybean genomes. In summary, these experiments demonstrate the potential of mPing-based mutagenesis for crop improvement and expanding crop genetic engineering capabilities.

*Facilitating gene discovery in soybean through mutagenesis: Identification of novel genes controlling the production of four-seeded pods

Cuong X. Nguyen[1], **Vikranth K. Chandrasekaran**[1], Manh V. Nguyen[1], Gary Stacey[1], Minviluz G. Stacey[1]

[1]Division of Plant Sciences, University of Missouri, vkc4kf@missouri.edu

The most important soybean agronomic trait targeted for crop improvement is increased yield, which can be achieved by increasing seed number and/or seed weight. Phenotypic screening of soybean fast neutron mutants identified a mutant line, designated MO27, that produced an increased number of four-seeded pods (4-SP), producing ~30% 4-SPs per plant compared to ~4% in wildtype. Genetic mapping and co-segregation analyses showed that the 4-SP phenotype in MO27 is controlled by the additive effects of at least two alleles located in Chr02 and Chr06. Based on the functional annotations of the deleted genes, we hypothesized that deletions of GmGATA1 on Chr. 2 and GmULT1-3 and GmULT1-5 on chr.6 are the putative causative mutations underlying the 4-SP trait. These genes encode putative transcription factors. Knock-out deletions in these genes via CRISPR/Cas9 confirmed their role in controlling the number of seeds per pod in soybean, likely through the CLAVATA3 (CLV3)-WUSCHEL (WUS) signalling pathway that coordinates stem cell proliferation with differentiation in floral meristems. Knock-out mutations of orthologous genes in Arabidopsis and tomato resulted in increased production of locules in siliques and fruits, respectively, suggesting functional conservation of GmGATA1-1 and GmULT1 in plants.

*Poster selected for oral presentation.

POSTER ABSTRACTS

P–1. Unraveling the Initial Signaling Cascades in Soybean Nodulation: Insights from Proteomic and Transcriptomic Analyses

Shin-ichiro Agake, Visiting Scholar, University of Missouri / Tokyo University of Agriculture and Technology, sagake@missouri.edu

Nod-factor receptor complexes, comprising NFR1, NFR5, and SymRK, play pivotal roles as essential kinase proteins in nodulation. Rhizobia employ a type III secretion system (T3SS) to enhance nodulation through the regulation of the Nod-factor triggered pathway. Notably, soybeans display delayed nodulation during the early growth stage when inoculated with T3SS knockout mutants. However, the specific initial signaling pathways governing these processes remain unclear. In this study, we sought to uncover the early signaling cascades using proteomic and transcriptomic analyses. To identify key components involved in the Nod-factor triggered pathway, we used proximal labeling with TurboID fused to the receptor kinases in the hairy root system. Subsequent LC-MS/MS analysis revealed 31 enriched proteins associated with NFR1, 11 proteins with NFR5, and 17 proteins with SymRK. Additionally, we conducted a kinase-client assay utilizing purified SymRK, which resulted in the enrichment of 69 phosphopeptides. These findings present promising candidates that may interact with Nod-factor receptors during the initial plant response. Furthermore, we investigated the T3SS triggered pathway by analyzing RNA-seq data obtained from isolated root hairs inoculated with T3SS mutant. At 12 hours post-inoculation, we identified 471 differentially expressed genes upregulated in response to T3SS, indicating a strong activation of crucial T3SS effectors at this time point. In conclusion, our comprehensive approach using proteomic and transcriptomic analyses has shed light on the early signaling events in nodulation. These findings deepen our understanding of the complex interplay between Nod-factor receptors and the T3SS machinery, providing valuable insights into the regulatory mechanisms underlying soybean-rhizobia symbiosis.

P–2. Uncovering the genomic diversity of the Soybean Cyst Nematode (*Heterodera glycines*) through pangenome analysis

Lucas Borges dos Santos, Doctoral Student, University of Illinois at Urbana-Champaign

Authors: Santos, L.B., Showmaker K., Walden K.O.K., Masonbrink R., Hernandez A., Severin A., Mitchum M., Hudson M.

The soybean cyst nematode (SCN) poses a significant threat to soybean yield globally. Despite its harmful impact, the mechanisms driving its virulence and adaptability largely remain a mystery. Previous investigations have employed single reference genomes to decode SCN genetic diversity, but this method risks missing or misinterpreting vital genetic variants absent in the reference. Pangenome analysis emerges as a solution, capturing the entire range of genomic diversity within a species, thus circumventing the bias tied to singular reference genomes.In this ongoing research, we aim to shed light on the structure and genomic diversity of SCN populations, which may potentially expose new directions for developing control measures. By leveraging high-quality metagenome assemblies from seven distinct SCN populations, we have gained a deeper understanding of the SCN genomic landscape. Our analysis identified 27,987 ortholog gene clusters, divided into 42% core and 58% non-core gene families. This distinction between core and non-core gene families elucidates the separation between fundamental genes found in all populations and those potentially contributing to adaptability and variation.Our functional annotation of these gene clusters has revealed critical cellular processes that core SCN genes engage in, offering insights into the nematode's survival strategies and biology. By laying the groundwork for an SCN pangenomic framework, we enable a more comprehensive understanding of the genetic mechanisms behind its pathogenicity. This enhanced understanding is a pivotal step towards the development of effective and targeted nematode control strategies.

P–3. Phenotyping and Yield of Soybean Treated with an Archaeal Antioxidant

Jeremy Brown, Graduate Student, Biochemistry, University of Nebraska–Lincoln, jbrown353@huskers.unl.edu

ArA is an antioxidant from archaeal origin with a redox potential similar to Glutathione (GSH). Previous studies have found that application of antioxidants have the potential to increase growth parameters and ameliorate abiotic stresses in plants. Growth trials with *Glycine max* (Thorne) at three weeks of growth showed increased biomass vs. untreated controls in a greenhouse setting. To determine if ArA has potential for agricultural applications, we grew G. max to seed

at the UNL greenhouse phenotyping facility with and without application of ArA to do phenotyping image analysis and collect yield data. Using the phenotyping facility allows for daily imaging with RGB, NIR, Fluorescence, and hyperspectral cameras for an accurate growth estimation and phenotype while the plants grow. With these images we have extracted information of plant height and approximate leaf area over the course of the plant growth to determine growth over time. Results of one growth trial showed an increase in yield and growth rate with application of 1mM ArA. Control plants between trials showed differences in yield due to seasonal differences. We also found that application of ArA changed light harvesting dynamics and treated plants displayed a smaller degree of photo protection but a faster response. We are currently extracting information from the hyperspectral images and managing a field experiment for application of ArA.

P–4. Soybeans Engineered for Enhanced Vitamin E and Effects on Oil Antioxidant Properties in Polyunsaturated Fatty Acid-Rich Gerplasm

Edgar Cahoon, Professor, University of Nebraska–Lincoln, ecahoon2@unl.edu

Engineered soybean oils enriched in nutritionally valuable omega-3 PUFAs have lower oxidative stability than conventional soybean oil, resulting in increased rancidity and associated off-flavors for food products. Oxidative stability of PUFA-enriched oils is typically enhanced by adding chemical antioxidants following extraction. This study examined biofortification of PUFA-enriched seeds with lipid-soluble vitamin E antioxidants to increase oil stability. We engineered soybeans for seed-specific expression of a barley homogentisate geranylgeranyl transferase (HGGT) transgene alone and with a soybean γ-tocopherol methyltransferase (γ-TMT) transgene, resulting in an 8- to 10-fold increase in total vitamin E, principally as tocotrienols. With γ-TMT co-expression, δ- and γ-tocochromanols were shifted largely to the more nutritionally valuable α- and β-tocochromanols. To test whether the high vitamin E trait improves oxidative stability of conventional and PUFA-enhanced seed oils, selected high vitamin E lines were crossed with a stearidonic acid (SDA, 18:4Δ6,9,12,15)-producing line, resulting in progeny with oil enriched in SDA and α- or γ-linoleic acid (ALA, 18:3Δ9,12,15 or GLA, 18:3Δ6,9,12), from transgene segregation. Oil from HGGT-expressing lines had ≥6-fold increase in free radical scavenging activity compared to controls. However, oxidative stability index of the oil was ~15% lower than that of oil from non-engineered seeds and only modestly increased in oil from the GLA, ALA and SDA backgrounds. We show that soybean is an effective platform for producing high levels of free-radical scavenging vitamin E antioxidants, but this trait may have negative effects on oxidative stability of conventional oil or only modest improvement of the oxidative stability of PUFA-enhanced oil.

P–5. SoyBase.org: Integrate genetics, genomics and breeding data to advance soybean research

Jacqueline Campbell, Faculty, Corn Insects and Crop Genetics Research Unit, USDA-ARS, Jacqueline.Campbell@usda.gov

SoyBase (https://soybase.org) is the USDA-ARS soybean resource hub for the soybean community, by providing a comprehensive collection of data, analysis tools, and links to external resources of interest to soybean researchers. SoyBase partners with other plant data resources (eg. LegumeInfo, PhyloGenes, Phytozome and SoybeanBase) to provide links to data sets not housed at SoyBase. Currently, SoyBase hosts seven annotated genomes from multiple *Glycine max* and *Glycine soja* cultivars, including the most recent Williams 82 genome (Wm82_ISU01.a2.v1). The soybean reference genome (Glyma.Wm82) GBrowse has numerous data tracks available to view including genome organization, gene annotation and expression, markers, methylation and sequence variants (SoySNP50K and GmHapMap projects). SoyBase is an actively curated database, with new data regularly being incorporated, including parentage information for Uniform Soybean Tests from both Northern and Southern regions, gene expression, GWAS QTLs, and genome sequences and annotations. The latest set of tools added to SoyBase include the Pan-Genome Sequence Search and the SequenceSever for both nucleotide and protein BLAST. The SequenceSever includes more output options and the ability to download all the hits as FASTA files and alignment of the hit and tabular reports of the hits. SoyBase provides easy access to download data including SoySNP50K marker data, QTL positions, pan-genome datasets, genome, CDS and protein sequences. A major goal of SoyBase is to assist researchers in discovering important trait, genomic, and genetic information within the vast amount of data available.

P–6. *Tabula Glycine: The *Glycine max* single-cell resolution transcriptome atlas

Sergio Alan Cervantes-Perez, Postdoctoral Research Associate, Department of Agronomy and Horticulture, University of Nebraska–Lincoln, alan.cervantes@unl.edu

Soybean (*Glycine max*), one of the most important crops in the world, is an essential source of protein and oil with high nutritional value for human and animal consumption. It also has the capability to establish symbiotic interactions with nitrogen-fixing soil bacteria. Synthetic biology offers an opportunity to improve important soybean agronomic traits. However, the development of well-sounded synthetic biology strategies requires a deep understanding of the gene expression and their associated regulatory mechanisms in each cell/cell type composing the plant. Here, we present "Tabula Glycine" the *Glycine max* single-cell resolution transcriptome atlas. This atlas is composed of ~133,000 nuclei isolated from 11 organs using the single-nuclei RNA-sequencing approach. Our analysis revealed 172 different groups of nuclei clustered based on their transcriptomic profile. Using spatial transcriptomic technology and comparative genomic approaches, we functionally annotated ~80% of the clusters composing the Tabula Glycine. Focusing on the transcriptional patterns of the soybean transcription factor (TFs) genes, we observed that their activity is sufficient to define a cell type, supporting the idea that TFs genes are key regulators of cell identity. Among the soybean epidermal cells, the "root hair cell" cluster is characterized by its unique transcriptome, likely a reflection of its biological specialization and polar elongation. Notably, we found 93 TFs co-expressed in the soybean root hair including orthologs to Arabidopsis TFs controlling root hair cell development and to Medicago TFs controlling the early stages of infection by rhizobia. The Tabula Glycine is a high-resolution functional genomic resource for the soybean community.

*Poster selected for oral presentation.

P–7. * Facilitating gene discovery in soybean through mutagenesis: Identification of novel genes controlling the production of four-seeded pods

Cuong X. Nguyen[1], **Vikranth K. Chandrasekaran**[1], Manh V. Nguyen[1], Gary Stacey[1], Minviluz G. Stacey[1]

[1]Division of Plant Sciences, University of Missouri, vkc4kf@missouri.edu

The most important soybean agronomic trait targeted for crop improvement is increased yield, which can be achieved by increasing seed number and/or seed weight. Phenotypic screening of soybean fast neutron mutants identified a mutant line, designated MO27, that produced an increased number of four-seeded pods (4-SP), producing ~30% 4-SPs per plant compared to ~4% in wildtype. Genetic mapping and co-segregation analyses showed that the 4-SP phenotype in MO27 is controlled by the additive effects of at least two alleles located in Chr02 and Chr06. Based on the functional annotations of the deleted genes, we hypothesized that deletions of GmGATA1 on Chr. 2 and GmULT1-3 and GmULT1-5 on chr.6 are the putative causative mutations underlying the 4-SP trait. These genes encode putative transcription factors. Knock-out deletions in these genes via CRISPR/Cas9 confirmed their role in controlling the number of seeds per pod in soybean, likely through the CLAVATA3 (CLV3)-WUSCHEL (WUS) signalling pathway that coordinates stem cell proliferation with differentiation in floral meristems. Knock-out mutations of orthologous genes in Arabidopsis and tomato resulted in increased production of locules in siliques and fruits, respectively, suggesting functional conservation of GmGATA1-1 and GmULT1 in plants.

*Poster selected for oral presentation.

P–8. Phosphorylation-mediated signalling at high temporal resolution in cold-stressed soybean seedlings

Ive De Smet, Group Leader, VIB-UGent Center for Plant Systems Biology, Technologiepark-Zwijnaarde 71 - 9052 Ghent - Belgium, ive.desmet@psb.vib-ugent.be

Low temperature stress limits the overall growth of soybean and leads to yield reduction. Therefore, unravelling the mechanisms associated with low temperature perception, signaling and response can provide valuable information for breeding and improving soybean yield. However, very little is known about this in soybean. To address this knowledge gap, we focused on protein phosphorylation cascades, which are responsible for transducing environmental and cellular

signals. To assess how signaling pathways dynamically rewire upon environmental cues we exposed cold-sensitive and tolerant soybean seedlings to control and cold conditions, harvested leaves of five-day-old seedlings every six minutes for one hour, and performed phosphoproteomics. Next, to shed light on phosphorylation cascades in soybean seedlings upon cold treatment, we used functional annotations from our newly developed PF-NET in combination with Bayesian network principles (NetPhorce) to predict kinase and phosphatase-mediated signaling cascades using time series phosphoproteomics. Our novel network inference approach allowed us to predict key temperature response regulators in soybean, that could be checked against a range of field-grown soybean at two temperatures (10 °C and 20 °C). These response regulators are ideally suited as breeding markers or targets.

P–9. Virulence Diversity of Soybean Cyst Nematode in Minnesota

Lauren Docherty, Master's Student, University of Minnesota, doche014@umn.edu

Soybean cyst nematode (SCN) (*Heterodera glycines*) is the most damaging pest affecting soybean crops, causing an estimated $1 billion in yield loss annually. The most effective method for managing SCN is the use of resistant soybean cultivars. SCN populations, however, hold the potential to overcome soybean resistance in what is known as a virulence shift. This project examines the virulence diversity of SCN in Minnesota to better understand the potential for virulence shifts. This will be done by testing the virulence of 180 inbred lines of SCN on six indicator soybean lines with diverse resistance genes. Many of these soybean lines are not part of the standard virulence test, thus their effectiveness is unknown and may include highly effective resistance to a broad sample of SCN populations. Results obtained so far indicate that many of the SCN lines can overcome the PI 88788 source of resistance. PI 90763 and PI 438489B displayed the broadest resistance, with very few SCN lines overcoming its resistance. The results also show that there is little overlap in virulence among different soybean lines, suggesting different virulence genes within SCN are required to overcome specific sources of host plant resistance. The information provided by this project will help farmers make management decisions and help soybean breeders develop cultivars with durable SCN resistance. In future research, the data from this project will be combined with genomic data to identify regions of the SCN genome associated with virulence.

P–10. SNP and small INDEL genomic variants underlying adaptive traits in soybean

Gezahegn Girma, Research Scientist, and **Yong-qiang Charles**, Research Molecular Biologist, Donald Danforth Plant Science Center, GTessema@danforthcenter.org

Significant efforts have been made to discover and validate genes and alleles important to soybean improvement. However, the complex nature of quantitative traits and limitations of discovery tools impede identification of loci associated with soybean adaptation to diverse environments. Our laboratory has been developing integrative data-driven technologies to discover genes and genetic alleles for soybean improvement. As
part of the efforts, more than 12,000 accessions with whole genome sequence data have been retrieved from public database. A subset of 1402 accessions, including both *Glycine soja* and *Glycine max*, was selected. Variant calling analysis identified 11,101,803 SNPs and small indels. We have implemented both single and multi-locus GWAS models to identify significant loci underlying soybean adaptation based on geographic information. A total of 26 genomic regions representing 12 indels and 14 SNPs were identified for their significant association with soybean adaptation to wider environments. Annotation of the significant genomic variants resulted in nine unique genes. Population differentiation was also measured based on latitudinal information to identify new beneficial alleles that becomes fixed in each population. We will further utilize available tools such as Meta-GWAS r-package and SoyBase to discover novel genes by comparing with previous studies. Understanding the molecular basis underlying soybean adaptation to diverse environments is crucial for designing effective strategies to develop new, climate-resilient soybean varieties.

P–11. Studying iron deficiency chlorosis: using soybean to turn the models right side up

Michelle Graham, Faculty, Corn Insects and Crop Genetics Research Unit, USDA-ARS, Michelle.Graham@usda.gov

Iron deficiency chlorosis (IDC) negatively affects crop quality and yield. Studies from model species demonstrate shoots control or influence of iron uptake in roots. In this study, grafting of near-isogenic soybean lines Clark and IsoClark (iron stress tolerant and susceptible, respectively), demonstrated the Clark rootstock drives phenotypic responses in

IsoClark leaves two weeks after iron stress. RNA-seq analyses from homo- and hetero-grafted plants 30 and 120 minutes after iron stress identified 518 and 846 differentially expressed genes (DEGs) in leaves and roots, respectively. Grafts with a Clark rootstock induced genes involved in iron uptake and utilization at 30 minutes in the root and by 120 minutes in the leaves, regardless of the leaf genotype. This suggests a mobile signal, initiated in Clark roots, regulates iron stress responses in soybean leaves. Better understanding of the complex differences between crop and model species will aid in the development of crops with improved IDC tolerance.

P–12. Dissecting function of the gene model that governs the soybean QTL cqSeed protein-003

Ming Guo, Research Assistant Professor, Department of Agronomy and Horticulture, University of Nebraska–Lincoln, mguo2@unl.edu

A major seed protein quantitative trait locus (QTL) in soybean that was mapped to chromosome 20 over 30 years ago, cqSeed protein-003, was fine mapped to gene model Glyma.20G085100 (Gm20P). Gm20P contains a transposon footprint in most of the domesticated soybean genotypes that interrupts the CONSTANS, CONSTANS-like (CO-like), and timing of CAB expression1 (TOC1) (CCT) domain present in the non-domesticated genotypes. The Gm20P paralog gene model resides on chromosome 10, Glyma.10G134400 (Gm10P). Using genome editing reagents, two lines were developed that harbor INDELs in both Gm10P and Gm20P alleles in the soybean genotype Thorne. One edited line carries a 200 bp deletion in the exon2 of Gm20 and small INDEL in the 5' UTR of GM10P. The second edited line carries INDELs in Gm10P and Gm20P, a 59 base pair insertion in the Gm20P exon2 and a 4 bp deletion in the Gm10P exon2. Both edited lines display delayed maturity relative to control Thorne, with the second edited line displaying a more prominent delayed maturity under field conditions. NIR analysis from the field plots revealed seed protein content is significantly decreased in the Gm10P and Gm20P double mutant, while oil content remains similar among both mutants and wild type Thorne. Wet bench analyses on seed suggested fiber content is significantly reduced in the mutants, while starch content is increased.

P–13. Enhanced Bioactivity of Day-4 Soybean Sprouts: A Potential Functional Food for Inflammation-Associated Diseases

Rajnee Hasan, Graduate Student, Biochemistry, University of Nebraska–Lincoln, rhasan2@huskers.unl.edu

Soybean, a prominent source of proteins and phytochemicals, has sparked interest in germination as a means to enhance health-beneficial effects through increased peptides and phytochemicals. In the context of health-beneficial activities, gastrointestinal digestion plays a crucial role in releasing bioactive compounds from ingested foods that can exert anti-inflammatory effects in the body. This study aimed to investigate how 0, 2, and 4 days of sprouting influence the release of bioactive compounds and anti-inflammatory capacity of soybean in gastrointestinal tract. Major monosaccharides and amino acids significantly accumulated in Day-4 sprouts and those subjected to in vitro gastrointestinal digestion. While the digestibility of soybean sprouts was reduced over 4 days of sprouting, the peptide content was significantly increased in these digests. In vitro anti-inflammatory assays demonstrated that treatment with Day-4 sprout digests significantly reduced the induced inflammatory responses in Caco-2 intestinal cell line, showing their anti-inflammatory capacity. In a mouse model of chronic inflammation, Day-4 sprout supplementation significantly reduced body weight and fasting blood glucose levels in a dose-dependent manner. Moreover, Day-4 sprouts supplementation reduced the expression of IL-6, suggesting its potential therapeutic value in treating inflammation-associated diseases. Di- and tripeptides with hydrophobic amino acids in N-terminal and polar amino acids in C-terminal including IP, ILR, IVR, IVK, LIK, LLR, LYK and MQ accumulated in Day-4 sprout digests, indicating their potential as anti-inflammatory molecules. In conclusion, Day-4 soybean sprouts likely possess a combination of bioactive compounds with anti-inflammatory potential, making them a promising candidate of functional food to prevent inflammation-related disorders.

POSTER ABSTRACTS

P–14. *An integrated single-cell comparative transcriptomic and evolutionary analysis of the legume membrane microdomain-associated protein-coding genes during the nodulation process

Md Sabbir Hossain, Doctoral Student, Department of Agronomy and Horticulture, University of Nebraska–Lincoln, mhossain8@huskers.unl.edu

Legumes have the unique ability to fix atmospheric dinitrogen through a mutualistic symbiosis with rhizobia. This symbiosis starts with the infection of the legume root hair cell and ultimately leads to the development of a new root organ, the nodule. The rearrangement of the plasma membrane is a pre-requirement for rhizobial infection. For instance, membrane microdomain-associated proteins (MMAPs), including FW2.2-LIKEs (FWLs), flotillins (FLOTs), prohibitins (PHBs), and remorins (REMs), play a crucial role in the initiation and development of the infection threads, a tube-like structure that allow rhizobia to infect the plant cells. In this poster, using state-of-the-art single-cell transcriptomics technology, we evaluate the co-expression pattern of the MMAPs in each cell type composing the *Glycine max* and *Medicago truncatula* nodules. As a result, we identified two GmFWLs, one GmFLOT, two GmPHBs, and three GmREMs, and, and two MtFWLs, two MtFLOTs, three MtPHBs, and one MtREM genes preferentially expressed in the infected cells of the soybean and Medicago, respectively. Expanding our analysis to the entire soybean single-cell transcriptomic atlas, we confirmed the specific expression of these soybean MMAPs in the infected cells of the nodule. The phylogenetic analysis of the legume MMAPs revealed that most of the nodule-infected cell-specific MMAPs co-cluster together suggesting their early functional allocation to regulate the nodulation process.

*Poster selected for oral presentation.

P–15. Bolstering virus-induced gene silencing and foreign protein expression in soybean with a cowpea severe mosaic virus-based vector

Feng Qu, Seung Hyun Yang, Fides Angeli Zaulda, **Junping Han**, Anne Dorrance, Department of Plant Pathology, The Ohio State University, han.393@osu.edu

Soybean gene functions cannot be easily interrogated through transgenic disruption (knock-out) of genes-of-interest, or overexpression of proteins-of-interest, because soybean transformation is time-consuming and technically challenging. An attractive alternative is to administer transient gene silencing or overexpression with a plant virus-based vector. However, existing virus-induced gene silencing (VIGS) and/or overexpression vectors suitable for soybean have various drawbacks that hinder their widespread adoption. We describe the development of a new vector based on cowpea severe mosaic virus (CPSMV). This vector, designated FZ, incorporates a cloning site in the CPSMV RNA2 cDNA, permitting insertion of nonviral sequences. When paired with an optimized RNA1 cDNA (QUIN), FZ readily infects both *Nicotiana benthamiana* and soybean. As a result, FZ constructs destined for soybean can be first delivered to *N. benthamiana* to propagate the modified viruses to high titers. FZ-based silencing constructs induced robust silencing of phytoene desaturase genes in *N. benthamiana*, multiple soybean accessions, and cowpea. Moreover, FZ-mediated expression of the Arabidopsis transcription factor MYB75 caused *N. benthamiana* to bear brown leaves and purple, twisted flowers, indicating that MYB75 retained the function of activating anthocyanin synthesis pathways in a different plant. The new CPSMV-derived FZ vector provides a convenient and versatile soybean functional genomics tool that is expected to accelerate the characterization of soybean genes controlling crucial productivity traits.

P–16. Genetic Diversity in the North Dakota State University Soybean Breeding Program

Forrest Hanson, Master's Student, North Dakota State University, forrest.hanson@ndsu.edu

Soybean is a relatively new crop in North Dakota; however, it has become the number one crop in the state for acres planted and production value. Public breeding efforts began in 1986 through North Dakota State University, and during this time, 40 varieties in maturity groups 00 and 0 have been released. Although yields have increased during this time, the yield gains and genetic diversity of the program have not been studied. It is important to understand these components of a breeding program to ensure yields do not become stagnant. We would like to determine the amount of genetic diversity in this program to improve yields further. This knowledge will allow our program and other breeders in the maturity group 00 and 0 environment to continue to improve yields.

POSTER ABSTRACTS

P–17. Single-trait and multiple-trait QTL analyses for seed oil and protein contents of soybean populations with elite background

Tu Huynh, Doctoral Student, The Ohio State University, huynh.177@osu.edu

Soybean seed oil and protein contents are negatively correlated, posing a challenge in breeding efforts to enhance both traits. Previous studies have identified hundreds of oil and protein QTLs mainly via single-trait QTL analyses. Multiple-trait QTL methods for correlated traits have been shown to improve detection power and mapping precision compared to single-trait methods but have not been applied to seed oil and protein contents. Our study conducted both single- and multiple-trait multiple interval mapping (ST-MIM and MT-MIM, respectively) for oil and protein contents using three recombinant inbred line populations with advanced breeding line background tested in four environments. We detected seven ST-MIM QTLs on chromosomes 1, 8, 6, 15, 19, and two on 20, five of which were confirmed by MT-MIM. Our findings show that, unlike multiple-trait QTL analyses for other traits and crops, MT-MIM did not outperform the single-trait approach for our traits of interest. All loci exerted opposite effects on oil and protein contents, but the protein-to-oil additive effect ratio varied (-0.6 to -48.8). We calculated the allelic effects on estimated processing values (EPV) using the National Oilseed Processors Association (NOPA) and High Yield + Quality (HY+Q) methods. Oil-increasing alleles of QTLs on chromosomes 6, 15, 19, and 20 increased both EPVNOPA and EPVHY+Q, while oil-increasing alleles of QTLs on chromosomes 1 and 8 increased EPVNOPA and decreased EPVHY+Q, which penalizes low protein meal. With the populations' elite pedigree, selected lines can be used to determine the allelic effects on yield and directly integrated into breeding programs.

P–18. *Development of EPA- and Astaxanthin-Enriched Soybean Germplasm for Aquaculture Feedstocks

Hyojin Kim, Postdoctoral Researcher, Center for Plant Science Innovation, University of Nebraska–Lincoln, hkim20@unl.edu

The omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and carotenoids such as astaxanthin are recognized for their health-promoting qualities. Marine fish and fish oil currently provide the main sources of EPA/DHA and astaxanthin for human consumption, but require this lipophilic compounds in their feed for aquaculture production. To provide a land-based source of these high-value feed components, we introduced the EPA and astaxanthin biosynthetic pathways in soybean by gene stacking. Our first version of aqua-soybean stacking EPA and astaxanthin biosynthetic genes showed poor seed quality such as reduced seed oil (<20% of seed weight), abnormal seed shape, low germination rate, decreased ABA level, and less EPA level (<2% of total fatty acids). From the design–build–test–learn (DBTL) cycle, the first version of aqua-soybean was crossed with high-alpha-linolenic acid soybean to improve EPA level. These crosses showed the enhanced seed quality such as high EPA level (~10% of total fatty acids) and normal seed shape, and the unexpected results such as improved germination rate. We generated only EPA soybean line or astaxanthin soybean line, separately. Our EPA soybean line accumulated up to 15% EPA of total fatty acids in seed with normal seed quality and germination rate. In addition, we developed astaxanthin-producing lines by introduction of biosynthetic genes (AaCBFD2 and AaHBFD1) with/without phytoene synthase gene (ZmPSY). Overall, our work represents a step toward viable soybean-based sources of astaxanthin-enriched fish oil for aquaculture production.

*Poster selected for oral presentation.

P–19. Characterization of soybean events with enhanced expression of the microtubule-associated protein 65-1

Panya Kim, Postdoctoral Research Associate, Center for Plant Science Innovation, University of Nebraska–Lincoln, kim3@unl.edu

Microtubule-associated protein 65-1 (MAP65-1) protein plays an essential role in plant cellular dynamics through impacting stabilization of the cytoskeleton by serving as a crosslinker of microtubules. The role of MAP65-1 in plants has

been associated with phenotypic outcomes in response to various environmental stresses. The Arabidopsis MAP65-1 (AtMAP65-1) is a known virulence target of plant bacterial pathogens and thus a component of plant immunity. Soybean events were generated that carry transgenic alleles for both AtMAP65-1 and GmMAP65-1, the soybean AtMAP65-1 homolog, under control of cauliflower mosaic virus 35S promoter. Both AtMAP65-1 and GmMAP65-1 transgenic soybeans are more resistant to challenges by the soybean bacterial pathogen *Pseudomonas syringae pv. glycinea* and the Oomycete pathogen P*hytophthora sojae*, but not the soybean cyst nematode, *Heterodera glycines*. Soybean plants expressing AtMAP65-1 and GmMAP65-1 also display a tolerance to the herbicidal oryzalin, that has a mode of action that destabilizes microtubules. In addition, GmMAP65-1 expressing soybean plants show reduced cytosol leakage under freezing conditions hinting that ectopic expression of GmMAP65-1 may enhance cold tolerance in soybean.

P–20. Deciphering protein rebalancing for desired seed composition traits in soybean

Ritesh Kumar, Postdoctoral Research Scientist, Department of Agronomy and Plant Genetics, University of Minnesota, Kumar797@umn.edu

Seed protein content is the critical factor determining soybean's nutritional and market value. Soybean varieties in the U.S. have around 36–38% protein. A higher percentage of the total soybean protein is constituted by the two main storage proteins, beta-conglycinin and glycinin. Based on their sedimentation coefficients, they are mainly named 7s (ß-conglycinin) and 11s (Glycinin) globulins. The 7S globulins are comprised of three subunits, alpha prime (α'), alpha (α), and beta (β). Meanwhile, five genes, gy1, gy2, gy3, gy4, and gy5, encode the 11s globulins. A slight increase in protein content significantly augments soybean crop value. The protein content regulation and subsequent protein composition are believed to be a multilevel process. To understand proteome plasticity at the molecular level, we need to identify and utilize seed storage machinery in a new fashion. In the current study, we used time-efficient and modern genome editing tools to create several storage protein family mutants in a single genotype and used multiple omics approaches to identify the common machinery involved in protein rebalancing. The common key regulators among different mutants will be tested to optimize the protein rebalancing and soybean seed composition traits.

P–21. Breeding for Resistance to Soybean Seedling Diseases

Feng Lin, Academic Specialist-Research, Michigan State University, fenglin@msu.edu

Soybean seedling disease is one of the most destructive diseases, causing 25-64 million bushels yield loss in the U.S. Seedling diseases is caused by multiple oomycetes and fungi, including Phytophthora spp, Pythium spp, Fusarium spp., Rhizoctonia spp., Phomopsis spp., etc. *Pythium irregulare* and *Pythium sylvaticum* are the most prevalent and aggressive Pythium species in soybean (Rojas et al 2017), causing seed rot, seedling damping off, and root rot. Partial resistance is the mostly known type of resistance to Pythium. Remarkably, pleiotropic QTLs have been identified (Scott et al. 2019, Clevinger et al. 2021). *Phytophthora sansomeana* E.M. Hansen & Reeser was differentiated from P. megasperma species complex, where *P. sojae* was once part of. *P. sansomeana* infects a wide range of plant species such as soybean, corn, clover, wild carrot, Douglas fir, and some weed species, causing sever symptoms of seed and root rot (Hansen et al. 2009; Hansen et al. 2012; Rojas et al. 2017). Unlike the resistance to *P. sojae*, little is known about the host resistance to P. sansomeana. In this poster, we are presenting our updates identifying resistance sources and mapping of quantitative and qualitative resistance genes/QTLs.

P–22. Temporal transcriptome profiling uncovers gene regulatory networks driving the progression of embryonic and post-germinative development

Jer-Young Lin, Assistant Research Fellow, Agricultural Biotechnology Research Center, Academica Sinica, jeryoung@gate.sinica.edu.tw

Morphogenesis occurs during both embryonic and post-germinative development in higher plants. These two morphogenesis events are connected by a period of developmental arrest, characterized by cessation of cell differentiation, and, simultaneously, a series of biological processes, including maturation, desiccation, dormancy, and germination.

However, what genes are sequentially expressed, and how are genes coordinately regulated to progress the transitions of developmental programs between the two morphogenesis events in soybean seeds are relatively unknown. To address this, we analyzed the transcriptomes from 12 soybean seed stages bridging the two morphogenesis events. Analysis of this considerable time course enabled the identification of consecutively active gene sets and gene regulatory networks promoting the transitions of developmental processes. For instance, a predicted gene regulatory network is preferentially provoked in early desiccation and contains many transcription factors involved in the response to abiotic stress. The function of one transcription factor highly active in this desiccation-related gene network was examined to validate the predictive regulatory network. This study illustrates the genetic basis underlying the transitions of developmental programs across embryogeny and post-germination in soybean seeds.

P–23. Maintaining and Fine-Tuning a Gene Editing Pipeline for Soybean Trait Improvement

Junqi Liu, Postdoctoral Research Scientist, Department of Agronomy and Plant Genetics, University of Minnesota, liuxx162@tc.umn.edu

Gene editing technologies have proved to be a powerful tool for crop improvement in recent years. In particular, CRISPR/Cas9 mediated gene editing technology has evolved very rapidly. A wide variety of tool kits have been developed and made readily available to researchers in academia. Despite the abundant technological progress, targeting multiple genes simultaneously and/or stacking desired mutations are still a challenging task in the process of improving soybean germplasm. We have utilized a modular vector system for cloning multiple gRNAs in the same vector and integrated it into our whole plant transformation (WPT) pipeline. The virulence of the Agrobacterium strain and the authenticity of modular vectors are periodically assessed and monitored to maintain the efficiency of the soybean mutagenesis pipeline. So far we have successfully mutated several target genes related to important agronomic traits and stacked multiple mutations in individual soybean lines.

P–24. Identification and Genetic Mapping of Quantitative Trait Loci Controlling Seed Protein Content Stability in Soybean

Drew Mitchell, Doctoral Student, Michigan State University, mitch987@msu.edu

Soybean seed protein content is a complex physiological trait regulated by many small-effect genes that show significant environmental interaction. Protein content stability across diverse growth environments is therefore often low in soybean. The identification of genomic regions associated with protein content stability may contribute to the development of stable high-protein cultivars, as well as to the further elucidation of mechanisms underlying physiological stability in soybean. In this work, 218 recombinant inbred lines (RILs) were derived from the intraspecific cross of the high protein accession PI-555396 (BARC-6) and the low protein MSU breeding accession E14077 for the investigation of quantitative trait loci (QTL) associated with protein content and stability across multiple years and test locations. Indices for absolute and relative protein content stability were used to estimate phenotypic variation across soybean production regions representing 4 unique year/location environments. Composite interval mapping returned one QTL associated with protein content on chromosome Gm20 explaining approximately 17.8% of phenotypic variation, and one QTL associated with both relative and absolute protein stability on chromosome Gm18, explaining approximately 6.5-7.3% of phenotypic variation, respectively. These identified QTLs are being used to inform continued genomic investigation for the prospect of developing highly stable, high-protein content soybean cultivars.

P–25. Cryptic isoprene synthase and its regulation under wounding and burning conditions in soybean

Mohammad Golam Mostofa, Postdoctoral Researcher, Michigan State University

Isoprene emission from some plants constitutes the major hydrocarbon flux from the biosphere to the atmosphere. Isoprene contributes to the formation of ozone and aerosols. However, plants can also benefit from the signaling roles of isoprene, particularly under stressful conditions. In plants, isoprene is synthesized from MEP pathway metabolite dimethylallyl diphosphate (DMADP) by isoprene synthase (ISPS). It was thought that ISPS had been lost from soybean as a

result of domestication pressure. In silico analysis revealed the presence of ISPS in soybean having high sequence similarity with the ISPS of known legume isoprene emitters. The phenylalanine (F), serine (S), and asparagine (N) residues, which are unique to ISPS, are conserved in soybean ISPS. We report that soybeans can make isoprene, but only transiently, when the same or nearby leaves are wounded or burned. Meanwhile, photosynthetic rate, stomatal conductance, and internal CO2 declined while isoprene was emitted from the undamaged part of the leaves. Metabolomics data showed that there is a surge in pyruvate and increases of most of the MEP pathway metabolites. ISPS of soybean, like kudzu, exhibited very strong cooperativity so that it is possible to have almost no isoprene emission at levels of DMADP sufficient for other required isoprenoid synthesis but significant isoprene emission at moderate to high DMADP. Jasmonic acid (JA) and JA-related metabolites were significantly upregulated in wounded leaves, suggesting a possible crosstalk between isoprene and the JA-signaling pathway. We conclude that soybean can make isoprene under certain stresses, when metabolic flux through the CBC to MEP pathway increases, highlighting the cryptic nature of GmISPS. Soybeans retained ISPS; future climate-related environmental pressure may result in isoprene emission from soybean, which may improve soybean resilience to various stresses.

P–26. Determining genetic mechanisms of maturity in North Dakota: expanding the molecular model for MG 00 and 0

Clara Mvuta, Graduate Student, Department of Plant Sciences, North Dakota State University, clara.mvuta@ndsu.edu

Soybean production has expanded significantly in North Dakota(ND), yet the state's yield averages remain among the lowest in the Midwest. Maturity is a crucial agronomic trait that affects yield potential, and since ND experiences a short growing season due to frost, early maturing soybean varieties are necessary. The dominant maturity groups in the state are MG 00 and 0, and it is possible to "fine-tune" maturity to the region/environment in order to maximize yield potential. The genetic mechanisms behind soybean maturity, particularly the E1, E2, and E3 genes, have been well characterized. Functional alleles of these genes result in late maturity, while null/semi-functional alleles lead to early maturity. Variations of the non-functional/semi-functional alleles of these genes create the MG 00 or 0 phenotype. However, it remains unclear which combination of alleles is most favorable for breeding purposes, potentially impacting yield. Additionally, it is hypothesized that other genes play a significant role in this specific environment, even if they have a minor effect in other maturity groups with functional E genes. The aim of this research is to identify the major effect maturity alleles present in MG 00 and 0 germplasm in ND. This will be accomplished through the examination of known maturity gene alleles in the breeding program at NDSU to determine the favored alleles.

P–27. Evaluation of Rps1c candidate gene in soybean against *Phytophthora sojae* using cowpea severe mosaic virus-induced gene silencing

Nghi Nguyen, Doctoral Student, The Ohio State University, nguyen.1759@osu.edu

Breeding soybean lines for resistance is the most effective strategy against the oomycete pathogen *Phytophthora sojae* which causes yield-limiting soybean root and stem rot disease. In a previous study using the resistance gene enrichment sequencing (RenSeq) method, we discovered several novel Rps1c candidate-resistant gene sequences. We investigated the function of one of these genes using a newly developed plus-strand RNA cowpea severe mosaic virus (CPSMV) vector to silence these genes in resistant soybean backgrounds. Hypocotyl inoculation of soybean line Williams 79 (contains Rps1c) with *P. sojae* race 1 after virus silencing resulted in an estimated 10% increase in lesion length expansion in the resistant backgrounds compared to the non-silence plants. This indicated that this candidate gene played a significant role in the resistant mechanisms of the soybean against *P. sojae*. Moreover, our study demonstrated that CPSMV is an effective and versatile tool to study gene function in soybeans.

P–28. Turning complex genomic data into action for trait development

Jason Nichols, Principal Scientist, Syngenta, jason.nichols@syngenta.com

The cost of DNA and RNA sequencing has decreased substantially in recent years, and as a consequence for any trait of interest the sequencing of relevant genomes and the generation of functional genomic datasets describing the target trait

has become much more accessible and commonplace. However, translating that wealth of genomic data into something actionable - i.e., a prioritized set of candidate loci that can be validated and advanced through biotechnology or breeding methods - remains a major hurdle for trait, especially complex trait, development. The output of such efforts should not simply be to hyper-describe a system, but it must predict that variation which is most likely to affect the desired trait and, ideally, in which direction must a locus be 'pushed' to affect that trait. Here we discuss advancements in tools and approaches that combine high precision genetic and genomic annotations with estimations of variant functional effects as a means to direct and prioritize hypothesis testing against target traits. Finally, we discuss some outstanding challenges, as well as opportunities, including combinatorial predictions and enabling selection of promising loci with genetic context in mind.

P–29. Investigating novel QTL to improve iron deficiency tolerance

Jamie O'Rourke, Faculty, Corn Insects and Crop Genetics Research Unit, USDA-ARS, Jamie.Orourke@usda.gov

Early quantitative trait loci (QTL) mapping studies identified major regions of the genome controlling traits of interest in agronomically important lines. Continued crop improvement requires the identification and utilization of alternative genomic regions, likely in novel germplasm. Fiskeby III is a soybean line with high tolerance to abiotic stresses, including iron deficiency, a perennial problem in the upper Midwest of the United States. A previous study identified a novel iron deficiency tolerance QTL on chromosome Gm05 in Fiskeby III. Using virus induced gene silencing (VIGS), we t argeted candidate genes in the Williams 82 genome sequence associated with the QTL. A single gene resulted in phenotypic changes under iron deficient and sufficient conditions. Using RNAseq we have examined gene expression patterns which have revealed Fiskeby III induces transcriptional reprogramming within 24 hours of iron stress, similar to other tolerant soybean varieties. While Fiskeby III induces all the canonical soybean iron deficiency responses, the individual genes and timing of these responses differs from other iron deficiency tolerant lines. Identifying the genes and understanding the pathways and timing utilized by Fiskeby III provides novel targets for improving abiotic stress tolerance in elite soybean lines.

P–30. Metabolomic Study of Aquatic-Feed Transgenic Soybean to Enhance Sustainability and Nutritional Value for Aquaculture Feedstock

Duyen Pham[1], Research Assistant, Umesh Yadav[1], Cintia L. Arias[1], Leah McHale[2], Edgar B. Cahoon[4], Tom E. Clemente[4], Truyen Quach[3], Hyojin Kim[4], Kiyoul Park[4], Hae Jin Kim[4], and Ana P. Alonso[1]

[1] BioDiscovery Institute, University of North Texas, Denton, TX, USA. [2] Department of Horticulture and Crop Sciences, The Ohio State University, Columbus, OH, USA. [3] Department of Agronomy and Horticulture. University of Nebraska, Lincoln, NE, USA. [4] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, USA.

An increased consumption of fish products results in higher demands for fish feedstock. However, the current feedstocks are wild-caught fisheries that are not sustainable. Soybean has emerged as a promising alternative to replace fish meals in aquaculture. The objective of this study is to develop new soybean cultivars synthesizing novel compounds that best fit for aquaculture. Nine-gene stack assembly was introduced to enhance the production of omega-3 fatty acids, tocotrienols and astaxanthin in soybean seeds, normally provided to the aquacultures by the fish meal or additional supplements. Unfortunately, these transgenic events showed a non-desired concomitant reduction of the total oil content. For further optimizations aiming to reach the desired levels of novel compounds without altering soybean general agronomic performance, a better understanding of carbon partitioning in these embryos is required. Therefore, we dissected soybean embryos at five developmental stages and determined their chlorophyll and carotenoid content, biomass composition, and metabolome. The most significant differences in the transgenic events were: i) the successful production of new compounds, such as omega-3 fatty acids and carotenoids at the detriment of total oil content; ii) the transient accumulation of starch was reduced by approximately 50%; iii) the content of chlorophyll a and b were drastically reduced; and iv) a significant increase in amino acid production. Identifying metabolic bottlenecks in carbon distribution in developing embryos will guide future engineering efforts to tailor soybean lines more valuable for aquafeeds.

POSTER ABSTRACTS

P–31. Genome editing of Glycinin and β-conglycinin family members in soybean (*Glycine max* Merr. (L.)

Truyen Quach, Research Assistant Professor, Center for Plant Science Innovation, University of Nebraska–Lincoln, tquach2@unl.edu

The glycinin and β-conglycinin seed storage proteins collectively account for approximately 55% of soybean seed protein content. Genome editing reagents were designed to specifically create null mutations in gene models Glyma.13G123500 and Glyma.10G246300, annotated as glycinin and ß-conglycinin family members, respectively. The goal being to investigate the phenotypic outcomes in seed storage reserves, and impact on expression of stacked transgenic alleles in the double null mutant background. Homozygous edited lines carrying INDELs that manifest predicted premature stop codons in each of the gene models have been developed. Imaging of total protein on SDA-PAGE gels revealed that the predicted translational products of the two gene models are devoid in the derived dual homozygous edited lines. The edited homozygous lines are rebalanced in protein content, with no observed changes in total oil or amino acid profile. A small-scale field study showed that the plot weights were reduced in the edited lines, with no change in 100 seed weight, relative to control plots. To assess if expression levels of transgenic alleles are impacted when stacked with the edited alleles, transgenic alleles designed to increase carbon flux to lipids, and for the accumulation of leghemoglobin were crossed into the edited lines for down-stream characterizations.

P–32. *A Genomic Selection Pipeline for Public Soybean Breeding Programs

Vishnu Ramasubramanian, Postdoctoral Associate, University of Minnesota, vramasub@umn.edu

Genomic selection has become an important part of plant and animal breeding programs to accelerate genetic gain. We've implemented a GS pipeline designed for soybean public breeding programs in the US Midwest using open source tools that are currently available. Herein, we describe the steps and tools in the pipeline and discuss results for the Northern Uniform Soy Trial Population. Soybeanbase, an instance of breedbase hosted by BreedingInsight is used for the storage of both genotypic and phenotypic data. Filtering of markers and lines are done in the R environment using rTASSEL and custom R scripts. Imputation using LD – K-nearest neighbors imputation (LDKNNi) implemented in rTASSEL often showed the highest imputation accuracy in our test data. An optimized training subset selected using the STPGA package demonstrated higher accuracies in 5-fold cross-validations compared to a randomly selected subset for certain combinations of candidate and training set sizes. In this version, we've implemented genomic prediction models using the rrBLUP, BWGR, BGLR and SOMMER packages for single trait and multiple traits across single or multiple environments. We've also implemented models that include GxE interactions and environmental covariates using the SOMMER and envRtype packages. Our preliminary results indicate that modeling GxE interactions significantly improved prediction accuracies for a subset of the NUST population and including environmental covariates improved prediction accuracies only for some conditions. In the future, we plan to refine these methods and optimize the GS pipeline.

*Poster selected for oral presentation.

P–33. Breeding Soybean for Intercropping with Pennycress: Genetic Variation of Target Traits

Lucas Roberts, Graduate Student, Department of Agronomy and Plant Genetics, University of Minnesota, robe2110@umn.edu

Pennycress, (*Thlaspi arvense*) is a newly domesticated winter oilseed adapted to the Midwest US. Due to its winter annual life history, pennycress is planted in the fall between rotations of maize and soybeans. In this intercropping system, pennycress provides the benefits of a cover crop while producing valuable oilseeds. The shorter growing season in Minnesota necessities that the following summer row crop overlap in time and space with pennycress 4 – 8 weeks. Soybean, (*Glycine max*) fits into this cropping system due to its high plasticity. Competition for resources during this overlap period is high and there is a need to develop adapted soybean varieties to ensure the adoption of pennycress into the cropping landscape. Our objectives include quantifying genotype-by-cropping system interactions and characterizing soybean traits relevant to winter oilseed intercropping. Towards identifying the genetic variation in soybean responses

to intercropping, 40 soybean genotypes were planted into the pennycress variety MN-106 in 2020, 2021, and 2022. Field experiments in western and southern Minnesota utilized a split block design with two cropping treatments – soybeans intercropped into pennycress and monocropped soybeans. Intercropping with pennycress only reduced soybean yield in 2 of the 5 environments. Additionally, there were significant genotype-by-treatment interactions.

P–34. Enhancing plant water use efficiency under overexpression of photosystem II subunit S

Seema Sahay, Postdoctoral Research Associate, Biochemistry, University of Nebraska–Lincoln, ssahay2@unl.edu

Limited water availability is the most constraint on crop production. Improving water use efficiency (WUE), which means reducing the amount of water required for per unit carbon gain has been a key target for crop improvement. The photosystem II subunit S of protein (PsbS) is a pigment binding protein and ubiquitous in all vascular plants and plays a role in the non-photochemical quenching (NPQ), a photoprotective mechanism of chlorophyll fluorescence.

Here, we hypothesized that PsbS has a potential vitality in the plants' water use efficiency via modify the signal for stomata opening and heat dissipation in light together with drought. We generated transgenic tobacco with increased levels of PsbS protein than wildtype. Tobacco plants accumulated more PsbS protein in drought conditions than control condition and showed higher NPQ, water use efficiency, lower chloroplastic quinone A (QA) state, and less stomata opening which resulted into increased carbon gain at ~20-30 less water consumption relative to wildtypes. We are currently investigating the same proof-of-concept in soybean, since PsbS is universally present in all plants, and we are hypothesizing that manipulating PsbS expression should be effective in other crops.

P–35. Identification and evaluation of soybean lines conferring major resistance to *Phytophthora sansomeana*

Muhammad Salman, Graduate Student, Michigan State University, salmanm3@msu.edu

Several soybean (*Glycine max*) diseases caused a loss of $95.48 billion over the last two decades in the USA. *P. sansomeana*, an oomycete closely related to *P. sojae*, has been reported as causing extensive root rot and in some cases more severe than the latter. *P. sansomeana* has a wide host range including corn, plum, and Christmas trees. Very little is known about the genetic sources of resistance. In this study, 135 improved soybean lines from MSU and 470 germplasm lines were screened with *P. sansomeana* isolate MPS17_22 using the hypocotyl inoculation method. Surprisingly, none of the MSU improved lines showed complete resistance. Nevertheless, screening of the 470 germplasm lines identified 16 resistant lines, 39 intermediate resistant lines, and 415 susceptible lines. Replicated tests confirmed stable resistance from seven lines, including T-286, Colfax, L77-1727, Bergerac, Serda 231A, NE1900, and NE2701. Challenging these lines with 15 more *P. sansomeana* isolates revealed different interaction patterns and found that L17-1727 conferred resistance to all the isolates. To validate the resistance in the field conditions, two resistant lines, Colfax and NE2701 were field inoculated with MPS17-22 at the MSU plant pathology farm. Resistant lines protected 60–80% of yield, while the susceptible controls lost 87–93% of yield. These resistant germplasm lines can be used for breeding.

P–36. Effect of QDRL-18 for quantitative resistance to *Phytophthora sojae* on seed nutrient composition

Maria Santiago Padua, Master's Student, and Christian Vargas-Garcia, Stephanie Karhoff, Sungwoo Lee, Anne Dorrance, Rouf Mian, Leah McHale The Ohio State University, santiago-Padua.1@osu.edu

Quantitative disease resistance (QDR) to *Phytophthora sojae*, causal agent of phytophthora root and stem rot (PRSR), is critical to maintaining yield in soybean fields with diverse populations of this pathogen. The molecular mechanisms of QDR are largely unknown and likely variable, with potential for pleiotropy impacting traits such as yield or seed composition. We used a previously identified major QDR locus in chromosome 18 (QDRL-18) to investigate pleiotropy in both disease conducive and non-conducive conditions. QDRL-18 was identified in recombinant inbred line (RIL) populations derived from crosses between OX-20-8 (S) and PI 427105B (R) or PI 427106 (R). Three sets of near-isogenic lines (NILs) varying only for the QDRL-18 allele were derived from the populations. Trials using NILs were previously conducted in

three environments conducive and four environments not conducive to PRSR. Previous studies showed that the QDRL-18 resistance alleles enhanced yield by 13 to 29% under PRSR conducive field conditions, with no significant impact on yield in the absence of *P. sojae* pressure. However, beyond yield stability, nutritional content of soybean seed is critical for the food and feed industries. In the present work, harvested seeds were analyzed by near-infrared reflectance spectroscopy and seed nutritional composition evaluated by ANOVA. Among the variation in nutrient content observed, QDRL-18 allele affected protein content of lines grown under disease conditions. These findings suggest that QDRL-18 may directly impact seed nutrient composition through a pleiotropic response, or indirectly through biotic stress from *P. sojae*. Further research will confirm and extend these findings.

P–37. Mapping and characterizing oval leaflet shape in soybean associated with low number of seeds per pod

Alina Smolskaya, Graduate Student, University.of Minnesota, smols001@umn.edu

An interesting relationship has been found between leaflet shape and number of seeds per pod (NSPP) in soybean. Leaf shape in soybean is typically characterized as ovate, oval, and lanceolate (narrow). Lanceolate leaflet soybean lines have a high NSPP with small seeds, while oval leaflet lines produce low NSPP. Decoupling seed size from NSPP by improving our understanding of genetic architecture underlying these traits is promising for enhancing future yields. In this study, we are fine-mapping and characterizing loci causing oval leaflets and low NSPP and comparing them to the previously mapped lanceolate locus casual for high NSPP and narrow leaflets. Fine mapping for the oval trait is being done by genotyping plants with KASP markers in a segregating backcross population. Leaflet shapes are being differentiated using the MuLES image analysis macro in ImageJ. NILs with oval and lanceolate leaves sharing a common recurrent parent will be grown in a multi-location yield trial to test for differences in yield between lines with low and high NSPP. The goal of this study is to better understand the genes underlying leaflet shape and NSPP traits in soybean for enhancing future soybean yields.

P–38. Shoot architecture traits are important determinants of canopy coverage and light Interception in soybeans

Suma Sreekanta, Post-doctoral Research Associate, Allison Haaning, Austin Dobbels, Riley O'Neill, Anna Hofstad, Kamaleep Virdi, Fumiaki Katagiri, Robert M. Stupar, Gary J. Muehlbauer and Aaron J. Lorenz. University of Minnesota, sreek002@umn.edu

Shoot Architecture (SA) is a result of complex interplay between many traits. In crops such as maize and wheat, altering SA is associated with enhanced yield. However, study of SA has been limited to a few traits because measuring SA traits has traditionally been a slow, low throughput process. Our study aims to characterize the genetic variation in diverse soybean accessions and to understand variation in SA traits that may have accompanied soybean breeding for yield in the past several decades. We use a combination of high-throughput technologies including an unmanned aircraft system as well as inexpensive smartphone images to parameterize SA in terms of multiple individual leaf, branch and whole plant traits of field grown plants. Our studies show heritable variation for many of the SA traits and that the traits defining the distal portion of the plant encompassing the top four nodes significantly affect light interception and canopy coverage. We have identified QTLs for SA traits impacting canopy coverage and that a major QTL for branch angle coincides with a QTL identified for canopy coverage. Our current work is examining the SA traits in a panel of important MG I public and private variety releases ranging from 1944 to 2017. Varieties were selected based on pedigree and organization diversity, importance in production, and uniform distribution through the years within each decade. The results of this study will be useful in identifying SA traits that enhance the yield potential of soybean by optimizing canopy coverage and light interception.

POSTER ABSTRACTS

P–39. Assembly and utilization of a single haplotype reference genome for soybean

Robert Stupar, Professor, University of Minnesota, stup0004@umn.edu

This report details the assembly of a new reference genome of the soybean genotype Williams 82 and its use in comparative genomics. The genome was derived from sampling a single plant of Williams 82, known as the sub-line 'Williams 82-ISU-01' (Wm82-ISU). The genome was assembled using Pacific BioSciences HiFi reads and integrated into chromosomes using HiC. The Wm82-ISU genome adds 59.5 Mb of sequence and reduces contig number from 9,202 to 36 total contigs. The new annotation includes a significant amount of full-length cDNA sequencing which has reduced the gene count from 52,872 to 48,387. Williams 82 was derived from backcrossing genotype Kingwa into the background of Williams, leading to heterogeneous introgressed segments that persists in modern Williams 82 sub-lines. The Wm82-ISU assembly shows clean Kingwa introgression segments, reflecting its derivation from a single sub-line DNA source. In addition to Wm82-ISU assembly, we also assembled the genome of soybean line 'Fiskeby III,' a rich resource for abiotic stress resistance genes. We used these assemblies to study the genomic variation between 'Fiskeby III' and the Wm82-ISU reference within a fine-mapped QTL for iron deficiency chlorosis resistance, revealing candidate sequence polymorphisms that may be underlying the QTL variation.

P–40. Let the bees do the work: using biotechnology to convert soybean from a self-fertilizing to an outcrossing plant

Nicole Szeluga, Doctoral Candidate, Cornell University, nms244@cornell.edu

Developing a mass hybrid seed production system in soybean [*Glycine Max* (L.) Merr.] has been a consistent goal for decades since the mainstream utilization of hybrid vigor for crop improvement. However, the small size of soybean flowers and their predisposition towards self-fertilization results in a low percentage of outcrossing and hinders the large-scale production of hybrid seeds. The implementation of rescuable male sterility overcomes the barrier of self-fertilization but is not sufficient to amplify the production of hybrid seeds without the recruitment of insect vectors to facilitate outcrossing. Can soybean flowers be phenotypically altered to increase pollinator visitation and outcrossing? Combined with the barnase/barstar sterility rescue system, this study aims to use biotechnology to alter floral phenotype and transform soybean from a self-fertilizing to an outcrossing plant.

P–41. *Nuclear retention of transcripts as regulatory mechanism of protein translation in soybean root and nodule cells

Sutton Tennant, Graduate Student Researcher, Department of Agronomy and Horticulture, University of Nebraska–Lincoln, sutennant@huskers.unl.edu

The central dogma of molecular biology follows a simple path, DNA is transcribed into transcripts in the nucleus, and transcripts are then translated into proteins in the cytosol. However, many studies reported that protein production is not solely impacted by the level of expression of genes, but by many other regulatory processes. The number of studies exploring these post-transcriptional regulatory processes in plants is sparse. Here, combining the use of single-nucleus transcriptomic and high-resolution fluorescent in situ hybridization technologies, we provide a new perspective on the role of the nuclear retention of transcripts as a central mechanism to control RNA biology and the biology of plant cells. The analysis of confocal microscopic images of transcripts at the sub-cellular resolution combined with the use of a specifically designed Image J software package, clearly revealed the differential nuclear retention of transcripts between genes, cell types, and organs of the soybean root and nodule. This work reveals the influence of the sub-compartmentalization of transcripts as another regulatory mechanisms of protein translation and a new understanding of the central dogma of molecular biology.

*Poster selected for oral presentation.

POSTER ABSTRACTS

P–42. Genomic prediction for sudden death syndrome (SDS) in soybean

Raju Thada Magar, Doctoral Student, Michigan State University, thadaraj@msu.edu

Sudden death syndrome caused by Fusarium virguliforme, is a major soybean disease prevalent in Northern United States. Genetic resistance is the most eco-friendly and economical approach to control this disease. Genomic selection (GS) has been successfully used to achieve genetic gain for various traits in soybean including disease resistance. GS utilizes all available molecular and phenotypic markers train models to calculate genomic estimated breeding value (GEBV), then trained models are used to predict GEBV of new individuals based on their genotypic information. In this study, the ridge regression best linear unbiased prediction (rrBLUP) model is employed to estimate the genomic estimated breeding value of 367 AYT(Advance Yield Trial) soybean lines for disease index, disease severity, and disease indices. With 10-fold cross-validation, the prediction accuracy is found to be 0.61 for disease incidence, 0.76 for disease severity, and 0.56 for disease indices. These findings demonstrate that genomic prediction is a viable option for facilitating selection against sudden death syndrome.

P–43. Ureide partitioning affects drought stress response in soybean

Sandi Win Thu, Doctoral Student, Washington State University, sandiwin.thu@wsu.edu

Legumes are able to access atmospheric di-nitrogen (N2) through a symbiotic relationship with bacteria residing in root nodules. In soybean [*Glycine max* (L.) Merr.], ureides are the products of N2 fixation and represent the primary long-distance nitrogen (N) transport compounds. Ureide transport is mediated by membrane-localized UPS ureide permeases and recent work in our lab has shown that UPS1 overexpression (UPS1-OE) in soybean leads to increased nodule-to-shoot N allocation with positive consequences for plant growth and seed development. In addition, it was demonstrated that enhanced N export from nodules positively affects N2 fixation and nodule metabolism. On the other hand, drought stress has an inhibitory effect on N2 fixation, probably due to decreased ureide transport out of, and their associated accumulation in, nodules. Here, we hypothesized that in UPS1-OE soybean plants down-regulation of N2 fixation under drought can be reduced and nodule-to-sink N allocation maintained. Soybean plants were exposed to medium and severe water-stress conditions, and we found that ureide movement from nodule to shoot in the xylem as well as from leaf to sink in the phloem was enhanced in water-stressed UPS1-OE versus wildtype plants. In addition, leaf chlorophyll content, photosynthetic rates, and sucrose phloem transport were higher in the transgenic plants under drought. The combined changes in N and carbon partitioning in UPS1-OE plants resulted in increased shoot and nodule biomass, and improved nodule numbers. Overall, the results support that ureide transport processes from nodules to sinks are essential for regulating ureide tissue levels and subsequently drought stress tolerance in soybean.

P–44. * Increasing Sulfur Content in Soybean Seed Protein

Trish Tully, Postdoctoral Associate, Donald Danforth Plant Science Center
TLA Tully[1], D Duressa[3], V Veena[1], TP Durrett[3], DK Allen[1,2]
[1.] Donald Danforth Plant Science Center, St. Louis, MO, 63132; [2.] United States Department of Agriculture, Agricultural Research Science, St. Louis, MO, 63132; [3.] Kanas State University, Manhattan, KS, 66506

Protein is one of the most valuable biomass components of soybean seeds and accounts for ~40% of seed biomass. However, soy protein is not optimal for animal meal-based diets as it is deficient in sulfur containing amino acids (cys + met). In the past, attempts to increase sulfur content in soy protein have been focused on the protein level, including heterologous protein expression and overexpression of endogenous storage proteins with high sulfur content. Unfortunately, these approaches have had limited success. Here we illustrate the first steps in increasing sulfur content of soy protein at the metabolic level. In wildtype soybean, low molecular weight thiols downstream of cysteine (γEC and hGSH) accumulate over the course of development. This sequesters sulfur in non-proteogenic compounds rather than in the protein found in meal. To increase cysteine availability for protein synthesis, we have generated RNAi-knockdown lines targeting CGL; the enzyme responsible for diverting cysteine towards γEC and hGSH biosynthesis. RNAi-knockdown lines show decreased expression of all CGL homologues with seeds that are morphologically similar to wildtype. Observed levels of free amino acids and sulfur intermediates show an increase in free cysteine and a decrease in both γEC and hGSH in

CGL-knockdown lines relative to wildtype. CGL-knockdown lines also show an increase in total protein as well as an increase in protein-bound cysteine relative to wildtype. Further work will combine the CGL-knockdown lines with protease-knockdown lines; combining a push and a protect to result in a hypothesized greater increase in both total protein and protein-bound cysteine.

*Poster selected for oral presentation.

P–45. Developing Robust and Durable Resistance to Soybean Rust

Rao Uppalapati, Biotech Disease Resistance Trait Portfolio Leader, Corteva Agriscience, rao.uppalapati@corteva.com

Asian soybean rust (ASR), caused by the fungus Phakopsora pachyrhizi (Pp), is one of the most economically important soybean disease. Farmers in Brazil spend US$1.7B on fungicides per year to control ASR and these chemical options are becoming less effective due to the emergence of fungicide resistance. Native soybean resistance from Rpp1-7 is identified but is reported to be overcome by one or more isolates from Brazil. To overcome these challenges, Corteva in collaboration with 2Blades identified a novel resistance gene from pigeon pea to provide robust and durable genetic resistance for ASR control. Cloning of CcRpp1 (Cajanus cajan Resistance against Phakopsora pachyrhizi 1) was previously reported (Kawashima et al., Nat Biotech, 2016). More recently, using a combination of cytology, transcriptomics, and metabolomics, we show that resistance is expressed rapidly, and Pp infection is arrested early, within 24-36 hours after inoculation and CcRpp1 shows excellent field level effiacy. The early resistance responses serve either to block initiation of haustoria formation or to inhibit maturation of incipient fungal feeding structures and effectively provide immune-level resistance against ASR.

P–46. Genomic diversity and engineering of soybean trypsin inhibitor gene family

Zhibo Wang, Postdoctoral Researcher, Donald Danforth Plant Science Center, zhibowang@danforthcenter.org

Trypsin inhibitors (TIs) have diverse biological functions. They accumulate in soybean seeds and are considered as anti-nutritional proteins that can severely reduce the digestibility of soybean meal. It is critical to understand genomic basis underlying production of TIs for developing soybean cultivars containing desirable seed TI activities without a negative effect on soybean performance. Genome-wide analysis showed that soybean contained 47 Kunitz TI (KTI) genes and 12 Bowman-Birk TI (BBTI) genes. We examined that each TI gene had a distinct temporal and spatial expression pattern, which is correlated with their phylogenetic relationship. A subfamily of 8 BBTI genes that were preferentially expressed in seeds over the course of seed maturation. The 47 KTI genes were clustered into three subfamilies and were expressed in both seed and non-seed tissues. We further identified KTI1 and KTI3 as two seed-specific TI genes. Mutant kti1 and kti3 alleles carrying small indels were created using CRISPR/Cas9-mediated genome editing approach. The KTI content and TI activity both remarkably reduced in the gene edited seeds. We further developed markers to co-select the mutant alleles of kti1/3 using a gel-electrophoresis-free method. The kti1/3 mutant line and associated markers will assist in accelerating the introduction of low TI trait into elite soybean cultivars in the future.

P–47. Rdm3 Locus – a Major QTL Underlying Resistance to Southern Stem Canker in Elite Soybean Germplasm

M Habib Widyawan, Doctoral Student, University of Georgia, widyawan@uga.edu

Soybean southern stem canker (SSC) caused by *Diaporthe aspalathi*, is an economically important disease in the southern United States. Five loci conferring resistance to SSC, namely Rdm1-5, have been named based on segregation analysis and reactions with different isolates. The Rdm3 locus carried by the SSC-resistant cultivar Crockett provides good comparable resistance to SSC when compared to the cultivars possessing multiple Rdm loci. However, the genomic location of this locus is unknown, and sources of resistance to SSC used in the breeding program are undetermined. This study aims to map the Rdm3 locus from Crockett and determine the key sources of resistance to SSC in the Georgia Soybean Breeding Program. Using a RIL population derived from a cross of G81-2057 ? Crockett, genetic mapping identified the

Rdm3 locus on chromosome 14 that explained 55% of phenotypic variation. To determine the key sources of resistance to SSC in elite germplasm, a panel consisting of 485 experimental lines from the Georgia Soybean Breeding Program was selected to perform a genome-wide association analysis. The resistant allele at the Rdm3 locus provides a major source of resistance to SSC in this elite germplasm pool. GSM_975, a marker tightly linked with the Rdm3 locus, could accurately distinguish soybean lines based on their SSC resistance provided by the Rdm3 locus. The results revealed the prevalence of the Rdm3 locus resistance allele in the elite soybean germplasm. The QTL and flanking marker information will provide useful information and tools to assist breeders in developing SSC-resistant cultivars.

P–48. An Efficient and Practical Fixation for Plant Single Nucleus RNA-seq

Hengping Xu[1], Sandra Thibivilliers[1], Sergio Alan Cervantes-Perez, and Marc Libault[1*], Center for Plant Science Innovation, Beadle Center, University of Nebraska–Lincoln, [1] These authors contribute equally to this work [*]Corresponding author: marc.libault@unl.edu

Single-nucleus RNA sequencing (snRNA-seq) technology is emerging as a robust alternative to single-cell RNA sequencing (scRNA-seq) in plant biology. Fixation of nuclei has been successfully tested on animal samples before conducting snRNA-seq. Unlike aldehyde-based fixatives or DSP [dithiobis (succinimidyl propionate)], methanol fixation has been validated when conducting single-cell RNA sequencing (scRNA-seq). Such fixation reduces potential RNA loss and enhances RNA quality improving the outcome of snRNA-seq experiments. However, little is known about the impact of methanol fixation on plant nuclei isolation and snRNA-seq experiments. In this study, we test the effect of a methanol fixative on snRNA-seq experiments conducted on Arabidopsis and Sorghum root samples. We found that methanol fixation is an efficient and convenient option to conduct with success plant snRNA-seq in transcriptomic studies.
Key words: Methanal fixation, nuclei, single nucleus RNA sequencing (snRNA-seq)

P–49. Mapping Active Pathways in Developing Thorne Embryos Using 13C-Labeling

Umesh Yadav, Research Scientist, University of North Texas, umeshprasad.yadav@unt.edu

13C-metabolic flux analysis aims to elucidate the distribution of carbon in a living organism, and ultimately, identify potential bottleneck(s) that can be targeted through metabolic engineering. *Glycine max* cv. Thorne variety was chosen because it is less recalcitrant for transformation than others with similar seed composition, which allows identified metabolic targets to be evaluated in vivo in the same cultivar. The construction of a flux map requires: i) the establishment of in vivo embryo culture conditions that mimic the in planta ones, and ii) the utilization of isotope tracers to map the flow of carbon through metabolic pathways. For this purpose, funiculi and endosperm tissues were collected, and the most abundant carbon and nitrogen substrates were identified to establish a suitable culture medium for developing soybean embryos. The efficiency with which Thorne embryos convert carbon substrates into biomass was found to be lower than other soybean cultivars. Then, carbon and nitrogen sources from the media were replaced with 13C-labeled ones, and the incorporation of 13C-labeling in intracellular compounds was monitored using LC-MS/MS. Our results demonstrate that there is no significant action of the phosphoenolpyruvate carboxykinase, and no significant gluconeogenesis neither. However, there is labeling evidence for a flow of carbon through the plastidic malic enzyme to support de novo fatty acid synthesis. We anticipate that the construction of embryo's flux map will allow the identification of critical control points in central metabolism that govern protein and oil content. Ultimately, those bottlenecks will be tested using plant metabolic engineering by generating transgenic lines.

P–50. Understanding the roles of soybean aphid effectors in soybean and soybean aphid interaction

Dandan Zhang, Graduate Student, and Gustavo MacIntosh, Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, ddzhang@iastate.edu

The production of soybean, an economically important crop, has been threatened by both direct and indirect damages caused by soybean aphid, which is a specialist colonizing only soybean plants. Our current soybean aphid management strategies rely heavily on the application of insecticides. However, the emergence of aphid populations with insecticide resistance has made development of novel aphid control strategies an urgent need. Undoubtedly, understanding the mechanisms underlying host and pest interaction is key to provide insights for the achievement of this goal. In this

study, we examined soybean responses induced by aphid infestation including the activation of soybean mitogen-activated protein kinases (MAPKs) and the production of reactive oxygen species (ROS), which are two of the most conserved plant immune responses to pathogen attack. Our study found that soybean aphid infestation activated MPK4 and MPK6 in soybean dynamically at different time points post aphid infestation. Colonization by aphids also inhibited chitin-induced ROS production in soybean but failed to suppress flg22-dependent induction. Three soybean aphid effector candidates predicted by our transcriptome analyses-based pipeline were selected and tested for their potential to influence aphid-triggered MAPKs activation and ROS production. C002, one of the putative effectors, was found to be able to inhibit both chitin and flg22 induced ROS production, and another putative effector, MP10 showed the ability to enhance both chitin and flg22 induced ROS production. These findings provide a deeper understanding of how soybean responds to aphid infestation and the potential roles of two effectors in soybean and soybean aphid interaction.

SOY2023 REGISTRANTS

This list includes attendees registered prior to program publication.

Andrea Acuna
Molecular Lab Operations
University of Arkansas
macunaga@uark.edu

Shin-ichiro Agake
Visiting Scholar
University of Missouri
Tokyo University of Agriculture and Technology
sagake@missouri.edu

Doug Allen
Computational Biologist
USDA Agricultural Research Service
doug.allen@usda.gov

Ana Paula Alonso
Professor
University of North Texas
Anapaula.Alonso@unt.edu

Sahand Amini
Postdoctoral Research Associate
Department of Agronomy and Horticulture
University of Nebraska–Lincoln
samini2@unl.edu

Ruthie Angelovici
University of Missouri
angelovicir@missouri.edu

Shveta Bagga
Protein and Analytical Core Technology Lead
Corteva Agriscience
shveta.bagga@corteva.com

Andre Belo
NBT Manager
GDM Seeds
abelo@gdmseeds.com

Francois Belzile
Professor
Universite Laval
francois.belzile@fsaa.ulaval.ca

Andrew Bent
Professor
University of Wisconsin–Madison
afbent@wisc.edu

Arthur Bernardeli
Graduate Student
arthurbernardeli@gmail.com

Janel Bettis Sr.
Research Associate
Corteva Agriscience
janel.bettis@corteva.com

Siddhi J Bhusal
University of Minnesota
Postdoctoral Researcher
sjbhusal@umn.edu

Lucas Borges dos Santos
PhD Graduate
University of Illinois Urbana-Champaign
lucasb@illinois.edu

Jeremy Brown
Graduate Student
Biochemistry
University of Nebraska–Lincoln
jbrown353@huskers.unl.edu

Edgar Cahoon
Director, Center for Plant Science Innovation
Professor, Biochemistry
University of Nebraska–Lincoln
ecahoon2@unl.edu

Jacqueline Campbell
Corn Insects and Crop Genetics Research
Geneticist Plants
Corn Insects and Crop Genetics Research Unit, USDA-ARS
Jacqueline.Campbell@usda.gov

Sergio Alan Cervantes-Perez
Postdoctoral Research Associate
Department of Agronomy and Horticulture
University of Nebraska–Lincoln
alan.cervantes@unl.edu

Sivakumar Chamarthi
Postdoctoral Fellow
University of Missouri
schamarthi@missouri.edu

Vvikranth Chandrasekaran
Postdoctoral Fellow
University of Missouri
vkc4kf@umsystem.edu

Dheeraj Chatti
Postdoctoral Researcher
Kansas State University
dheerajc@ksu.edu

Tom Clemente
Professor
Department of Agronomy and Horticulture
University of Nebraska–Lincoln
tclemente1@unl.edu

Hyeon-Je Cho
Technical Manager
Corteva Agriscience
hyeon-je.cho@corteva.com

Maria Luiza de Oliveira
Soy Transformation Production Lead
Plastomics
maria@plastomics.com

Ive De Smet
Group Leader
VIB-UGent Center for Plant Systems Biology
Ive.DeSmet@psb.ugent.be

Jennifer Derkits
Graduate Student
University of Nebraska–Lincoln
jenn@derkits.com

Ryan Disney
PhD Student & Graduate Research Assistant
University of Illinois Urbana–Champaign
rdisney2@illinois.edu

Lauren Docherty
Graduate Student
University of Minnesota
doche014@umn.edu

Luke Dojack
Master's Student
University of Guelph
ldojack@uoguelph.ca

SOY2023 REGISTRANTS

Anne Dorrance
Professor
Dept. of Plant Pathology
The Ohio State University
dorrance.1@osu.edu

Timothy Durrett
Associate Professor
Kansas State University
tdurrett@ksu.edu

Ben Fallen
Research Geneticist
USDA-ARS
Ben.Fallen@USDA.GOV

Liliana Florez-Palacios
Program Associate
University of Arkansas
sandrafp@uark.edu

Felix Fritschi
C. Alice Donaldson Professor in Bioenergy Crop Physiology and Genetics
Division of Plant Science and Technology
University of Missouri
fritschif@missouri.edu

Jonathan Mendoza Garcia
Visiting scholar
Undergraduate student
University of Arkansas
jm199@uark.edu

Zhengxiang Ge
Research Technologist II
Center for Plant Science Innovation
University of Nebraska–Lincoln
zge4@unl.edu

Luis Bienvenido Gomez Luciano
lgomez-luciano@danforthcenter.org

Rafael Goncalves Marmo
Visiting Scholar
University of Arkansas
rafaelg@uark.edu

Michelle Graham
Faculty
Corn Insects and Crop Genetics Research Unit
USDA-ARS
Michelle.Graham@usda.gov

David Grant
USDA Collaborator & Affiliate Associate Professor
USDA-ARS & Iowa State University
macgrant@gmail.com

Ming Guo
Research Assistant Professor
University of Nebraska–Lincoln
mguo2@unl.edu

Junping Han
Technician
OSU
han.393@osu.edu

C. Nathan Hancock
Associate Professor
University of South Carolina Aiken
nathanh@usca.edu

Forrest Hanson
Master's Student
North Dakota State University
forrest.hanson@ndsu.edu

Rajnee Hasan
Graduate Student
Biochemistry
University of Nebraska–Lincoln
rhasan2@huskers.unl.edu

Kristin Haug Collet
Research Scientist
Corteva
Kristin.HaugCollet@Corteva.com

Md Sabbir Hossain
Graduate Student
Department of Agronomy and Horticulture
University of Nebraska–Lincoln
mhossain8@huskers.unl.edu

Tu Huynh
Doctoral Student
The Ohio State University
huynh.177@osu.ed

Ugochukwu Ikeogu
Postdoctoral Researcher
Kansas State University
uni3@ksu.edu

Justin Jantes
SRA II Technician
Corteva
jantes@gmail.com

Jen Jaqueth
Research Scientist
Corteva Agriscience
jennifer.jaqueth@corteva.com

Sarah Johnson
lsaverbio@gmail.com

Rupesh Kariyat
Associate Professor
University of Arkansas
rkariyat@uark.edu

Aamir W Khan
Postdoctoral Fellow
University of Missouri
maky74@umsystem.edu

Hyojin Kim
Postdoctoral Research Associate
Center for Plant Science Innovation
University of Nebraska–Lincoln
hkim20@unl.edu

Panya Kim
Postdoctoral Researcher
University of Nebraska–Lincoln
pkim3@unl.edu

Kyle Kocak
Research Scientist
Corteva
kyle.kocak@corteva.com

Felipe Krause
Doctoral Student
University of Nebraska–Lincoln
fkrause2@huskers.unl.edu

SOY2023 REGISTRANTS

Ritesh Kumar
Research Scientist (R6)
University of Minnesota
Kumar797@umn.edu

Naoufal Lakhssassi
Associate Scientist
Southern Illinois University
naoufal.lakhssassi@siu.edu

Zenglu Li
Professor
University of Georgia
zli@uga.edu

Marc Libault
Associate Professor
Department of Agronomy and Horticulture
University of Nebraska–Lincoln
marc.libault@unl.edu

Mason Lien
Soybean Breeding Support Specialist
Syngenta
mason.lien@syngenta.com

Feng Lin
Postdoc Academic Specialist-Research
Michigan State University
fenglin@msu.edu

Jer-Young Lin
jeryoung@gate.sinica.edu.tw

Junqi Liu
Researcher
University of Minnesota
liuxx162@tc.umn.edu

Zhan-Bin Liu
Corteva Fellow
Corteva Agriscience
zhan-bin.liu@corteva.com

Neeta Lohani
Post Doctoral Associate
Donald Danforth Plant Science Center
nlohani@danforthcenter.org

John Long
Senior Research Associate
Corteva Agriscience
john.long-2@corteva.com

Aaron Lorenz
University of Minnesota
lore0149@umn.edu

Jianxin Ma
maj@purdue.edu

Sai Subhash Mahamkali
Research Assistant
University of Nebraska–Lincoln
smahamkalivenkatas2@huskers.unl.edu

Shin-Yi Marzano
Research Molecular Biologist
USDA-ARS
shinyi.marzano@usda.gov

Jon Massman
jon.massman@corteva.com

Michaela McGinn
Program Manager
Smithbucklin/USB
mmcginn@smithbucklin.com

Leah McHale
Professor
The Ohio State University
mchale.21@osu.edu

Jonathan Mendoza Garcia
Visiting Scholar
University of Arkansas
jm199@uark.edu

Olivia Meyer
Research Technologist II
Department of Agronomy and HorticultureUniversity of Nebraska–Lincoln
ofiala2@unl.edu

Ian Miller
Chief Development Officer
Pairwise
imiller@pairwise.com

Samuel Mintah
Graduate Research Assistant
University of Illinois Urbana Champaign
smintah2@illinois.edu

Esmaeil Miraeiz
National Soybean Research Center
emiraeiz@illinois.edu

Drew Mitchell
Ph.D. Researcher
Michigan State University
mitch987@msu.edu

Melissa Mitchum
Professor
University of Georgia
melissa.mitchum@uga.edu

Mohammad Golam Mostofa
Michigan State University
mostofam@msu.edu

Leandro Mozzoni
Soybean Product Development Scientist
Bayer
leandro.mozzoni@bayer.com

Clara Mvuta
Graduate Student
North Dakota State University
clara.mvuta@ndsu.edu

Rex Nelson
Faculty
Corn Insects and Crop Genetics Research Unit, USDA-ARS
Rex.Nelson@usda.gov

Hanh Nguyen
Researcher
ntmhanh76@gmail.com

Manh Nguyen
Research Assistant
University of Missouri
mvnpcm@umsystem.edu

Nghi Nguyen
Graduate Student
The Ohio State University
sonibecool@gmail.com

SOY2023 REGISTRANTS

Jason Nichols
Principal Scientist
Syngenta
jason.nichols@syngenta.com

Toshihiro Obata
Associate Professor
University of Nebraska–Lincoln
tobata2@unl.edu

Jamie O'Rourke
Faculty
Corn Insects and Crop Genetics Research Unit
USDA-ARS
Jamie.Orourke@usda.gov

Wayne Parrott
Professor
University of Georgia
wparrott@uga.edu

Gunvant Patil
Assistant Professor
Texas Tech University
gunvant.patil@ttu.edu

Kerry Pedley
Research Molecular Biologist
USDA ARS
kerry.pedley@usda.gov

Duyen Pham
Research Assistant University of North Texas
duyen.pham@unt.edu

Wrojay Bardee Potter Jr.
Research Officer I
Central Agricultural Research Institute
potterwrojay@gmail.com

Feng Qu
Professor
The Ohio State University
qu.28@osu.edu

Truyen Quach
Research Assistant Professor
Center for Plant Science Innovation
University of Nebraska–Lincoln
tquach2@unl.edu

Vishnu Ramasubramanian
Postdoctoral Associate
University of Minnesota
vramasub@umn.edu

Camila Ribeiro
NBT Scientist
GDM Seeds
cribeiro@gdmseeds.com

Lucas Roberts
Graduate Student
University of Minnesota
robe2110@umn.edu

Seema Sahay
Postdoctoral Research Associate
Biochemistry
University of Nebraska–Lincoln
ssahay2@unl.edu

Muhammad Salman
Graduate Student
Michigan State University
salmanm3@msu.ed

Sanju Sanjaya
Director
WVSU Energy and Environmental Science Institute
West Virginia State University
sanjaya@wvstateu.edu

Maria Santiago Padua
Master's Student
The Ohio State University
santiago-Padua.1@osu.edu

Shirley Sato
Lab Manager
University of Nebraska–Lincoln
ssato1@unl.edu

James Shannon
Professor Emeritus
University of Missouri
shannong@missouri.edu

Bo Shen
Senior Research Manager
Corteva Agriscience
Bo.shen@corteva.com

Wenhao Shen
Danforth Center
Wshen@danforthcenter.org

Lovepreet Singh
PhD Student
University of Minnesota Twin Cities
sing1135@umn.edu

Alina Smolskaya
Graduate Student
University.of Minnesota
smols001@umn.edu

Suma Sreekanta
Research Associate
University of Minnesota
sreek002@umn.edu

Bing Stacey
Professor
University of Missouri
staceym@missouri.edu

Gary Stacey
Professor
University of Missouri
staceyg@missouri.edu

Adam Steinbrenner
Assistant Professor
University of Washington
astein10@uw.edu

Robert Stupar
Professor
University of Minnesota
stup0004@umn.edu

Nicole Szeluga
Ph.D. Candidate
Cornell University
nms244@cornell.edu

Sutton Tennant
Graduate Student Researcher
University of Nebraska–Lincoln
sutennant@huskers.unl.edu

Gezahegn Tessema
Post Doc
Donald Danforth Plant Science Center
gezgrm@yahoo.com

SOY2023 REGISTRANTS

Raju Thada Magar
Graduate Research Assistant
Michigan State University
thadaraj@msu.edu

Sandi Win Thu
Graduate Student
Washington State University
sandiwin.thu@wsu.edu

Trish Tully
Postdoctoral Associate
Donald Danforth Plant Science Center
ttully@danforthcenter.org

Benjamin Turc
Postdoctoral Research Associate
Biochemistry
University of Nebraska–Lincoln
bturc2@unl.edu

Rao Uppalapati
Biotech Disease Resistance Trait
Portfolio Leader
Corteva Agriscience
rao.uppalapati@corteva.com

Tri Vuong
vuongt@missouri.edu

JINBIn WANG
Purdue University
Postdoctoral Researcher
wang5549@purdue.edu

Dechun Wang
Professor
Michigan State University
wangdech@msu.edu

Yang Wang
Research Laboratory
Supervisor
Corteva Agrisciences
yangsc.wang@corteva.com

Zhibo Wang
zhibowang@danforthcenter.org

Jackie Weiss
Research Director
SmithBucklin for United
Soybean Board
Jweiss@smithbucklin.com

Steve Whitham
Professor
Iowa State University
swhitham@iastate.edu

Habib Widyawan
Graduate Student
University of Georgia
widyawan@uga.edu

Cole Williams
North Dakota State University-
cole.a.williams@ndsu.edu

Robert Williams Sr
Director
Inari
bwilliams@inari.com

John Woodward
Research Scientist
john.woodward@corteva.com

Wenjuan Wu
Purdue University
Postdoctoral Researcher
wu2014@purdue.edu

Hengping Xu
Research Technologist I
Center for Biotechnology
University of Nebraska–Lincoln
hxu28@unl.edu

Umesh Yadav
Research Scientist
University of North Texas
umeshprasad.yadav@unt.edu

Brad Zamft
X (Google)
zamft@google.com

Dandan Zhang
ddzhang@iastate.edu

Nengyi Zhang
BASF
nengyi.zhang@basf.com

NOTES

THANK YOU FOR PARTICIPATING IN SOY2023.