Soy2025: 19th Biennial Conference on Molecular & Cellular Biology of Soybean

July 23 – 26, 2025

University of Wisconsin-Madison

SCHEDULE

July 23	Wednesday Afternoon	
11:00 AM – 7:00 PM	**Registration**	**Annex Room**
11:00 AM – 4:00 PM	**Poster Display Room Open**	**Tripp Commons**
12:30 PM – 2:15 PM	**Tour of Wisconsin Crop Innovation Center**	**Meet at the Memorial Union**
2:15 PM – 4:00 PM	Free Time – On Your Own Three Iconic Walks Three-Iconic-Walks.pdf Follow this link to view activities around Madison: Madison Events | Concerts, Festivals & Fun in Madison WI	
4:00 PM – 6:00PM	**Session I – Conference Opening Session** Session Chair: Andrew Bent	**Great Hall**
4:00 PM	Welcome and Key Information	Andrew Bent
4:05 PM	Soybean translational genomics: from natural variation to edited mutation	Jianxin Ma
4:30 PM	The impact of stress combination on soybean yield under a changing climate	Ron Mittler
4:55 PM	Supercharging Soybean Development: How Comprehensive Genomic Resources Accelerate Trait Discovery and Breeding	Aamir Khan
5:20 PM	Keynote Talk	Tom Clemente
6:00 PM -9:00 PM	**Poster Gathering**	**Tripp Commons & Main Lounge**
6:20 PM – 7:00 PM	Poster Session: Authors stand by Even Numbered Posters	Tripp Commons & Main Lounge
7:00 PM	**Dinner Begins**	**Tripp Commons & Main Lounge**
7:30 PM – 8:10 PM	Poster Session: Authors stand by Odd Numbered Posters	Tripp Commons & Main Lounge
8:15 PM	**Soybean Serenade**	**Tripp Commons**

July 24	Thursday Morning	
7:30 AM – 5:00 PM	**Registration**	**Annex Room**
8:30 AM – 10:00 AM	**Session II – Approaches** Session Chair: Michelle Graham	**Great Hall**
8:30 AM	Spatial Genomics and Metabolomics Reveal Cell-Specific Regulatory Programs in Soybean	Bob Schmitz
8:55 AM	Defining cell type and cell state. What can we learn from the soybean single-cell transcriptome atlas?	Marc Libault
9:20 AM	Soybean-based platform for sustainable and efficient production of betalain pigments	Soyoung Jung
9:40 AM	Improving Soybean Seed Sucrose Content using Tilling by Sequencing Analyses of The Soybean Sucrose Synthase Gene Family	Dounya Knizia
10:00 AM	A vision for soybean research	Bob Stupar
10:15 AM	**Refreshment Break**	**Reception Room**
10:45 AM – 12:00 PM	**Session III – Genotype Phenotype Mechanisms** Session Chair: Gunvant Patil	**Great Hall**
10:45 AM	Engineering Soybean Root Traits for Climate Change Mitigation	Wolfgang Busch
11:10 AM	Cis-Regulatory Mutations Drive Tissue-Specific Subfunctionalization of sRNALoci Regulating Soybean Seed Color during Domestication	Young Cho
11:30 AM	Inari Agriculture: The SEEDesign™ company	Marlies Wouters
11:50 AM	Engaging Undergraduates in the Functional Characterization of Soybean Mutants	Nathan Hancock
12:10 PM – 1:30 PM	**Lunch & Poster Session – Provided by Soy2025**	**Tripp Commons & Main Lounge**
12:10 PM	**Lunch Begins**	**Tripp Commons**
12:45 PM – 1:05 PM	Poster Session: Stand by your poster if first letter of First name A-L	Tripp Commons & Main Lounge
1:05 PM – 1:25 PM	Poster Session: Stand by your poster if first letter of First name M-Z	Tripp Commons & Main Lounge

July 24	Thursday Afternoon	
1:30 PM – 3:00 PM	**Session IV – Plant Microbe Interactions I** Session Chair: Asela Wijeratne	**Great Hall**
1:30 PM		Xuelu Wang
1:55 PM	The biochemistry of nodulation	Gary Stacey
2:20 PM	Engineering a functional copy of Inceptin Receptor (INR) into soybean confers herbivory recognition	Di Wu
2:40 PM	Discovery of Novel Resistance Resources Independent of *rhg1* and *Rhg4* for Broad-based SCN Resistance in Soybean	Sushil Chhapekar
3:00 PM – 3:30 PM	**Refreshment Break**	**Reception Room**
3:30 PM – 5:00 PM	**Session V – New Technologies** Session Chair: Wayne Parrott	**Great Hall**
3:30 PM	Pleiotropic functions of a gibberellin receptor gene in the regulation of plant architecture, yield and nitrogen fixation in soybean	Yan Li
3:55 PM	As Designed by Nature: Unexpected Soybean Transformation Results from Virulence Plasmid Launched-Transfer DNA	Ray Collier
4:15 PM	Advancing Soybean Transformation: A Journey at Corteva	Hyeon-Je Cho
4:35 PM	Turbocharging Genomics to Fuel Advances in Plant Biology	Kristin Bilyeu
5:10 PM – 5:50 PM	**Session VI – Early Career DeepStrike Lightning Talks:** Kavya Sekar Susitha, Lauren Docherty, Sachini Lakshika KK, Yu-Hyeon Park, Lucille Owens, Sachleen Singh	**Great Hall**
6:00 PM	**Dinner – On your own**	**Union, State St., Capitol Square, other**
7:00 PM – 12:00 AM	**Music on the Union Terrace (optional)**	**Memorial Union Terrace**

July 25	Friday Morning	
7:30 AM – 5:00 PM	**Registration**	**Annex Room**
8:30 AM – 10:15 AM	**Session VI – Plant Microbe Interactions II** Session Chair: Adam Steinbrenner	**Great Hall**
8:30 AM	New players in soybean resistance and SCN virulence	Melissa Mitchum
8:55 AM	Exploring the Role of AATRhg1 in Soybean Cyst Nematode Resistance and Amino Acid Dynamics	Yulin Du
9:15 AM	Exploring Novel Genetic Resistance to *Heterodera glycines* and *H. sojae* Using a Diverse Soybean Germplasm Panel	Kyung Do Kim
9:35 AM	Effects of increasing atmospheric carbon dioxide levels on soybean responses to diverse pathogens	Steve Whitham
9:55 AM	Conferring broad spectrum disease resistance in soybean using dual action elicitor peptide variant(s)	Ambika Pokhrel
10:15 AM – 10:45 AM	**Refreshment Break**	**Reception Room**
10:45 AM – 12:00 PM	**Session VII – Genetics/Genomics** Session Chair: Jianxin Ma	**Great Hall**
10:45 AM	From Code to Crop: Harnessing Soybean Genomics for Gene Cloning, Editing, and More	Bob Stupar
11:10 AM	Selection, pangenomes and structural diversity in soybean and a soybean pathogen.	Matt Hudson
11:30 AM	Targeting Induced Local Lesions in Genomes “TILLING” to improve agronomically importance traits in soybean	Khalid Meksem
11:50 AM	SoyBase: Here’s to another 30 years	Jacqueline Campbell
12:10 PM – 1:30 PM	**Lunch & Poster Session – Provided by Soy2025**	**Tripp Commons & Main Lounge**
12:10 PM	**Lunch Begins**	**Tripp Commons**
12:40 PM – 1:20 PM	**Posters Open for Viewing**	**Tripp Commons & Main Lounge**
1:20 PM – 2:45 PM	**Poster Take Down**	**Tripp Commons & Main Lounge**

July 25	Friday Afternoon	
1:30 PM – 3:00 PM	**Session VIII – Abiotic Stress** Session Chair: Bing (Minviluz) Stacey	**Great Hall**
1:30 PM	It's getting hot in here: gene expression responses to extended heat stress	Jamie O'Rourke
1:55 PM	Drought Does Not Mitigate the Effects of Ozone on Soybean Photosynthesis and Yield	Lisa Ainsworth
2:20 PM	Unraveling Molecular and Cellular Insights in Soybean-AMF Symbiosis	Leonidas D'agostino
2:40 PM	Genome-wide Association Studies Revealed Seven Putative Drought Tolerance Genes in Soybean	Madan Bhattacharyya
3:00 PM – 3:30 PM	**Refreshment Break**	**Reception Room**
3:30 PM – 5:00 PM	**Session IX – Agronomic Traits** Session Chair: Andrew Scaboo	**Great Hall**
3:30 PM	Transcriptome-wide association uncovers lncRNAs controlling seed weight in soybean	Qingxin Song
3:55 PM	Identification of novel loci related to amino acid contents in soybean by combination of network analysis and genome-wide association study	Kyujung Van
4:15 PM	Comprehensive Transcriptomic Analysis of Environmentally Modulated Seed Protein Content in Soybean: Implications for Western Canada and Northern Regions	Bahram Samanfar
4:35 PM	Investigating a putative susceptibility gene towards Phytophthora sojae in soybean	Leah McHale
5 PM – 6:45 PM	Free Time – On Your Own Three Iconic Walks Three-Iconic-Walks.pdf Follow this link to view activities around Madison: Madison Events | Concerts, Festivals & Fun in Madison WI	
6:45 PM – 8:45 PM	**Banquet Dinner**	**Tripp Commons**

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Table of Contents

26 Conferring broad spectrum disease resistance in soybean using dual action elicitor peptide variant(s)
Ambika Pokhrel, Principal Investigator Dilip Shah

27 From Code to Crop: Harnessing Soybean Genomics for Gene Cloning, Editing, and More
Robert Stupar

28 Selection, pangenomes and structural diversity in soybean and a soybean pathogen.
Matthew Hudson

29 Targeting Induced Local Lesions in Genomes "TILLING" to improve agronomically importance traits in soybean
Erdem Anil, Mallory A Cullen, Abdelhalim El Baze, Tarek Hewezi, Dounya Knizia, Naoufal Lakhssassi, Shiming Liu, Khalid Meksem, Henry Nguyen, Sarbottam Piya, Yasser Salhi, Zhou Zhou

30 SoyBase: Here's to another 30 years
Jacqueline Campbell, Connor Cameron, Alan Cleary, Sudhansu Dash, Andrew Farmer, Wei Huang, Scott Kalberer, Simon Novak, Chen Prom, Nathan Weeks, Steven Cannon, Rex Nelson

31 Title TBD
Ron Mittler

32 Drought Does Not Mitigate the Effects of Ozone on Soybean Photosynthesis and Yield
Lisa Ainsworth, Dr. Duncan Martin, Dr. Shuai Li, Dr. Elise Aspray, Professor Andrew Leakey

33 Unraveling Molecular and Cellular Insights in Soybean-AMF Symbiosis
Leonidas D'agostino, Dr. Lenin Yong-Villabos, Dr. Luis Herrera-Estrella, Dr. Gunvant Patil

34 Genome-wide Association Studies Revealed Seven Putative Drought Tolerance Genes in Soybean
Dr. Atit Parajuli, Ramesh Chethri, Iman Saha, Micheline N. Ngaki, Cecelia Ryden, Madeline Thompson, Qingfeng Xing, Liang Dong, Madan Bhattacharyya

35 Transcriptome-wide association uncovers lncRNAs controlling seed weight in soybean
Qingxin Song, Xinyu Jiang

36 Identification of novel loci related to amino acid contents in soybean by combination of network analysis and genome-wide association study
Kyujung Van, Sungwoo Lee, Rouf Mian, Leah McHale

37 Comprehensive Transcriptomic Analysis of Environmentally Modulated Seed Protein Content in Soybean: Implications for Western Canada and Northern Regions
Bahram Samanfar, Julia Hooker, Elroy Cober, Ashkan Golshani

38 Investigating a putative susceptibility gene towards Phytophthora sojae in soybean
Leah Mchale, Anne Dorrance, Stephanie Karhoff, Christian Vargas-Garcia, Feng Qu, Chee Gang Ngui

P101 Exploration of Fast Neutron Breeding Pipeline in Soybean
Cheryl Adeva, Francia Seconde Ravelombola, Maiara de Oliveira, Jessica Argenta, Harmeet Singh, Jenish Simon, Jaqueline Lopes Rodrigues, Destiny Hunt, Feng Lin

P102 Genomic and biochemical comparison of allelic triple-mutant lines derived from conventional breeding and multiplex gene editing
Junqi Liu, Ritesh Kumar, Samatha Gunapati, Steven Mulkey, Yinjie Qiu, Yer Xiong, Vishnu Ramasubramanian, Jean-Michel Michno, Praveen Awasthi, Daniel D. Gallaher, Thi TThao Nguyen, Won-Seok Kim, Hari B. Krishnan, Aaron J. Lorenz, Robert M. Stupar

P103 UNL PTCRF Soybean Transformation and Genome Editing: lab-to-field trait development
Ming Guo, Truyen Quach, Hanh Nguyen, Zhengxiang Ge, Olivia Meyer, Nathaniel Butler

P104 Introgression of drought and flood tolerance genes into four elite soybean cultivars through backcrossings
Ramesh Chethri, Atit Parajuli, Iman Saha, Madan K. Bhattacharyya

P105 Genomic and transcriptomic data to identify causal variants of aphid resistance in soybean
Lori Croghan, Yinjie Qiu, Aaron J. Lorenz, Robert L. Koch, James P. Menger, Robert M. Stupar

P106 A comprehensive survey of genomic variation in soybean through resequencing and pangenome construction
Ji Hun Hwang, Yang Jae Kang, Tae-Hwan Jun, Tae-Ho Lee, Kyung Do Kim

P107 Multi-Environment Performance of Genomic Selection Models in Soybean Breeding
Maiara De Oliveira, Yu Wu, Marcelo Ferraz, Francia Ravelombola, Cheryl Adeva, Harmeet Singh, Jessica Argenta, Destiny Hunt, Jaqueline Rodrigues, Jenish Simon, Julia Passos, Feng Lin

P108 Identifying Virulence Loci in Soybean Cyst Nematode
Lauren Docherty, Vishnu Ramasubramanian, Cory Hirsch, Aaron Lorenz, Senyu Chen

P109 Soybean resistance to southern root-knot nematode reduces nematode population density under field conditions
Emanuel Ferrari do Nascimento, Andrea Acuña, Mariola Usovsky, Amanda Pominville, Travis Faske[1], Zenglu Li, Melissa G. Mitchum, Henry T. Nguyen, Grover Shannon, Caio Canella Vieira

P110 Discovering cqSCN-007, a Novel Type of Resistance to Soybean Cyst Nematode
Susana Ferrer Orgaz, Dr. Jeysika Zayas-Rivera, Dr. Andrew Bent

P111 Investigating Genotype by Environment Dynamics in Soybean Iron Deficiency Chlorosis
Isabella Fiore, Mjay Espina, Aaron Lorenze, Robert Stupar

P112 Targeted editing of GmSNAP14 enhances the soybean cyst nematode resistance in Soybean
Vinavi A. Gamage, Mariola Usovsky, Anser Mahmood, Elizebeth J. De Meyer, Clinton G. Meinhardt, Bishnu Dhital, Qijian Song, Andrew Scaboo, Melissa G. Mitchum

P113 Editing of the of the gene model underlying the major protein quantitative trait loci (QTL), cqSeed protein-003, and its paralog in soybean
Ming Guo, Truyen Quach, Hanh Nguyen, Olivia Meyer, Shirley Sato, Vikranth Chandraskaren, Lauren A. Lail, C. Nathan Hancock, Wayne Parrott, Minviluz Bing Stacey, Matthew E. Hudson, Brian W. Diers, Tom Elmo Clemente, Chi Zhang, Nathaniel Butler

P114 Association Mapping and Genomic Prediction of Mid-Season Flood Tolerance in Soybean
Derrick Harrison, Dr. Chengjun Wu, Dr. Andrea Acuna, Dr. Liliana Florez-Palacios, Daniel Rogers, Rafael Marmo, Emanuel Ferrari do Nascimento, Dr. Heng Ye, Dr. Henry T. Nguyen, Dr. Grover Shannon, Dr. Diego Jarquin, Dr. Caio Canella Vieira

P115 Determine the cell-type-specific role of membrane nanodomain-associated proteins in the infection processes of nodule formation
Md Sabbir Hossain, Sandra Thibivilliers, Sahand Amini, Sergio A C Perez, Marc Libault

P116 Integrative omics analysis elucidates the genetic basis underlying seed weight and oil content in soybean
Xiaobo Yuan, Xinyu Jiang, Mengzhu Zhang, Longfei Wang, Wu Jiao, Huatao Chen, Junrong Mao, Wenxue Ye, Qingxin Song

P117 Development of EPA- and Astaxanthin-Enriched Soybean Germplasm for Aquaculture Feedstocks
Hyojin Kim, Truyen Quach, Shirley Sato, Hanh Nguyen, Kiyoul Park, Thomas Clemente, Edgar Cahoon

P118 Development of a Reduced-Allergen Soybean Line and Preclinical Validation of Anti-Allergenicity
Namgeol Kim, Yung Choon Yoo, Inhye Lee, Yo-han Yoo, Seuk-Ki Lee, Kwang-soo Cho, Youngkwang Ju, Min-Jung Seo

P119 Identifying regulatory elements that control Rhg1 α-SNAP abundance in SCN resistance
Paulami Koley, Andrew Bent

P120 Multi-Locus GAWS Mapping Kudzu Bug Resistance in Soybean (Glycine max L. Merr.)
Ms. Rikki Locklear, Dr. Abdelmajid Kassem, Ms.Sheree Wright, Dr. Elvira de Mejia, Dr. Ben Fallen, Dr. Guoliang Jiang, Dr. Jiazheng (John) Yuan

P121 Screening of advanced high-yield MU-FDREEC soybean lines for resistance against Stink Bugs Complex
Jaqueline Lopes Rodrigues, Jessica Argenta, Jenish Simon, Francia Ravelombola, Cheryl Adeva, Maiara de Oliveira, Ivair Valmorbida, Jeff Davis, Feng Lin

P122 Genetic Mapping of Novel QTL for Seed Protein Stability in Food Grade Soybean (Glycine max)
Drew Mitchell, Feng Lin, Heng Ye, Tri Vuong, Zixiang Wen, Dechun Wang

P123 Assessing Fungal Synthetic Community Function in Soybean Growth and Drought Stress Adaptation
Lucille Owens, Dr. Abdul Khan, Waqar Ahmad

P124 Pathogenicity Evaluation and High-Throughput Molecular Detection of Calonectria ilicicola in Soybean Using an HRM Marker
Krishna Raj Pandey, Steven J. Clough

P125 Development of SNP markers for the identification of soybean varieties
Soyeon Park, Kyungdo Kim

P126 Elevated seed oil phenotype associated with a single nucleotide polymorphism in GmNFR1α
Sri Veda Patibandla, Karen Hudson, Militza Carrero-Colon, Qijian Song

P127 Advancing Soybean Carbohydrate Profiles through Gene Editing and Next-Generation Mutation Breeding
Ramkumar Thakku, Gaurav Raturi, Kaushik Ghose, Haiying Shi, Khalid Meksem, Henry Nguyen, Gunvant Patil

P128 GS4PB: An R/Shiny Application to Facilitate a Genomic Selection Pipeline for Plant Breeding
Vishnu Ramasubramanian, Dr. Cleiton Wartha, Mr. Lovepreet Singh, Dr. Paolo Vitale, Dr. Sushan Ru, Dr. Aaron Lorenz

P129 Decoding tripartite symbiotic relationships: Insights into AMF and Rhizobium Interactions for sustainable agriculture
Gaurav Raturi, Leonidas D'Agostino, Gunvant Patil

P130 Selecting Resilient Soybean Genotypes Using Multi-Trait Stability Index Under Drought Stress
Francia Ravelombola, Harmeet Singh, Cheryl Adeva, Jessica Argenta, Maiara de Oliveira, Jenish Simon, Jaqueline Rodrigues, Destiny Hunt, Feng Lin

P131 Are the Phytophthora resistance Rps6 and Rps13 genes allelic?
Iman Saha, Atit Parajuli, Dr. Madan K Bhattacharyya

P132 Mechanistic Insights into a Novel Transcriptional Regulator Conferring Broad-Spectrum Resistance to Soybean Cyst Nematode
Sonam Singh, Dr. Vikas Devkar, Dr. Sushil Chhapekar, Dr. Gunvant Patil, Prof. Henry Nguyen

P133 Identification of novel genomic regions and candidate genes associated with soybean nodulation for future breeding programs.
Kuber Shivashakarappa, Dr. Korsi Dumenyo, Dr. Ali Taheri

P134 Characterization of genes involved in sitosterol to stigmasterol conversion in soybean
Sana Shakoor, Dr. Walter P. Suza

P135 Evaluation of Elite Soybean Lines from the MU-FDREEC Breeding Program for Resistance to Phytophthora spp.
Julia Silva Passos Dos Santos, Jessica Agrenta, Bekhzod Khayrullayev, Jaqueline Lopes Rodrigues, Jenish Simon, Nannan Li, Cheryl Adeva, Francia Seconde Ravelombola, Maiara de Oliveira, Harmeet Singh, Peng Tiam, Feng Lin

P136 Genome-Wide Association Study Reveals Novel Genetic Loci for Resistance to Phytophthora sojae in Soybean
Jenish Simon, Jessica Argenta Argenta, Allen Wu, Bekhzod Khayrullayev, Julia Santos, Jaqueline Rodrigues, Francia Ravelombola, Cheryl Adeva, Maiara Oliveira, Harmeet Singh, Peng Tian, Lin Feng

P137 Developing genotype-by-environment interaction (GxE) genomic prediction models for soybeans capable of predicting breeding values
Lovepreet Singh, Vishnu Ramasubramanian, Eliana Monteverde, George Graef, Matthew Hudson, David Hyten, Carrie Miranda, Katy Rainey, Nicolas Federico Martin, Leah McHale, Andrew Scaboo, William Schapaugh, Asheesh Singh, Dechun Wang, Aaron Lorenz

P138 From Sky to Canopy: Integrating UAV Imaging and Photosynthetic signatures for Soybean Drought-Resilience Breeding
Harmeet Singh-Bakala, Francia Ravelombola, Maiara Oliveira, Cheryl Adeva, Jacob Washburn, Jessica Argenta, Grover Shannon, Feng Lin

P139 Mapping and characterizing oval leaflet shape in soybean associated with low number of seeds per pod
Alina Smolskaya, Robert Stupar, Aaron Lorenz

P140 From host plant immunity to agro-ecological IPM: a case study in Phaseolus vulgaris
Adam Steinbrenner, Dr. Natalia Guayazán-Palacios, Dr. Patrick Grof-Tisza, Dr. Di "Woody" Wu, Dr. Betty Benrey, Dr. Ted Turlings

P141 Bioengineering of the Rice Pikm-1 NLR Protein for Creating an Artificial Effector Triggered Immunity System Against the Fungal Pathogen Fusarium virguliforme
Fariha Tasnim, Atit Parajuli, Micheline Ngaki, Madan K. Bhattacharyya

P142 Discovery of Novel Resistance Resources Independent of rhg1 and Rhg4 for Broad-based SCN Resistance in Soybean
Sushil Chhapekar, Dr. Vikas Devkar, Dr. Naoufal Lakhssassi, Dr. Heng Ye, Dr. Sonam Singh, Dr. Aamir Khan, Dr. Tri Vuong, Dr. Gunvant Patil, Dr. Khalid Meksem, Dr. Henry Nguyen

P143 A Comprehensive Evaluation of Spatial Adjustment and Genomic Prediction Models for Selecting Sudden Death Syndrome Field Resistance in Soybean (Glycine max)
Raju Thada Magar

P144 An Effective Genomic Prediction Workflow to Optimize Early-stage Soybean Selection
Andre L. Tischler, Vishnu Ramasubramanian, Siddhi Bhusal, Aaron J. Lorenz

P145 High-Throughput Gene Knockout in Nicotiana benthamiana via TRV-Delivered CRISPR and Agrobacterium Infiltration
Wai Tan Wong, Matthew Hudson

P146 Optimizing root system performance in crops through functional-structural root modeling, target discovery and gene editing at Inari
Marlies Wouters, Joris Jourquin, Veerle De Schepper, Daniel Leitner, Tobias Selzner, Xavier Draye, Kerstin Nagel, Andrea Schnepf, Sonali Quantius

P147 Improved genomic prediction accuracy for complex seed composition traits using highly replicated, multi-environment phenotypic data in soybean (Glycine max L.)
Hee Jin You, Professor Sungwoo Lee

P148 GAANTRY launched gene editing T-DNA results in higher plant transformation efficiency relative to the binary plasmid
Guifen Li, Ed Williams, Nathalie Walter, Shawn Kaeppler, Ray Collier

P149 Seed RUBYv1: Optimization of a tool facilitating non-destructive sorting of null segregant from transgenic soybeans.
Robert Sears, Edward Williams, Nathalie Walter, Shawn Kaeppler, Ray Collier

P150 The GAANTRY System outperforms the binary plasmid in deliverable DNA cargo size, plant transformation efficiency, and transgenic plant quality
Ray Collier, Shawn Kaeppler, Roger Thilmony, James Thomson, Edward Williams, Guifen Li, Nathalie Walter

P151 Genetic improvement of soybean for increased oil production
Edgar B. Cahoon, Bagyalakshmi Muthan, Sanju Sanjaya

P152 CRISPR/Cas9-Mediated Genome Editing to Enhance Soybean Mosaic Virus (SMV) Resistance in USDA and African Originated Soybean (Glycine max) Lines.
Zaheer Ahmed, Muhammad Moaz Latif, Raheela Rehman, Azeem Iqbal Khan

P153 Pangenome analysis of nine Soybean Cyst Nematode genomes reveal hidden variation contributing to diversity and adaptation.
Lucas Borges Dos Santos, Kurt C. Showmaker, Rick Masonbrink, Kimberly K.O. Walden, João P. Gomes Viana, Khee-Man Kwon, Alvaro G. Hernandez, Christopher J. Fields, Thomas R. Maier, Andrew Severin, Thomas J. Baum, Melissa G. Mitchum, Matthew Hudson

P154 Research Status of Soybean Red Crown Rot in Taiwan
Hao-xun Chang, Dr. Ping-Hu Wu, Dr. Kuo-Lung Chou, Dr. Da-Gin Lin

P155 Early Identification of High-Yielding and Stable Soybean Genotypes
Rafael Goncalves Marmo, Andrea Acuña, Liliana Florez-Palacios, Derrick Harrison, Chengjun Wu, Daniel Rogers, Emanuel Ferrari do Nascimento, Qijian Song, Caio Canella Vieira

P156 Identification of causal R genes involved in soybean response to Phytophthora sojae infection
Katerina Holan, Dr. Michelle Graham, Dr. Jamie O'Rourke

P157 Identification of QTLs and Candidate Genes Associated with Yield and Yield Stability in Soybean
Guo-Liang Jiang, Dr. Patrick Mireku, Mr. William Townsend, Mr. David Johnson, Ms. Amanda Seow, Dr. Qijian Song

P158 Epigenetic Regulation of Soybean Defense Responses During Phytophthora sojae Infection
Sachini Lakshika, Shyaron Poudel, Brett Hale, Sachleen Singh, Alison Robertson, Clarice Schmidt, Asela Wijeratne

P159 Pleiotropic functions of a gibberellin receptor gene in the regulation of plant architecture, yield and nitrogen fixation in soybean
Jiajun Tang, Shuxuan Li, Xiuli Yue, Shancen Zhao, Junyi Gai, Yan Li

P160 Searching for alternative hypotheses: Investigating sequence diversity in novel genes differentially expressed between lines varying for resistance to Phytophthora sojae conferred by QDRL-18 in soybean
Chee Gang Ngui, Leah McHale

P161 Analysis of Genetic Diversity, Population Structure, and Virulence of Cercospora sojina Isolates Causing Frogeye Leaf Spot Disease of Soybeans Over 42 Years
Raymond Parcon, Dr. James Buck, Dr. Zenglu Li, Dr. Shavannor Smith

P162 Application of Crop Pest Classification System based on Deep Learning Vision Algorithms in Soybean (Glycine max (L.) Merr.).
Yu-Hyeon Park, Sang-Gyu Kim, Insoo Choi, Tae-Hwan Jun

P163 *Detection of Symptoms of Bacterial Disease and Development of Damage Assessment Algorithms based on Deep Learning Vision Technology in Soybean (Glycine max (L.) Merr.)*
Yu-Hyeon Park, Hakyung Kwon, Tae-Hwan Jun

P164 Can We Breed Soybeans For Intercropping: Characterizing Interactions and Ideotypes for a Pennycress Relay System
Lucas Roberts, Samantha Wells, Russ Gesch, Seth Naeve, Aaron Lorenz

P165 Identification and Molecular Mapping of a Major Gene Conferring Resistance to Phytophthora sansomeana in Soybean 'Colfax'.
Muhammad Salman, Feng Lin, Zhanguo Zhang, Austin G. McCoy, Wenlong Li, Raju T. Magar, Drew Mitchell, Meixia Zhao, Martin I. Chilvers, Dechun Wang

P166 Characterization of Glyma.18G270900 and a Related Kinase Gene Family as Candidates for Phytophthora sojae Resistance in Soybean
Kavya Sekar Susitha, Leah McHale

P167 Deciphering Core and Context-Specific Gene Regulatory Networks in Soybean Through Multi-Study Transcriptomic Analysis
Sachleen Singh, Dr. Asela Wijeratne

P168 Assessing inoculation methods for screening soybean varieties against white mold under greenhouse conditions
Suneth Sooriyapathirana, Research Assistant Kelly G. Wang, Research Technologist Cuihua Gu, Research Associate Austin Mccoy, Graduate Research Assistant Raju Thada Magar, Field Technician Randall Laurenz, Field Technician Paige Tabit, Research Associate Muhammad Salman, Professor Martin Chilvers, Professor Dechun Wang

P169 Rhizosphere Microbiome Dynamics in Soybean Under Fusarium virguliforme Stress
Kipa Tamrakar, Emilio Soriano Chavez, Philip Winston Miller, Mahesh Bamunusingha, Brett Hale, Daniel Jackson, Nathan Williams, Maureen Dolan, Scott Mangan, Edward Brown, Asela Wijeratne

P170 Genomic Characterization and Phylogenetic Analysis of Calonectria ilicicola isolates from Illinois Soybean Fields
Sreya Venadan, Samuel Mintah, Joao Paulo Gomes Viana, Nancy McCoppin, Nicholas Greatens, Rachel A. Koch Bach, Steven J. Clough

P171 Not Assigned

P172 Molecular, genetic, and evolutionary study into enhancing seed quality traits and yields of US soybean
Mohammad Aslam, Dr. Wolfgang Goettel, Dr. H. Zhang, Dr. Neeta Lohani, Dr. Yong-qiang Charles An

P173 Cell-type-resolved transcriptomics sheds light on SCN-induced remodeling and resistance in soybean roots
Vikas Devkar, Leonidas D'Agostino, Sushil S. Chhapekar, Moises Frausto, Jiamei Li, Lenin Yong-Villalobos, Fiona L. Goggin, Luis Herrera-Estrella, Henry T. Nguyen, Gunvant B. Patil

P174 Supercharging Soybean Development: How Comprehensive Genomic Resources Accelerate Trait Discovery and Breeding
Aamir W. Khan, Heidi Chen, Sushil S. Chhapekar, Tiffany Duong, Heng Ye, Nolan T. Hartwick, Vanika Garg, Sonam Singh, Qijian Song, Claudio D. Carrasco, Grant Poole, Rajeev K. Varshney, Wolfgang Busch, Todd P. Michael, Henry T. Nguyen

P175 Advancing Spatial Transcriptomics for Soybean Research: High-Resolution, Cost-Effective Spatial Profiling
Ziliang Luo, Xuan Zhang, Robert Schmitz

P176 Climate-adaptive soybean: root architecture and plasticity tuning for nutrient capture and stress resilience
Dhandapani Raju, Dr. Heng Ye, Dr. Marcus Griffiths, Dr. Sourabh Palande, Dr. Gus Thies, Dr. Keith Duncan, Dr. Christopher N Topp, Dr. Henry T Nguyen[1]

P177 Dual stress response of near-isogenic soybean lines to Fusarium graminearum and iron deficiency
Natalia Sancho-Quiros, Dr. Michelle A. Graham, Dr. Jamie A. O'Rourke, Dr. Silvina Arias

P178 Branching out: How genotype and row spacing shape soybean branch angle
Lara Waldt, Suma Sreekanta, Aaron Lorenz, Gary Muehlbauer

P179 Genetic and Epigenetic Responses Insight into Soybean Resistance and Susceptibility to Phytophthora Sansomeana
Gwonjin Lee, Charlotte N. DiBiase, Beibei Liu, Tong Li, Austin G. McCoy, Martin I. Chilvers, Lianjun Sun, Dechun Wang, Feng Lin, Meixia Zhao

P180 Decoding soybean cyst nematode infection using single-cell technologies
Xuan Zhang, Ziliang Luo, Vinavi Gamage, Xunliang Liu, Robert Schmitz, Melissa Mitchum

P181 SCN resistance protein WI12Rhg1 modulates core defense and GA signaling, and may be 55 amino acids longer than annotated
Aaron Lowenstein, Yulin Du, Jorge El-Azaz Ciudad, Hiroshi Maeda, Andrew Bent

Oral Presentation #1

Soybean translational genomics: from natural variation to edited mutation

Jianxin Ma[1]

[1]Purdue University

Soybean ranks second, after corn, among the most-planted field crops in the United States. However, the genetic improvement of cultivated soybeans is hindered by a low level of genetic diversity, primarily due to the domestication bottleneck, which accounts for a ~50% reduction in diversity. To facilitate the utilization of untapped genetic variations in the wild ancestors for soybean improvement, it is essential to understand the genetic and molecular bases underlying the suite of domestication-related traits and to identify the 'neglected treasures in the wild' for enhancement of elite cultivars. Today, advances in genome editing technologies enable us to introduce targeted, precise changes within specific genes associated with desirable traits. In this presentation, I will summarize recent progress made by my lab and collaborators in unraveling the history and dynamic process of soybean domestication, as well as the discovery of genes and genetic elements underlying domestication-related and agronomically important traits. I will also highlight our recent discoveries of molecular mechanisms promoting soybean nodulation, including both rhizobium- and host plant-based mechanisms accelerating rhizobial infection before autoregulation of nodulation, acting as a "brake", begins to repress rhizobial infection. Furthermore, I will briefly describe how we are working to translate these findings into practical applications for soybean improvement using our soybean transformation and gene editing pipeline.

Oral Presentation #2

It’s getting hot in here: gene expression responses to extended heat stress

Michelle A. Graham[1,2], Jamie O'Rourke, Asheesh K. Singh[2], Arianna Spellman-Kruse[3], Liza Van der Kaan[2]

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Soybean yield is significantly impacted by heat stress events. In a previous study we examined gene expression responses in leaves from three different genotypes: two heat stress tolerant lines and a heat stress susceptible line which were grown under optimal and heat stress temperatures. In our current study we examine gene expression from the roots of the previous study to identify differentially expressed genes (DEGs) that could play important roles in prolonged heat stress responses. Identified DEGs were compared to previously identified quantitative trait loci (QTLs) for traits related to heat stress tolerance. We also used the RNAseq data to identify putative long non-coding RNAs (lncRNAs) that may be crucial for soybean heat tolerance. LncRNAs are important in a number of biological processes in plants including abiotic stress responses. Specifically, lncRNAs can target genes with roles in stress perception, epigenetic modulation, and transcriptional and translational regulation. A subset of our lncRNAs are unique to the heat tolerant lines. Understanding the role these genes and RNAs play in the soybean stress responses could dramatically improve tolerance and yield preservation in soybeans exposed to high heat.

Oral Presentation #3

Supercharging Soybean Development: How Comprehensive Genomic Resources Accelerate Trait Discovery and Breeding

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

[1]University of Missouri

Soybean faces major yield losses from diseases and environmental stresses, creating significant constraints on global production. To address these challenges, we developed a comprehensive genomic toolkit through a strategic approach. We first constructed a pangenome with more than 1200 lines from the USDA National Soybean Germplasm Collection and developed near-gapless reference genomes for the community. We created a high-resolution haplotype map using diverse cultivars, landraces and wild relatives, then sequenced representative soybean lines using long-read sequencing technology to build a graph pangenome that captures previously undetectable genetic variations. This enhanced framework enables genome-wide studies to identify genes controlling resistance to major diseases and tolerance to abiotic stresses. We further developed a cost-effective 6K AgriSeq genetic marker panel optimized for breeding programs. We recently started a pan-transcriptome using PacBio Iso-Seq and single-cell technologies as a resource for gene functional studies. These comprehensive genome resources provide insights into the genus' genomic architecture and evolutionary history, serving as a valuable resource for future breeding programs and functional studies.

Oral Presentation #4

Editing of the Gene Model Underlying the Major Protein Quantitative Trait Loci (QTL), cqSeed Protein-003, and Its Paralog in Soybean

Truyen Quach[1,2], Hanh Nguyen[1], Olivia Meyer[1,2], Shirley Sato[1], Weilong Yang[1,7], Vikranth Chandraskaren[3], Lauren McDaniel[4], C. Nathan Hancock[5], Wayne Parrott[4], Minviluz Bing Stacey[3], Matthew E. Hudson[6], Brian W. Diers[6], Tom Elmo Clemente[2], Chi Zhang[1,7], Ming Guo[1,2]

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A major seed protein quantitative trait locus (QTL) in soybean resides on chromosome 20, cqSeed protein-003, was recently mapped to gene model Glyma.20G085100 (*Gm20P*). Its paralog resides on chromosome 10 [Glyma.10G134400 (*Gm10P*)]. To gain insight on the mechanism by which *Gm20P* and *Gm10P* influence seed reserve content in the crop, genome editing reagents were designed to create null mutations in both genes. Two soybean edited lines were identified and characterized that contain INDELs in *Gm10P* and *Gm20P* and devoid of editing reagents. One, a *gm20p* edit and the second a *gm10p gm20p* double edit. Seed protein content of the *gm10p gm20p* was significantly lower relative to the *gm20p* mutant and wildtype, with a concomitant increase in oil and starch content. A delayed maturity phenotype was observed in both the single and dual edited lines under field settings across multiple environments. The delay in maturity phenotype was influenced by day length, wherein under a short-day environment the delay mirrored what was observed under field conditions, while under long day environment, no delay in maturity was observed, but changes in growth were detected in the gm10p gem20p line. The observed phenotypic changes along with datasets gathered from transcriptomic analyses on the edited lines suggest the possible co-involvement of *Gm10P* and *Gm20P* in influencing seed reserve content in the crop, that connects light sensing and changes in cellular hormonal status.

Oral Presentation #5

Spatial Genomics and Metabolomics Reveal Cell-Specific Regulatory Programs in Soybean

Robert Schmitz[1], *Xuan Zhang, Ziliang Luo, Alex Marand, Tao Zhang, Jazz Dickinson, Peter LaFayette, Wayne Parrott, Xunliang Liu, Vinavi Lakshman, Melissa Mitchum*

[1]University of Georgia

Understanding how genes are turned on or off in specific cells or in response to specific environments is key to improving important traits in crops. We used advanced single-cell and spatial genomics techniques to map gene activity and DNA accessibility across ten different soybean tissues, identifying 103 unique cell states and over 300,000 regulatory regions in the soybean genome. Nearly 40% of these regions were active in only specific cell types, helping us pinpoint the gene switches and transcription factors that control distinct cell identities. We also discovered new DNA motifs and traced how regulatory networks are conserved in legumes, especially those involved in nitrogen fixation. By following the development of key seed tissues—like the endosperm and embryo—we uncovered how cells transition over time and identified genes likely controlling sugar transport and embryo patterning. We are now applying these techniques to study how soybeans respond to cyst nematode infection and to better understand the genetic control of seed composition traits. In particular, spatial metabolomics—mapping the location of metabolites in specific cell types—is proving especially powerful for investigating the biochemical pathways that determine seed nutritional quality. Together, these tools provide a powerful foundation for improving soybean traits important to agriculture and food systems.

Oral Presentation #6

Defining cell type and cell state. What can we learn from the soybean single-cell transcriptome atlas?

Sandra Thibivilliers[1], Sergio Alan Cervantes-Pérez[2], Sutton Tennant[1], Olivier C. Martin[3], Marc Libault[1]

[1]University of Missouri-Columbia, [2]University of Arizona, [3]Universities of Paris-Saclay, Paris-Cité and Evry

Soybean (Glycine max) is an essential source of protein and oil with high nutritional value for human and animal consumption. To enhance our understanding of the biology of the soybean plant, it is essential to have accurate information regarding the expression of each of its 55,897 protein-coding genes and a clear understanding of the population of cell types composing the soybean plant.

To date, single-cell sequencing technologies help understand the cellular complexity of human and animal organisms, notably in response to disease and treatments. In plants, we currently lack such an assessment. Here, upon developing and mining “Tabula Glycine”, the soybean single-cell resolution transcriptome atlas that includes the transcriptome of nearly 120,000 nuclei isolated from 10 different organs, we share a first insight into the cellular diversity composing this major plant species.

We identified 157 different soybean cell types based on their distinctive transcriptomic profiles and functionally annotated many of them by analyzing various spatial transcriptomic datasets. The analysis of their transcriptome revealed a “biological” component shared between cell clusters with similar function, and an “organ” component that serves as a signature of the organ of origin of the cell type. Focusing on the transcriptional patterns of the soybean transcription factors (TFs), we observed that their combinatorial activities are sufficient to define most cell types and their organ of origin, supporting the idea that TFs are key descriptors of cell identity and function. For instance, in guard cells, we identified co-expressed TFs with strong and specific expression in the leaf. A comparative analysis of their expression across different species reveals the conservation of their molecular function during plant evolution. Our study confirms that, similarly to human and animal biological systems, a small number of TFs are the defining factors of plant cell identity.

Oral Presentation #7

Soybean-based platform for sustainable and efficient production of betalain pigments

Soyoung Jung[1], *Ray Collier*[2], *Marcos de Oliveira*[1], *Hiroshi Maeda*[1]

[1]University of Wisconsin-Madison, [2]Wisconsin Crop Innovation Center

Synthetic biology offers powerful tools for producing valuable compounds in plant chassis. However, most plant synthetic biology efforts have focused on *Nicotiana benthamiana*, due to its rapid agroinfiltration method, despite the enormous metabolic diversity among plant species. Betalains are tyrosine-derived natural pigments found in beets and are widely used in the food industry as natural alternatives to synthetic red and yellow dyes. Previously, we showed that debottlenecking the L-DOPA-4,5-dioxygenase (DODA) step, combined with additional tyrosine supply, boosts betalain production in *N. benthamiana*. Based on the findings, here, we tested the optimized betalain biosynthetic pathway with additional DODA enzyme ("pull") and tyrosine precursor supply ("push") on betalain production in three distinct plant chassis—Arabidopsis, tobacco and soybean. The "push+pull" lines produced higher betalain levels than the "pull" lines in all three species, even exceeding those found in beet roots. However, Arabidopsis and tobacco "push+pull" lines showed growth defects, likely due to high accumulation of tyrosine and an intermediate, L-DOPA. In contrast, soybean "push+pull" lines maintained normal growth and development despite enhanced tyrosine and L-DOPA levels, suggesting greater tolerance of soybean to these compounds. This study demonstrates that soybean is an effective chassis for betalain production, highlighting the importance of plant chassis selection for efficient target compound production.

Oral Presentation #8

Improving Soybean Seed Sucrose Content using Tilling by Sequencing Analyses of The Soybean Sucrose Synthase Gene Family

Dounya Knizia[1], Erdem Anil[1], Yasser Salhi[1], Haiying Shi[2], Abdelhalim El Baze[1], My Abdelmajid Kassem[3], Naoufal Lakhssassi[4], Henry T Nguyen[2], Khalid Meksem[1]

[1]Southern Illinois University, [2]University of Missouri, [3]Fayetteville State University, [4]Hampton University

Soybean seed quality is influenced by its soluble sugar composition, with high sucrose content being desirable for nutritional and industrial applications. In contrast, excessive raffinose and stachyose levels are considered undesirable due to their adverse effects on gastrointestinal function in humans and monogastric animals. Therefore, developing soybean mutant lines with elevated sucrose content and optimal raffinose and stachyose content is desirable. In this study, we characterized twelve sucrose synthase genes through a comprehensive phylogenetic tree analysis, synteny analysis, gene structure evaluation, and variations in conserved domains. Additionally, we conducted a TILLING by Sequencing approach to identify EMS mutations in the characterized sucrose synthase. Numerous mutations have been identified in soybean sucrose synthase that resulted in high sucrose content, including the sucrose synthase mutants SL446 and F1115 with a sucrose content of 9.5% and 9.1%, respectively. The obtained soybean mutants with enhanced sugar content can be useful in breeding programs to improve soybean nutritional quality without potential developmental trade-offs.

Oral Presentation #9

A Vision for Soybean Research

Robert Stupar[1], *Jamie O'Rourke*[2], *Michelle Graham*[2]

[1]University of Minnesota, [2]USDA-ARS

The soybean genomics research community is continually adapting to emerging challenges and threats to soybean production. In 2022, soybean researchers from public institutions, industry, and funding agencies across the United States convened to recognize recent accomplishments in soybean research and to establish priorities for future efforts. The group also focused on the development and maintenance of critical resources—including genomic datasets, germplasm collections, mutant populations, and workforce development—highlighting the financial investments essential to their sustainability. This initiative culminated in a five-year strategic plan published in 2024 (https://doi.org/10.1002/tpg2.20516). Concurrently, an international group of soybean researchers developed a decadal vision for soybean genomics, published in 2025 (https://doi.org/10.1016/j.molp.2025.01.004), with overlapping themes centered on future directions in -omics, functional genomics, and breeding. This talk will highlight the most salient content from these two papers. It will also emphasize the importance of sustaining a vibrant soybean research community and our collective responsibility to advocate for continued investment in soybean research.

Oral Presentation #10

Engineering Soybean Root Traits for Climate Change Mitigation

Wolfgang Busch[1]

[1]Salk Institute for Biological Studies

Climate change will soon profoundly and negatively affect the vast majority of our planet's biota, including most human beings. Despite the importance and urgency of addressing this problem, we still lack technologies to globally address the root cause of climate change – increased levels of CO2 in the atmosphere. Since plants are central agents in the earth's carbon cycle, fixing atmospheric carbon that then mostly gets released when they decompose, engineering plant traits that affect the decomposition rate of plant derived carbon molecules can potentially lead to a large and globally significant drawdown of atmospheric CO2. In particular, root systems and the rhizosphere are of interest for such approaches as soils are enormous carbon sinks. Since plants first colonized the earth's land surfaces, their carbon depositions have built up three times more carbon in the soil than is contained in the atmosphere. Specific root traits are important contributors to the accumulation and permanence of carbon in the soil. These include root depth, root biomass and the levels of refractory carbon compounds in root tissues. Row crops are the most probable way to scale root-based carbon dioxide removal within the timeframe that matters most, which is before mid-century. This is due to their vast acreage and the unmatched ability of the agricultural sector to scale rapidly. I will present our efforts to discover molecular and genetic mechanisms in soybean that have the potential to enhance its capacity to store more carbon for longer periods while also increasing stress resilience and improving soil quality.

Oral Presentation #11

Cis-Regulatory Mutations Drive Tissue-Specific Subfunctionalization of sRNALoci Regulating Soybean Seed Color during Domestication

Young Cho[1]

[1]University of Hawaii

Gene duplication and structural rearrangements have played a pivotal role in plant genome evolution, enabling functional divergence and the development of novel traits. In soybeans (Glycine max), the I locus, which regulates seed color through tissue-specific subfunctionalization of chalcone synthase (CHS) genes, offers a compelling example of these mechanisms. Using long-read sequencing, we investigated structural variations underlying four major alleles (I, i-i, i-k, and i) at the I locos. Our study revealed that large-scale rearrangements, including duplications, inversions, and deletions, within a 180-kb CHS repeat-rich region drive allele-specific RNA silencing through small interfering RNAs (siRNAs). The dominant I allele contains a DnaJ fragment upstream of CHS genes, while the i-i and i-k alleles feature subtilisin- and P450-driven siRNA loci, respectively. The recessive i allelelacks siRNA production due to deletions or inversions disrupting CHS gene clusters. Phenotypic analyses and RNA-seq confirmed allele-specific, tissue-dependent expression of siRNAs correlating with seed coat pigmentation patterns. This study highlights the evolutionary role of repeat-rich regions in generating regulatory innovations and phenotypic diversity. Our findings underscore the importance of structural rearrangements in domestication traits and demonstrate the power of long-read sequencing for resolving complex genomic regions, advancing both evolutionary biology and crop improvement.

Oral Presentation #12

Inari Agriculture: The SEEDesign™ company

Marlies Wouters[1]

[1]Inari Agriculture

Inari Agriculture is designing seeds to address the dual global challenges of food security and climate change. The Inari SEEDesign™ technology platform integrates AI-assisted Predictive Design and advanced Multiplex Gene Editing tools to unlock the full potential of seeds. In Predictive Design, we harness the power of data, computational modeling, and artificial intelligence to gain an understanding of the pathways and genes that underpin traits crucial for crop performance under varying environmental conditions. Once we have identified these genes and defined their editing strategy, we use our CRISPR/Cas Multiplex Gene Editing toolbox to deliver the desired changes into elite parental lines. Our toolbox consists of technologies that enable us to turn off/on genes, tune their expression up/down and facilitate sequence replacement with an initial focus on creating higher-yielding varieties of soybeans, corn and wheat that require fewer resources. Leveraging the SEEDesign™ technology platform Inari is revolutionizing crop breeding, accelerating breeding cycles to bring superior products to market at reduced costs.

Oral Presentation #13

Engaging Undergraduates in the Functional Characterization of Soybean Mutants

C. Nathan Hancock[1]

[1]University of South Carolina Aiken

Mutagenized soybean populations provide an opportunity to teach basic plant physiology, genetics, bioinformatics, and molecular biology to undergraduate students. Over multiple years, students analyzed two heritable phenotypes discovered in *mPing* transposition tagging soybean lines. The *y24* allele exhibited chlorotic leaves, smaller stature, weaker stems, and reduced root system while a dwarf allele resulted in severe stunting. Students phenotyped seedlings from F_2 populations to determine that both phenotypes are controlled by a single recessive allele. Alignment of whole genome Illumina sequencing reads to the soybean reference genome allowed us to identify the variants present in each mutant. SnpEff was used to identify variants that were likely to result in loss of protein function. Genes shown to have nonfunctional alleles in the soybean pangenome were excluded, resulting in manageable candidate lists. Amplicon sequencing of bulked segregants from F_2 populations further narrowed the list. Further confirmation of the responsible genes was achieved through literature review of homologs, publicly available expression datasets, and Arabidopsis transformation. In summary, modern sequencing and bioinformatics technologies are facilitating the training of the next generation of soybean scientists.

Oral Presentation #14

Title TBD

Xuelu Wang

Oral Presentation #15

The Biochemistry of Nodulation

Gary Stacey[1]

[1]University of Missouri, Columbia, MO

The long-term goal of our research is to further fundamental understanding of the agronomically important soybean N2 fixing symbiosis. Soybeans are the major source of nitrogen for livestock feed and are also processed into protein-rich products for human consumption. In 2020, soybean was grown on more than 90 million acres in the U.S. with an estimated value of more than $46 billion. Soybean is a major crop worldwide due to its ability to fix atmospheric N2 through its symbiotic relationship with soil bacteria. It has been estimated that more than 60 million metric tons of N2 are fixed by legumes annually with a fertilizer replacement value of $7-10 billion. Our research group has a specific focus on understanding unique areas of the rhizobial-legume symbiosis that are critical for nodule formation and nitrogen fixation. Data integration and system modeling of complex biological processes require detailed, functional genomic and biochemical data, the latter often overlooked in the current age of large-scale genomic analyses. While the early events in symbiotic establishment are well studied at a genetic level, much less is known about the detailed biochemical processes that define the rhizobial infection process. We utilize high-resolution sampling of root hair cells to explore in detail the molecular mechanisms leading to the establishment of a N2-fixing symbiosis. This approach has led to the identification of new components of the nodulation signaling cascade, which interact directly with the Nod factor receptors. Filling in the gaps in our understanding of the rhizobial-legume infection process will be critical to ongoing efforts to transfer this symbiosis to non-leguminous plants (e.g., maize).

Oral Presentation #16

Engineering a Functional Copy of Inceptin Receptor (INR) into Soybean Confers Herbivory Recognition

Di Wu[1], *Euan McCubbin*[1], *Adam Steinbrenner*[1]

[1]University of Washington

Plant defense mechanisms against pests and pathogens are controlled by diverse families of immune receptor genes. Inceptin Receptor (INR) is a legume-specific pattern recognition receptor which detects In11, a ubiquitous 11-amino acid peptide in the oral secretions of Lepidopteran larvae. In11 triggers robust direct and indirect anti-herbivore defenses in cowpea, common bean, mung bean, and several other legumes, but shows no activity in both wild and domesticated soybean (*Glycine max*). To search for novel pest management tactics, we generated transgenic soybean lines in the Williams 82 (W82) background expressing *INR* from common bean (*Phaseolus vulgaris*), termed PvINR-OE. Phenotypic analysis on multiple independent PvINR-OE lines demonstrates that presence of INR transgene restores In11 recognition, as evidenced by production of the defense hormone ethylene, expression of defense marker genes, activation of Mitogen-activated protein kinases, and reduced herbivore performance on PvINR-OE. Ongoing investigations are focused on assaying indirect defense traits of PvINR-OE and a multi-omics study examining In11-triggered reprogramming of transcriptome, phospho-proteome, and acetylome. Taken together, INR offers a promising addition to integrated pest management strategies for mitigating yield losses of soybean from Lepidopteran pests.

Oral Presentation #17

Discovery of Novel Resistance Resources Independent of *rhg1* and *Rhg4* for Broad-based SCN Resistance in Soybean

Sushil Chhapekar[1], Dr. Vikas Devkar[2], Dr. Naoufal Lakhssassi[3], Dr. Heng Ye[1], Dr. Sonam Singh[1], Dr. Aamir Khan[1], Dr. Tri Vuong[1], Dr. Gunvant Patil[2], Dr. Khalid Meksem[3], Dr. Henry Nguyen[1]

[1]University of Missouri, [2]Texas Tech University, [3]Southern Illinois University Carbondale

Soybean cyst nematodes (SCN) cause severe yield loss (>$1.0 billion) throughout soybean production regions in the United States. Currently, about 98% of resistant varieties carry the resistance from PI 88788 and Peking (either through rhg1 or Rhg4). Continuous use of rhg1-derived resistance caused changes in the virulence of SCN populations (race shift) as well as due to narrow genetic diversity; currently, about 80% of SCN populations can overcome the host resistance offered by rhg1. Therefore, it is imperative to identify and develop additional SCN-resistance sources independent of rhg1 and Rhg4 to combat this ever-changing pathogen. The whole-genome resequencing-derived pangenome analysis of 1200 soybean lines demonstrated six unique new sources of SCN-resistant germplasms independent of rhg1 and Rhg4. Haplotype, gene expression, and SCN phenotyping analysis identified several Glycine max accessions, such as PI 567516C, PI 407729, and two wild G. soja accessions, which contain unique haplotypes and are not related to any known SCN resistant loci (rhg1, Rhg4, and Rhg2). These novel soybean lines are resistant to multiple SCN populations (HG types), providing new genetic resources for durable SCN resistance. Soybean exotic line PI567516C carries two novel loci (qSCN10 & qSCN18) for SCN resistance and displays resistance mechanisms different from those of known genes. qSCN10 and qSCN18 were fine-mapped to 142-kbp and 130-kpb regions containing 20 genes and 15 genes, respectively. Based on gene expression, gene ontology, in-silico and haplotyping analysis, two candidate genes from qSCN10 and four candidates from qSCN18 were selected for further gene functional characterization through overexpression, CRISPR knockout and single-nucleus RNA-seq. Overexpression of candidate genes in the SCN-susceptible Williams 82 composite transgenic roots showed cyst count reduction by 52 to 65% compared to wild type. These novel germplasms serve as crucial genetic material for broadening the genetic diversity and developing next-generation soybean varieties for the U.S. farmers.

Oral Presentation #18

Pleiotropic functions of a gibberellin receptor gene in the regulation of plant architecture, yield and nitrogen fixation in soybean

Jiajun Tang[1], Shuxuan Li[1], Xiuli Yue[1], Shancen Zhao[2], Junyi Gai[1], Yan Li[1]

[1]Nanjing Agricultural University, [2]BGI Genomics, BGI-Shenzhen

The yield of soybean is far below that of major cereal crops. The green revolution increased the yield of cereal crops partially through high-density planting of lodging-resistant semi-dwarf varieties, but required more nitrogen fertilizers, posing an environmental threat. Genes that can improve nitrogen use efficiency need to be integrated into semi-dwarf varieties to avoid the overuse of fertilizers without the loss of dwarfism. Unlike cereal crops, soybean can assimilate atmospheric nitrogen through symbiotic bacteria. Here, we created new alleles of *GmGID1-2* (*Glycine max* GIBBERELLIN INSENSITIVE DWARF 1) using clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated nuclease 9 (Cas9) editing, which improved soybean architecture, yield, seed oil content, and nitrogen fixation, by regulation of important pathways and known genes related to branching, lipid metabolism, and nodule symbiosis. *GmGID1-2* knockout reduced plant height, and increased stem diameter and strength, number of branches, nodes on the primary stem, pods, and seeds per plant, leading to an increase in seed weight per plant and yield in soybean. The nodule number, nodule weight, nitrogenase activity, and nitrogen content were also improved in *GmGID1-2* knockout soybean lines, which is novel compared with the semi-dwarf genes in cereal crops. No loss-of-function allele for *GmGID1-2* was identified in soybean germplasm and the edited *GmGID1-2s* are superior to the natural alleles, suggesting the *GmGID1-2* knockout mutants generated in this study are valuable genetic resources to further improve soybean yield and seed oil content in future breeding programs. This study illustrates the pleiotropic functions of the *GID1* knockout alleles with positive effects on plant architecture, yield, and nitrogen fixation in soybean, which provides a promising strategy toward sustainable agriculture.

Oral Presentation #19

As Designed by Nature: Unexpected Soybean Transformation Results from Virulence Plasmid Launched-Transfer DNA

Ray Collier[1]

[1]Molecular Technologies Department, Wisconsin Crop Innovation Center, University of Wisconsin-Madison

For ~45 years transgenic plants have mostly been produced via *Agrobacterium tumefaciens* harboring a binary plasmid. However, compelling work from Stephen Farrand demonstrated that *Agrobacterium rhizogenes* strain NCPPB2659 (a.k.a. K599) induced more transgenic hairy roots from a wider range of soybean cultivars relative to other *A. rhizogenes* strains. The Farrand work was leveraged in the lab of Chris Taylor at the Danforth Center where we disarmed NCPPB2659 to produce Ar18r12v, which has for ~20 years outperformed *A. tumefaciens* in a wide range of target plant species, including soybean. Quality characteristics of Ar18r12v-derived transgenic plants informed strain choice for the GA*A*NTRY system that Roger Thilmony, James Thomson, and I co-invented at the USDA-ARS in Albany, California. GA*A*NTRY established the T-DNA launch point on the disarmed Ri plasmid of ArNCPPB2659 (called ArPORT1) in which T-DNA molecules are assembled via Recombinase Mediated Cassette Exchange (RMCE). At the Wisconsin Crop Innovation Center (WCIC) we have directly compared identical T-DNA launched from either the disarmed Ri or a binary plasmid and have found that pRi launched T-DNA results in remarkable improvements to plant transformation efficiency, especially with regard to gene editing constructs for dicots. The unexpected gene editing experiment result was sufficiently impactful and directional to lead the WCIC to no longer construct binary plasmids for dicots for gene editing; only the GA*A*NTRY system is used. Finally, building on the cargo size advantage GA*A*NTRY holds over binary plasmids, the WCIC has modified the GA*A*NTRY system to offer 2 T-DNA capability, enabling production of constructs which direct gene editing at designated loci (T-DNA # 1), with concurrent delivery of a modified (non-editable) copy of the targeted gene (T-DNA # 2), opening the door to new scientific approaches which were previously not possible due to the size limitations imposed by the binary plasmid cargo capacity.

Oral Presentation #20

Advancing Soybean Transformation: A Journey at Corteva

Hyeon-Je Cho[1]

[1]Corteva Agriscience

Soybean is one of the most important food, feed, and biofuel crops in the world and a strategic crop for Corteva. A key component of modern plant biotechnology, plant transformation plays a crucial role in developing and commercializing transgenic and gene-edited crops. Over the last four decades, the transfer of DNA into plant cells has been achieved by using several methods. In soybeans, the most frequently employed plant genetic engineering methods are *Agrobacterium*-mediated transformation and particle bombardment. Both systems have successfully been used in genetic transformation of soybean. Since the initial reports of fertile transgenic soybean production, various efforts have been made to improve the transformation efficiency and to produce transgenic soybean. Nevertheless, new methods have been developed for more efficient soybean transformation.

At Corteva, we have made significant progress in developing novel and efficient plant transformation technologies for soybean. We have discovered a novel bacterium, *Ochrobactrum haywardense* (Oh) H1 capable of efficient plant transformation. *Ochrobactrum* is a new host for *Agrobacterium*-derived *vir* and T-DNA–mediated transformation. Oh H1-8 successfully transformed elite Corteva soybean varieties, genotype independently, with T0 transformation frequencies up to 40% using soybean embryonic axes as a target explant via organogenesis. We also developed highly efficient *Agrobacterium*-mediated soybean suspension cultures and an immature cotyledon transformation system that produced fertile transgenic plants at a frequency of more than 350% (number of T0 events/immature cotyledon) via embryogenesis. This presentation will detail our journey in the development of soybean transformation systems and implementation of a production pipeline.

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Oral Presentation #21

Turbocharging Genomics to Fuel Advances in Plant Biology

Kristin Bilyeu[1]

[1]USDA/ARS Plant Genetics Research Unit and University of Missouri

Our vision is for species-independent applied genomics tools and resources that boost research to identify the alleles of genes that control phenotypes. As technology progresses, how we think about developing new crop varieties must evolve with those advances. One challenge for today's researchers is adopting technologies that will meet current needs and also position researchers for the next generation of advancement. I envision a scenario where new crop varieties will be designed as part of the breeding process. To implement this vision, we need to boost our knowledge of variations in genes that control phenotypes. We leverage prior genomic sequence investments to provide comprehensive solutions to select for soybean genes that control traits. The current landscape of technologies, particularly in the development and practical application of genomic sequence information, holds exciting promise for better crops of the future.

Oral Presentation #22

New Players in Soybean Resistance and SCN Virulence

Melissa Mitchum[1], Vinavi A. Gamage[1], Mariola Usovsky[2], Khee Man Kwon[1], Joao P.G. Viana[3], Kimberly K.O. Walden[3], Anser Mahmood[2], Elizabeth J. De Meyer[2], Clinton G. Meinhardt[2], Bishnu Dhital[2], Qijian Song[4], Matt E. Hudson[3], Andrew M. Scaboo[2]

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Genetic resistance is the primary approach for managing the soybean cyst nematode (SCN), which is the most economically important pathogen of soybeans. Despite the planting of resistant soybean cultivars, SCN still robs producers of $1.5B in yield annually. Moreover, the major gene for resistance from the donor source PI 88788, *rhg1-b*, has declined in its effectiveness against SCN due to decades of repeating planting that has selected for SCN capable of reproducing on these resistant cultivars. Consequently, research efforts have focused on understanding the genes and mechanisms of resistance offered by the donor source PI 548402 (Peking), also known to carry the *rhg1-a* allele which acts epistatically with *rhg2* and *Rhg4* to confer SCN resistance. However, SCN has also evolved around this resistance leading to virulence in field populations where this resistance is planted. The finding that *Rhg* genes code for atypical housekeeping proteins involved in vesicular trafficking (soluble NSF attachment proteins) and 1-C folate metabolism (serine hydroxymethyltransferase) suggests SCN is likely targeting these pathways for successful parasitism. Recent research has uncovered two additional vesicular trafficking proteins, *GmSNAP02* and *GmSNAP14*, as new players in SCN resistance that may function as potential virulence targets, as loss-of-function alleles enhance resistance to virulent SCN. Moreover, mapping studies have led to the identification of candidate SCN virulence genes that may be acting upon these host targets.

Oral Presentation #23

Exploring the Role of AAT_{Rhg1} in Soybean Cyst Nematode Resistance and Amino Acid Dynamics

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Soybean cyst nematode (SCN, Heterodera glycines) causes widespread reductions in soybean yields. The *Rhg1* locus is a key source of SCN resistance and contains three genes that contribute to resistance, including *Rhg1-GmAAT* (*Glyma.18G022400*), which encodes a putative amino acid transporter, AAT_{Rhg1}. The molecular function of AAT_{Rhg1} in SCN resistance has been unclear. In recent work we have demonstrated that silencing *Rhg1-GmAAT* in SCN-resistant *rhg1-b* plants compromises resistance against both HG 0 and HG 2.5.7 SCN populations, indicating that AAT_{Rhg1} still contributes against problematic HG 2.5.7 populations that partially overcome *rhg1-b*-mediated resistance. Transcriptomic and metabolomic analyses of the SCN infection zone on soybean roots revealed distinct transcript and metabolite profile changes between *Rhg1-GmAAT*-silenced and non-silenced *rhg1-b* plants, with notable impacts on ethylene signaling and amino acid homeostasis. While overexpression of *Rhg1-GmAAT* alone in susceptible or resistant soybean backgrounds did not enhance SCN resistance, it significantly increased betalain accumulation when co-expressed with the RUBY transgene cassette, potentially through its influence on tyrosine levels and amino acid homeostasis. Confocal microscopy demonstrated that AAT*Rhg1* localizes to the tonoplast in soybean root cells. Two single amino acid mutations, D122A and Y268L, were found to affect AAT_{Rhg1} protein function in opposite directions. Together, these findings provide a more detailed characterization of AAT_{Rhg1} and offer clues into the potential mechanisms by which AAT_{Rhg1} contributes to SCN resistance.

Oral Presentation #24

Exploring Novel Genetic Resistance to *Heterodera glycines* and *H. sojae* Using a Diverse Soybean Germplasm Panel

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Soybean cyst nematode (SCN), *Heterodera glycines*, is a major destructive pest significantly impacting global soybean production. More recently, *Heterodera sojae*, another soybean parasitic cyst nematode, has emerged as a significant threat, particularly in East Asia including Korea, where resistant cultivars are underdeveloped for both species. This study aimed to identify genetic factors associated with resistance to both *H. glycines* and *H. sojae* in Korean soybean germplasm. We utilized a panel of over 400 soybean accessions, including a core collection, for comprehensive analysis. Genotyping revealed that known *H. glycines* resistance types (PI88788 and Peking), primarily controlled by *Rhg1* and *Rhg4* genes, were present in only a minority of these varieties. Copy number variation (CNV) analysis of the *Rhg1* gene using Droplet Digital PCR (ddPCR) and *in silico* methods confirmed three distinct categories (high copy PI88788-type, medium copy Peking-type, and single copy susceptible-type), with consistent results between methods. Genome-wide association studies (GWAS) were conducted using whole-genome resequencing data coupled with phenotypic screening data for both nematode species. For *H. glycines*, GWAS identified associated SNPs, providing a basis for exploring resistance mechanisms. For *H. sojae*, GWAS identified 13 significant single nucleotide polymorphisms (SNPs). Haplotype analysis surrounding significant SNPs on chromosomes 1 and 18 revealed 16 candidate genes, several predicted to be root-specific based on expression atlases. Kompetitive allele-specific PCR (KASP) markers were developed from four significant SNPs associated with *H. sojae* resistance for efficient genotyping. This comprehensive research enhances our understanding of the genetic basis of resistance to both *H. glycines* and *H. sojae* in diverse soybean germplasm, identifying novel candidate genes and providing valuable SNP markers for developing broadly nematode-resistant soybean varieties.

Oral Presentation #25

Effects of increasing atmospheric carbon dioxide levels on soybean responses to diverse pathogens

Steve Whitham[1], Melissa Bredow[1], Ekkachai Khwanbua[1], Aline Sartor Chicowski[1], Yunhui Qi[1], Matthew Breitzman[1], Katerina Holan[2], Peng Liu[1], Michelle Graham[2]

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Elevated atmospheric CO_2 (eCO_2) has complex effects on plant-pathogen interactions, making it difficult to predict how rising CO_2 levels will impact crop disease dynamics. To investigate these effects, we examined disease development and molecular defense responses in soybean grown under ambient (419 ppm) and elevated (550 ppm) CO_2. Plants were challenged with bacterial, viral, fungal, and oomycete pathogens, and we assessed disease severity, pathogen growth, gene expression, and defense mechanisms. Under eCO_2, soybean was less susceptible to *Pseudomonas syringae* pv. *glycinea* but more susceptible to bean pod mottle virus, soybean mosaic virus, and *Fusarium virguliforme*, while susceptibility to *Pythium sylvaticum* remained unchanged, though greater biomass loss was observed. Reduced susceptibility to bacterial infection correlated with enhanced defense responses, whereas increased viral susceptibility was linked to diminished antiviral defenses. These findings provide key insights into how rising CO_2 levels may reshape soybean-pathogen interactions, underscoring the need to consider microbial threats to both shoots and roots in future climates. Understanding these responses is crucial for developing climate-resilient disease management strategies.

Oral Presentation #26

Conferring broad spectrum disease resistance in soybean using dual action elicitor peptide variant(s)

Ambika Pokhrel[1], *Principal Investigator Dilip Shah*

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Plant elicitor peptides (Peps) are signaling molecules produced by various plants and known to amplify or elicit the defense responses against various pathogens and insects. Previous studies have found that soybean (Glycine max) genome encodes eight 23-amino acids elicitor peptides (GmPeps). Of the eight GmPeps, three GmPeps (1, 2, and 3) are already characterized and shown to promote defense against soybean root-knot and cyst nematodes. Phylogenetics analysis of these GmPeps has shown that GmPep6 is closely related to Arabidopsis elicitor peptide AtPep1 known to enhance resistance against bacterial and oomycete pathogen. This research aims to elucidate the role of GmPep6 and its two synthetic variants (GmPep6_V1 and GmPep6_V2) in inducing the defense response and direct antimicrobial activity against soybean pathogens. The *in vitro* antimicrobial activity assays revealed that GmPep6_V2 has direct fungicidal activity against multiple fungal pathogens, *Botrytis cinerea, Fusarium virguliforme*, and *Cercospora sojina (Cs)*, but it is not active against a bacterial pathogen *Xanthomonas citri* pv. *glycines (Xcg)*. However, spray applications of both GmPep6_V1 and GmPep6_V2 on soybean leaves *in planta* significantly reduced frogeye leaf spot disease symptoms caused by *Cs* and bacterial pustule disease symptoms caused by *Xcg*. Modes of action studies using confocal microscopy revealed that GmPep6_V2 permeabilized the fungal plasma membrane and localized in the vacuole and nucleolus in fungal cells. Furthermore, in soybean leaves, confocal microscopy revealed that GmPep6_V2 was internalized into leaf epidermal and mesophyll cells when applied as a foliar spray. Gene expression studies using qRT-PCR and phytohormone analysis revealed that spray application of these peptides induced both the expression of jasmonic acid (JA) marker genes and the production of JA phytohormones respectively in soybean leaves. Overall, our findings indicate that GmPep6 variants can be used to enhance resistance against multiple diseases in soybean.

Oral Presentation #27

From Code to Crop: Harnessing Soybean Genomics for Gene Cloning, Editing, and More

Robert Stupar[1]

[1]University of Minnesota

Recent advances in biotechnology and the development of near-gapless reference genomes have enhanced our ability to apply genomic tools towards understanding and improving crops. In the soybean research community, this progress has accelerated gene cloning and editing efforts, including work targeting previously intractable loci and traits. This talk will highlight current efforts to uncover complex genetic variants underlying key agronomic traits, with a focus on iron deficiency chlorosis tolerance and variation in plant architecture. It will explore the factors that distinguish quantitative trait loci where causative DNA polymorphisms can be readily identified from those where they remain elusive. Finally, the implications for crop improvement will be discussed, particularly how biases in gene cloning may limit the effective application of molecular tools.

Oral Presentation #28

Selection, pangenomes and structural diversity in soybean and a soybean pathogen

Matthew Hudson[1]

[1]University of Illinois, Urbana-Champaign

Genetic diversity is critical to crop breeding and improvement, and dissection of the genomic variation underlying agronomic traits can both assist breeding and give insight into basic biological mechanisms. Although recent genome analyses in plants reveal that a lot of intraspecific variation is in the form of structural variants, most current studies of crop genetic variation are still dominated by single-nucleotide polymorphisms. Currently available reference genomes thus fall short in representing intraspecific diversity, impeding our understanding of soybean traits and their evolution, and virulence and host adaptation in pathogens. Graph-based pangenomes provide a potential solution to understanding evolution and selection of all genomic variation at a species level, and recent advances in sequencing technology and assembly algorithms have made such pangenomes feasible for the first time. Results from the sorghum and soybean cyst nematode pangenomes will be presented, as well as preliminary findings from the ongoing DOE funded soybean pangenome project. New software and algorithms for the analysis of selection in pangenomes will be presented, and the relative value of gene – trait associations versus selective analysis will be discussed.

Oral Presentation #29

Targeting Induced Local Lesions in Genomes “TILLING” to improve agronomically importance traits in soybean

Erdem Anil[1], Mallory A Cullen[1], Abdelhalim El Baze[1], Tarek Hewezi[2], Dounya Knizia[1], Naoufal Lakhssassi[1, 4], Shiming Liu[1, 6], Khalid Meksem[1], Henry Nguyen[3], Sarbottam Piya, Yasser Salhi[1], Zhou Zhou[1, 5]

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Developing soybean lines with improved agronomic traits is a primary goal of the soybean industry. Over the past two decades, we developed several Ethyl methanesulfonate (EMS) mutagenized populations and used traditional Targeting Induced Local Lesions in Genomes (TILLING) method to allow directed identification of mutations in soybean genes. Recently, we developed a TILLING by Target Capture Sequencing (Tilling-by-Sequencing+) technology, a versatile extension of the conventional TILLING by sequencing, and successfully identified thousands of ethyl methanesulfonate mutants at soybean seeds traits genes (Sugar, oil, proteins and antinutrient) and other agronomically important genes (Resistance to nematode).

Functional analysis of the identified mutants revealed an unprecedented role of several genes in several biosynthesis pathways. An update of the soybean oil and sugar biosynthesis pathway will be presented.

Oral Presentation #30

SoyBase: Here's to another 30 years

Jacqueline Campbell[1], Connor Cameron[2], Alan Cleary[2], Sudhansu Dash[2], Andrew Farmer[2], Wei Huang[1], Scott Kalberer[1], Simon Novak[3], Chen Prom[1], Nathan Weeks[1], Steven Cannon[1], Rex Nelson[1]

[1]USDA-ARS, [2]National Center for Genome Resources (NCGR), [3]HACU-USDA Intern, Michigan Technological University

SoyBase (https://soybase.org) is the USDA-ARS soybean genetics and genomics resource hub, providing a comprehensive collection of analysis tools, data, and links to external resources for the soybean research community. For over 30 years, SoyBase has been professionally curated, with new data regularly being incorporated. After a multi-year effort, we released a major SoyBase upgrade in October 2024. The upgrade includes new data organization, new infrastructure, and multiple user interface improvements while retaining the historical data that SoyBase team has been curating since 1992. SoyBase currently holds 67 annotated genomes from multiple Glycine max and Glycine soja cultivars, with tools for browsing and querying these genomes and annotations. Numerous JBrowse tracks are available for the Williams 82 soybean reference genome (assembly versions 1 through 6), including genome organization, gene expression, markers, genome methylation, and sequence variants (such as the SoySNP50K and GmHapMap projects). In the past year, several tools have been added to SoyBase including: a browser to view GWAS and QTL at the same time (ZZBrowse), a Gene Symbol Search, an Allele Search Tool, a Trait Search Tool, and Pedigree Search and Viewer Tool. Since its inception, the major goal of the SoyBase staff was to assist researchers in discovering important trait, genomic, and genetic information to improve soybean yield and quality while defending against pests, diseases and abiotic stress. We hope to remain an integral component of the soybean research community for the next 30 years.

Oral Presentation #31

The impact of stress combination on soybean yield under a changing climate

Ron Mittler

Oral Presentation #32

Drought Does Not Mitigate the Effects of Ozone on Soybean Photosynthesis and Yield

Lisa Ainsworth[1], *Dr. Duncan Martin, Dr. Shuai Li, Dr. Elise Aspray, Professor Andrew Leakey*

[1]University of Illinois Urbana-Champaign

The co-occurrence of elevated tropospheric ozone concentrations and drought in agricultural regions is anticipated to increase with climate change. Both stressors negatively impact leaf photosynthetic capacity and stomatal conductance, contributing to reductions in biomass and yield. The interaction of ozone and drought stress is complex and under-researched, particularly in field settings. Stomatal closure in response to soil drying may provide protection from high ozone influx to leaves. Conversely, the presence of elevated ozone may prevent drought-induced stomatal closure, leading to depletion of soil water resources, and exacerbation of drought stress. Here, we use Free Air Concentration Enrichment of ozone (100 ppb) and rainfall exclusion canopies (intercepting ~40% of seasonal rainfall) to test potential interaction effects of elevated ozone and drought stress on soybean leaf-level physiology and yield. Elevated ozone consistently reduced soybean Rubisco carboxylation capacity (-17%) and maximum electron transport capacity (-9%) across three years of study. Elevated ozone did not alter the relationships between soil moisture, abscisic acid and stomatal conductance. Thus, there was no evidence that ozone exposure prevented stomata from responding during drought. Yield was significantly reduced in soybeans exposed to elevated ozone, resulting from both fewer seeds per plot and reduced seed size. The reduced precipitation treatment only affected yields in the driest growing season. These findings suggest that the effects of elevated ozone and drought are additive rather than interactive, and dose dependent. The persistence of ozone damage under soil moisture depletion is likely to be exacerbated by global climate change.

Oral Presentation #33

Unraveling Molecular and Cellular Insights in Soybean-AMF Symbiosis

Leonidas D'agostino[1], *Dr. Lenin Yong-Villabos*[1], *Dr. Luis Herrera-Estrella*[1], *Dr. Gunvant Patil*[1]

[1]Texas Tech University

Beneficial microbes like arbuscular mycorrhizal fungi (AMF) present an opportunity for sustainable enhancements to nutrient uptake in legume crops. AMF-plant symbiosis increases nutrient and water accessibility through their hyphal-arbuscule networks, as well as resistance to drought and salinity stress. However, the complexity of signal perception, metabolic-flux, nutrient absorption and translocation at the sub-cellular level is elusive in soybean. In this study, we have employed single nucleus sequencing technology utilizing five distinct time points of AMF colonization to create a comprehensive atlas combining both structural and temporal gene expression. Comparative analyses have offered insights into cell-type specific changes in pathways such as organelle development, nutrient uptake, and senescence over time. Investigation so far has identified novel symbiosis-induced genes significant for establishing and maintaining cooperative relationships, as well as providing further evidence for previously established symbiosis related genes. Previously unstudied soybean specific genes have been chosen for functional validation experiments to further identify and characterize genes essential for soybean-AMF symbiosis. This resource provides valuable information for optimizing symbiotic relationships in agricultural practices, as well as a framework for genetic discovery in a non-model crop species.

Oral Presentation #34

Genome-wide Association Studies Revealed Seven Putative Drought Tolerance Genes in Soybean

Dr. Atit Parajuli[1,2], Ramesh Chethri[1,2], Iman Saha[1,2], Micheline N. Ngaki[1,2], Cecelia Ryden[1,2], Madeline Thompson[1,2], Qingfeng Xing[1,3], Liang Dong[1,3], Madan Bhattacharyya[1,2]

[1]Iowa State University, [2]Department of Agronomy, [3]Department of Electrical and Computer Engineering

Drought stress significantly limits soybean yield, especially if it occurs during flower and early pod development stages. To better understand the genetic mechanisms of drought tolerance in soybeans, we investigated a collection of 240 highly diverse soybean accessions. The genotypes were grown under either rainout shelters or no shelters. The leaf-flipping phenotypes were recorded with a digital camera. Genome-wide association study of the variation in the leaf-flipping trait identified three candidate drought tolerance genes: (i) a thaumatin-like protein gene, the tea homologue of which was shown to regulate the root hair development and drought tolerance in Arabidopsis; (ii) a chloroplast isopropyl malate synthase gene that plays an important role in root development and drought tolerance in Arabidopsis; (iii) a transcriptionally regulated gene encoding glycinol 2-dimethyltransferase. A subset of 47 genotypes were studied for leaf surface humidity and temperature using a wearable plant sensor to identify mechanisms involved in transpiration. The GWAS of the humidity and temperature ratios of the 47 accessions revealed two candidate transcriptionally regulated drought-responsive genes encoding α-tubulin and phosphoenolpyruvate carboxykinase (PCK). The α-tubulin was shown to control stomatal opening, while PCK improved water retention by closing stomata during drought stress. Thus, these two genes most likely regulate transpiration in soybeans. Two additional genes encoding (i) an uncharacterized DUF1118 containing protein and (ii) HAT5 homeodomain-leucine zipper protein, identified from another locus, may also regulate transpiration during drought stress. This study paved the way for better understanding the molecular basis of drought tolerance in legume species.

Oral Presentation #35

Transcriptome-wide association uncovers lncRNAs controlling seed weight in soybean

Qingxin Song[1], *Xinyu Jiang*[1]

[1]Nanjing Agricultural University

Long non-coding RNAs (lncRNAs) are emerging as key regulators of various biological processes in plants, yet their role in seed weight regulation remains underexplored. Here, we employed a comprehensive approach combining transcriptome-wide association studies (TWAS) and expression quantitative trait loci (eQTL) analysis to identify lncRNAs associated with seed weight using 238 soybean accessions. We identified 175 and 50 TWAS-significant lncRNAs at 14 and 21 days after flowering, respectively, and further validated the positive regulatory role of two lncRNAs in seed weight regulation. Additionally, we identified a lncRNA-lncRNA co-expression module significantly correlated with seed weight. The eQTL analysis revealed dynamic and static eQTLs that coordinately modulated lncRNA expression across seed developmental stages. Notably, integration of major-effect proximal and minor-effect distal eQTLs enhanced phenotype prediction of seed weight, underscoring the significant impact of distal eQTLs on phenotypic variation. These findings elucidate the intricate mechanisms by which lncRNAs contribute to seed weight regulation, offering novel insights for enhancing crop yield.

Oral Presentation #36

Identification of novel loci related to amino acid contents in soybean by combination of network analysis and genome-wide association study

Kyujung Van[1], Sungwoo Lee[2], Rouf Mian[3,4], Leah McHale[1]

[1]The Ohio State University, [2]Chungnam National University, [3]USDA-ARS, [4]North Carolina State University

The amino acid (AA) profile is important to the nutritional value of soybean seed, but is a complex, quantitative trait controlled by multiple interconnected genes and pathways. We used three genome-wide association study (GWAS)-based approaches to investigate the genomic regions controlling the AA content measured across a total of 621 soybean germplasm grown in five environments. Approach I applied a GWAS of 24 single AAs, identifying significant SNPs grouping into 16 linkage disequilibrium (LD) blocks from 18 traits under all environments combined. For Approach II, individual AAs were examined based on the sum, ratios, and interactions of AAs within the same biochemical family, identifying significant SNPs grouping into 35 LD blocks. These significant SNPs associated with traits from the same biochemical family often positioned on the same LD blocks. For Approach III, a correlation-based network analysis was performed and two groups were described by the network topology, Group 1: Ala, Gly, Lys, available Lys (Alys), and Thr and Group 2: Ile and Tyr. Significant SNPs associated with a ratio of connected AAs or a ratio of a single AA to its fully or partially connected metabolic groups were identified within 9 LD blocks for Group 1 and 2 LD blocks for Group 2. Among 40 identified QTL for AA or AA-derived traits, three genomic regions were novel in terms of seed composition traits (oil, protein, and AA content). An additional 24 regions had previously not been specifically associated with the AA content. Three specific genomic regions on chromosomes 5, 9, and 20 were significantly identified by all three approaches, but most associations between a genomic region and an AA trait were approach- and/or environment-specific. Our results confirmed loci identified from previous studies but also suggested that network approaches for studying AA contents in soybean seed are valuable.

Oral Presentation #37

Comprehensive Transcriptomic Analysis of Environmentally Modulated Seed Protein Content in Soybean: Implications for Western Canada and Northern Regions

Bahram Samanfar[1,2], *Julia Hooker*[1,2], *Elroy Cober*[1], *Ashkan Golshani*[2]

[1]Agriculture and Agri-Food Canada- Ottawa, [2]Carleton University

Soybean seeds are a rich source of oil and protein, but their composition is shaped by both genetic and environmental factors. Over the past two decades, a consistent trend has emerged: soybeans grown in western Canada exhibit lower seed protein concentrations (by approximately 1–5% of total seed weight) compared to those grown in eastern Canada, regardless of genotype. To investigate the underlying causes of this disparity, we cultivated multiple soybean genotypes with seed protein content ranging from 32.8% to 50.7% across four locations in Canada—one eastern control site and three western experimental sites. Using RNA sequencing and differential gene expression analyses, we generated 55 distinct differential expression datasets to examine genotype-specific responses to environmental variation. Differentially expressed genes were linked to key biological pathways, including amino acid biosynthesis, lipid metabolism, and circadian rhythm regulation. Notably, region-specific differences were observed in cysteine and methionine metabolism, likely contributing to the variation in seed protein content. Additionally, an upregulation of lipoxygenases in western-grown soybeans suggests a role in modifying seed composition in response to environmental stressors.

A striking discovery was the consistent upregulation of asparaginase and downregulation of asparagine synthetase in western-grown soybeans, highlighting a shift in nitrogen metabolism that correlates with reduced protein accumulation. This metabolic shift was further supported by a positive correlation between seed protein content at maturity and free asparagine levels in developing seeds. Furthermore, the downregulation of the E11 early maturity gene in western-grown soybeans points to a circadian response to regional photoperiod differences.

These findings provide valuable insights into the genetic and metabolic mechanisms underlying environmental influences on soybean seed composition. By identifying key pathways associated with protein content variation, this study offers a foundation for marker-assisted selection and breeding strategies aimed at improving soybean varieties for western Canada and other northern growing regions.

Oral Presentation #38

Investigating a putative susceptibility gene towards Phytophthora sojae in soybean

Leah Mchale[1], Anne Dorrance[1], Stephanie Karhoff[1], Christian Vargas-Garcia[2], Feng Qu[1], Chee Gang Ngui[1]

[1]The Ohio State University, [2]US Department of Agriculture, Agricultural Research Service

As genome-editing has become increasingly prevalent in crop improvement, recent research has turned toward identifying and disrupting susceptibility genes to enhance resistance to biotic stresses. One such challenge in soybean production is Phytophthora root and stem rot of soybean caused by the oomycete Phytophthora sojae. With highly complex field populations of P. sojae, no single deployed pathotype-specifc Rps (Resistance to Phytophthora sojae) gene confers resistance to all P. sojae isolates. Quantitative disease resistance (QDR) toward P. sojae is generally considered to be non-pathotype specific and, in combination with Rps-genes, has provided durable resistance to P. sojae. A major locus for QDR towards P. sojae, QDRL-18, was identified in a population derived from a cross with the resistant plant introduction 427105B. This locus and its candidate gene, Glyma.18g026900 which is predicted to encode a serine-threonine kinase, have the hallmarks of being a susceptibility gene. These hallmarks include apparent recessive gene action, decreased expression in resistant cultivars, and resistance associated with disruption of the predicted active site potentially. While these features are consistent with those of a susceptibility gene, functional validation of Glyma.18g026900 is still ongoing. Interestingly, evidence of positive selection acting on Glyma.18g026900 indicates that the gene may have adaptive roles in certain environments. This raises the possibility that the resistant allele, or loss-of-function variants, could be selected against under specific conditions. These findings highlight the potential for negative pleiotropic effects, which are common when susceptibility genes are disrupted. Further investigation is needed to fully understand the broader functional implications of inactivating Glyma.18g026900. Thus, while Glyma.18g026900 represents a promising candidate gene and potential genome editing target to improve resistance to P. sojae, additional functional assays are needed to clarify its precise role in disease susceptibility and broader plant performance.

Poster Presentation #101

Exploration of Fast Neutron Breeding Pipeline in Soybean

Cheryl Adeva[1], *Francia Seconde Ravelombola*[1], *Maiara de Oliveira*[1], *Jessica Argenta*[1], *Harmeet Singh*[1], *Jenish Simon*[1], *Jaqueline Lopes Rodrigues*[1], *Destiny Hunt*[1], *Feng Lin*[1]

[1]University of Missouri-Fisher Delta Research, Extension, and Education Center

Mutation breeding has a well-recognized history in the advancement of soybean varieties. This approach not only aids in the identification of new beneficial genes but also reduces the time required to develop new varieties with improved traits. The Soybean Breeding Group at the University of Missouri-Fisher Delta Research, Extension, and Education Center (MU-FDREEC) in Portageville, Missouri, plays an important role in advancing research in the field of soybean. Our program aims on establishing a mutation breeding pipeline by deploying the fast neutron mutagenesis technique, with a long-term goal of developing, selecting, and releasing mutant soybean lines that possess elite genetic backgrounds. In 2025, we used two of our elite soybean varieties, S19-10701 and S19-12537, which were exposed to a controlled fast neutron irradiation at a dosage of 30 Gray (Gy) units. The 10,000 M0 seeds are planted and grown at the MU-FDREEC and M0 lines will be selected and determined through field observations. The selection of M2:1 lines to M2:5 lines will be determined based on their seed yield performance as well as their agronomic and seed composition traits through yield trials. By the end of the project, the selected mutant lines are anticipated to exhibit improvements in one or more traits such as seed yield, seed composition, disease resistance, and resilience to abiotic stresses. We anticipate a significant reduction of years in releasing new and improved soybean lines through the mutation breeding pipeline.

Poster Presentation #102

Genomic and biochemical comparison of allelic triple-mutant lines derived from conventional breeding and multiplex gene editing

Junqi Liu[1], Ritesh Kumar[1], Samatha Gunapati[1,2], Steven Mulkey[1], Yinjie Qiu[1,3], Yer Xiong[1], Vishnu Ramasubramanian[1], Jean-Michel Michno[1], Praveen Awasthi[1], Daniel D. Gallaher[4], Thi Thao Nguyen[5], Won-Seok Kim[6], Hari B. Krishnan[6,7], Aaron J. Lorenz[1], Robert M. Stupar[1]

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Multiplex gene editing allows for the simultaneous targeting and mutagenesis of multiple loci in a genome. This tool is particularly valuable for plant genetic improvement, as plant genomes often require mutations at multiple loci to confer useful and/or novel traits. However, the regulation of gene editing can vary depending on the number of loci targeted. In this study, we developed triple-mutant soybean (Glycine max (L.) Merrill) lines using different crop improvement strategies, including conventional backcross breeding of standing variant alleles and clustered regularly interspaced short palindromic repeats-based multiplex editing to introduce new alleles. The mutations were targeted to genes encoding seed antinutritional components, as previously described in a triple null soybean carrying knockout alleles for a Kunitz trypsin inhibitor, a soybean agglutinin, and the allergen P34 protein. The products developed from these respective genetic improvement pipelines were tested for differences between the triple-mutant lines and their parental lines. Analyses included genomics, seed proteomics, trypsin inhibition, seed protein digestibility, and harvestable yield of the different lines. We observed that both multiplex gene editing and conventional breeding approaches produced essentially equivalent products in comparison to their parental lines. We conclude that the multiplex gene editing strategy is not inherently riskier than conventional breeding for developing complex mutant lines of this type.

Poster Presentation #103

UNL PTCRF Soybean Transformation and Genome Editing: lab-to-field trait development

Ming Guo[1,3], Truyen Quach[1,2,3], Hanh Nguyen[1,3], Zhengxiang Ge[1,3], Olivia Meyer[1,3], Nathaniel Butler[1,2,3]

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Since the first transgenic soybean plant was generated in 1988, soy has become the leading model crop among dicot species with trait development being the primary focus of genetic engineering work. Important technical hurdles have been overcome over the years in developing current soybean transformation protocols regarding Agrobacterium recalcitrance and regenerable starting material. However, throughput and achieving consistent efficiencies among elite germplasm, in addition to the ability to evaluate engineered events in regulated field locations, present formidable barriers to new trait development and accessibility of modern genetic engineering technologies to academic, government and industry research groups.

The PTCRF has operated since 1996 and has been a leader in publicly available soy transformation services since its inception. The PTCRF has achieved its success through building institutional knowledge and applying this institutional knowledge towards developing and cross-training an efficient team of scientists and technicians. UNL created the PTCRF early in the development of crop genetic engineering, resulting in break-through technologies currently being licensed as Roundup Ready®XTEND and has only increased its support through providing over 500 acres of regulated trial fields across the state, a state-of-the-art SPIDERCAM® field phenotyping facility, and LemnaTec 3D Scanalyzer for high-throughput greenhouse plant phenotyping. Furthermore, the PTCRF coordinates with other states (e.g. MO, SC, GA) for multi-state regulated trials and is currently developing traits relate to protein quality, insect and nematode resistance and improving use as feedstock. In summary, the PTCRF remains a vital public resource for soybean transformation services, leveraging modern field and controlled environment phenotyping technologies and institutional knowledge towards improving soy utilization and production.

Poster Presentation #104

Introgression of drought and flood tolerance genes into four elite soybean cultivars through backcrossings

Ramesh Chethri[1], *Atit Parajuli*[1], *Iman Saha*[1], *Madan K. Bhattacharyya*[1]

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In recent years, soybean production in the United States has faced increasing threats from climate-induced stresses—particularly drought and flooding. These extreme weather events are becoming more frequent and severe, contributing to annual yield losses exceeding $1 billion. To address this challenge, we are attempting to develop soybean cultivars with improved tolerance to both drought and flood stresses. We selected four elite cultivars—MN1606 (MG I), IAR1902 (MG I.5), IAR3001 (MG III), and Spencer (MG IV) for introgessing drought and flood tolerance genes through backcrossing. These lines already possess favorable agronomic traits and disease resistance genes. PI 567690, PI 567731, PI 416937, and PI 408105A were chosen to donate quantitative trait loci (QTL) underlying drought and flood tolerance genes. We targeted six key QTL: four for drought [qSW_Gm12 (7.8%, 27%), qSW_Gm10 (20%), qSW_Gm17 (13%), qSW_Gm19 (8%)], and two for flood tolerance FTS-11 (19.7%) and FTS-13 (16.1%). Crossing and generation advancement have been carried out by the Illinois Crop Improvement Association (ICIA) in Puerto Rico, where multiple generations can be completed annually. In early generations, we applied SSR markers to identify true F1s. We have developed and applied a SNP panel created in collaboration with AgriPlex Genomics to facilitate foreground selection of the selected stress tolerance loci. Background selection will be conducted using the Soy1K panel with 1,290 SNPs to retain most of the elite genomes while minimizing undesirable DNA linked to the stress tolerance genes. To accomplish our goal, we are conducting four backcrossing programs for each of the four recurrent parents to introgress four drought and two flood tolerance QTL. Following two backcrossings, the BC2F1s are crossed to pyramid the six stress tolerance genes into two F1s, which will subsequently be crossed to stack all six-stress tolerance QTL into each of the four elite lines.

Poster Presentation #105

Genomic and transcriptomic data to identify causal variants of aphid resistance in soybean

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Soybean aphid (*Aphis glycines*) is one of the most significant pests for soybean production in the U.S., and the use of aphid-resistant soybean varieties help reduce damages caused by this pest. Several *Resistance to Aphis glycines* (*Rag*) loci have been identified in soybean, and progress has been made towards fine mapping some of these loci. To identify causal genes or variants for this important trait, we have generated PacBio HiFi long-reads and Illumina short-reads whole-genome sequencing data for resistant and susceptible backcross soybean lines. We have generated assemblies for genotypes used in this study. However, several genetic variants, including single nucleotide polymorphisms and structural variants, were identified in the fine-mapped regions of these *Rag* loci, making candidate gene identification difficult. The ongoing RNA sequencing in resistant and susceptible backcross lines will assist in *de novo* gene annotation in those assemblies and help to identify candidate *Rag* genes/variants.

Poster Presentation #106

A comprehensive survey of genomic variation in soybean through resequencing and pangenome construction

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Genomic variation drives phenotypic diversity, including traits associated with plant adaptation and domestication. While single-nucleotide polymorphisms (SNPs) have been extensively studied due to their high frequency and ease of detection, structural variations (SVs) remain underexplored due to detection challenges and their relatively low frequency. To comprehensively characterize genomic variation in soybean (*Glycine max*), we analyzed two complementary datasets. First, we compiled whole-genome sequencing data for 2,737 globally distributed soybean accessions from previous studies. After filtering accessions based on sequence quality, coverage depth, read length, and mapping quality, variant calling was performed on the remaining 2,589 accessions, identifying approximately 62.3 million SNPs and 10.4 million indels. Next, to capture complex structural variation and identify genomic regions absent from the reference genome, we constructed a soybean pangenome using *de novo* assemblies of 12 genetically diverse accessions with HiFi long-read sequencing. Gene and genome assemblies showed over 98% BUSCO completeness. Each assembly was aligned to the Williams 82 reference to define core and variable genomic regions, revealing approximately 5.3 million SNPs and 96 thousand structural variants longer than 50 bp. Notably, we identified a 3-kb insertion in *CHX1*, linked to salt tolerance, and a 15-kb deletion in *E3*, involved in flowering time regulation, in multiple accessions. CNV analysis revealed copy number variation at the *Rhg1* and *GA2ox8* loci, associated with resistance to cyst nematode (*Heterodera glycines*) and plant height, respectively. Our combined approach enhances our understanding of genomic diversity in soybean and provides a foundation for linking genetic variation to agronomic traits.

Poster Presentation #107

Multi-Environment Performance of Genomic Selection Models in Soybean Breeding

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Genomic selection (GS) is a key tool in modern plant breeding, enabling the prediction of complex traits using molecular marker data. This study evaluated the predictive performance of two GS models—Bayesian Ridge Regression (BRR) and Reproducing Kernel Hilbert Spaces (RKHS)—using data from the University of Missouri's soybean breeding program. The dataset comprised 765 genotypes evaluated over five years (2017–2021) in advanced yield trials (AYT) and genotyped with a 6K SNP chip. Two cross-validation schemes were tested: CV1, simulating the prediction of untested genotypes across environments, and CV2, predicting genotype performance in environments where they were not observed. Predictive ability was assessed using the Pearson correlation between observed and predicted values across five folds. In CV1, both models showed low accuracy with similar performance, though RKHS yielded a slight improvement. In CV2, where partial genotype-environment data was available, RKHS outperformed BRR modestly, with average correlations of approximately 0.52 versus 0.50. These results suggest RKHS may better model non-linear genotype-by-environment interactions under partially observed conditions. Variance component analysis indicated that RKHS explained a greater proportion of phenotypic variance through genomic (G) and interaction (G×E) effects than BRR, reflecting enhanced model flexibility. Overall, RKHS provided slightly more robust predictions in multi-environment scenarios. Future work will expand this framework by incorporating machine learning models, environmental covariates, and drone-derived phenotypes to further enhance predictive accuracy in soybean breeding.

Poster Presentation #108

Identifying Virulence Loci in Soybean Cyst Nematode

Lauren Docherty[1], *Vishnu Ramasubramanian*[1], *Cory Hirsch*[1], *Aaron Lorenz*[1], *Senyu Chen*[1]

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Soybean cyst nematode (SCN) (*Heterodera glycines*) is a major pest affecting soybean and is primarily controlled by growing resistant cultivars. In both field and greenhouse experiments, SCN populations have demonstrated the ability to overcome known resistance loci in soybean. While significant work has been done to identify and characterize additional resistance loci in soybean, relatively little is known about SCN's genome. Field populations of SCN are very heterogeneous and individuals are microscopic, making it difficult to extract sufficient DNA for genomic analysis. To overcome these challenges, 178 inbred lines of SCN were developed for this research. These nematodes were originally collected from field populations and inbred using single-cyst descent for at least 10 generations. Following these 10 generations of full-sibling mating, the lines are on average 87% homozygous. The genome of each line was sequenced to an average depth of 40x, aligned to the TN7 reference genome, and variants were identified. The lines were also phenotyped for their virulence on six soybean indicator lines with diverse sources of resistance: Pickett (PI 548988), Peking (PI 548402), PI 88788, PI 90763, PI 438489B, and PI 567516C. A phylogenetic tree, principal component analysis, and admixture analysis suggest little population structure among the lines. However, the virulence phenotyping results show significant diversity is present. A genome-wide association analysis was conducted to identify loci associated with virulence on the six tested indicator lines. Sixteen variants were identified, and all but one were uniquely associated with a single indicator line. The results of this project could be used to develop a molecular test for virulence in SCN populations, allowing for more efficient tracking of virulence shifts across landscapes. They also provide insights into SCN's virulence mechanisms and can inform breeding efforts for more durable soybean resistance.

Poster Presentation #109

Soybean resistance to southern root-knot nematode reduces nematode population density under field conditions

Emanuel Ferrari do Nascimento[1], *Andrea Acuña*[1], *Mariola Usovsky*[2], *Amanda Pominville*[3], *Travis Faske*[1], *Zenglu Li*[4], *Melissa G. Mitchum*[4], *Henry T. Nguyen*[2], *Grover Shannon*[2], *Caio Canella Vieira*[1]

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The southern root-knot nematode [SRKN, *Meloidogyne incognita* (Kofoid & White) Chitwood] is one of the most yield-limiting soybean pathogens worldwide. With limited control options, genetic resistance remains the primary management strategy. Suppressing nematode population density through the use of resistant cultivars is essential to mitigate damage in subsequent host crops. This study aims to evaluate the effectiveness of a major quantitative trait locus (QTL) on chromosome 10 in reducing SRKN population density under field conditions. A total of 76 advanced breeding lines from maturity groups (MG) IV and V were assessed. Based on the presence of the major QTL on chromosome 10, 38 breeding lines were classified as resistant and 38 as susceptible to SRKN. Field experiments were conducted in 2018 and 2019 using a randomized complete block design (RCBD) with three replications under high nematode pressure. Soil samples were collected from the two center rows of each four-row plot for nematode extraction and quantification. Nematode population density was expressed as the number of second-stage juveniles (J2) per 100 cm³ of soil. Over the two-year study, SRKN-resistant breeding lines had, on average, 1,620 SRKN-J2 per 100 cm³ of soil, while the susceptible group averaged 2,126 J2 per 100 cm³ of soil (p-value < 0.001). However, in 2019, prolonged off-target dicamba exposure significantly increased nematode density across all lines. Dicamba-tolerant breeding lines consistently exhibited lower SRKN-J2 densities than dicamba-susceptible lines, regardless of their SRKN resistance. Therefore, soybean resistance suppresses nematode reproduction, allowing effective crop rotation management. However, prolonged off-target dicamba exposure imposes additional stress that may compromise the plant's defense response to SRKN, leading to increased nematode density in the soil.

Poster Presentation #110

Discovering *cqSCN-007*, a Novel Type of Resistance to Soybean Cyst Nematode

Susana Ferrer Orgaz[1], *Dr. Jeysika Zayas-Rivera, Dr. Andrew Bent*[1]

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Soybean cyst nematode (SCN) is by far the most damaging disease of soybean in the U.S. Although genetic resistance is the most effective strategy against SCN, most commercially grown soybeans rely on single-locus *rhg1-b* resistance that is losing efficacy as SCN populations slowly evolve. Wild ancestors of domesticated plants offer naturally occurring new alleles. One example is *cqSCN-007*, derived from *G. soja* PI 468916, which confers quantitative but substantial resistance to various SCN populations, and is additive with *rhg1-a* or *rhg1-b*. Brian Diers and colleagues introgressed *cqSCN-007* into elite soybean genotypes and delimited the locus to an 11-gene genetic interval. The genes within this genetic interval do not include obvious candidates based on their annotation or polymorphisms between resistant and susceptible haplotypes. Initial efforts to identify the functional gene via RNAi or CRISPR knockouts in detached transgenic roots were impeded by undetectable resistance in transgenic root petri plate assays. To identify the causal gene, we are now conducting reverse genetics using composite plants (transgenic roots on non-transgenic shoots) and investing in the generation of transgenic soybean lines. We are also identifying SCN populations and resistance combinations that most strongly reveal the quantitative *cqSCN-007* resistance phenotype. Determining the causal gene(s) will facilitate breeding for this QTL, new allele discovery and genetic engineering for improved disease control of this major agricultural disease.

Poster Presentation #111

Investigating Genotype by Environment Dynamics in Soybean Iron Deficiency Chlorosis

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Iron deficiency chlorosis (IDC) is a nutritional deficiency common in the Upper Midwest, primarily caused by high pH and calcareous soils. While these soil conditions are known to trigger IDC in soybeans, the timing and severity of IDC onset can vary from year to year, even within the same field. We grew plants in field conditions prone to IDC in 8 locations across 3 states. In our analysis, we began by correlating various soil properties to IDC scores across locations as well as different resistant and susceptible genotypes. We also found a best-fit model leveraging soil and weather data to predict IDC scores across our locations. Based on preliminary results, we are seeing a real and pervasive genotype by environment interaction in IDC and beginning to understand how different environmental factors interact to create IDC symptoms. These findings provide valuable insights for breeding programs aimed at developing IDC-resilient soybean varieties.

Poster Presentation #112

Targeted editing of *GmSNAP14* enhances the soybean cyst nematode resistance in Soybean

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Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most destructive soybean pathogen, causing annual yield losses exceeding $1.5 billion in the United States. SCN management primarily relies on resistant cultivars and crop rotation with non-host species.

Most commercial soybean cultivars derive resistance from two major sources: PI 88788, which requires the *rhg1-b* allele on chromosome 18, and Peking, which depends on epistatic interactions among *rhg1-a* (*GmSNAP18*) on chromosome 18, *Rhg4* (*GmSHMT08*) on chromosome 8, and *rhg2* (*GmSNAP11*) on chromosome 11. However, unlike Peking, which is moderately resistant to SCN HG type 1.2.5.7 (Race 2), the soybean cv. Pickett, which derived this three-gene combination from Peking, is susceptible to SCN type 1.2.5.7. To investigate this, a bi-parental mapping population derived from a cross between Peking and Pickett was developed and used for mapping studies. Fine mapping led to the identification of *GmSNAP14* on chromosome 14 as a candidate gene contributing to SCN HG type 1.2.5.7 resistance in conjunction with *GmSNAP18* and *GmSNAP11*. Additionally, GWAS quantitative trait nucleotides (QTNs) associated with SCN resistance on chromosome 14 determined *GmSNAP14* as a candidate gene due to its proximity with the QTN. *GmSNAP14* on chromosome 14 is one of five soybean *α-SNAP* genes and is a close paralog of *GmSNAP02* sharing high sequence similarity. Recently, *GmSNAP02* on chromosome 2 was identified as a novel contributor to resistance to SCN type 1.2.5.7 through a loss-of-function mechanism in PI 90763 and PI 437654. Like *GmSNAP02*, whole-genome re-sequencing revealed multiple *GmSNAP14* alleles harboring structural variations in the form of insertions and deletions. To further assess the function of *GmSNAP14*, a CRISPR-Cas9 gene-editing approach was employed to knock out *GmSNAP14* in a susceptible background similar to Pickett. Knocking out *GmSNAP14* enhanced resistance to SCN HG type 1.2.5.7, demonstrating its utility for developing soybeans with broader resistance to SCN.

Poster Presentation #113

Editing of the of the gene model underlying the major protein quantitative trait loci (QTL), cqSeed protein-003, and its paralog in soybean

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A major seed protein quantitative trait locus (QTL) in soybean resides on chromosome 20, cqSeed protein-003, which was recently mapped to gene model Glyma.20G085100 (*Gm20P*), with its paralog Glyma.10G134400 (*Gm10P*) on chromosome 10. To gain insight into how Gm20P and Gm10P influence seed reserve content in the crop, genome editing reagents were designed to create null mutations in both *Gm20P* and *Gm10P*. Two soybean lines were identified and characterized, possessing INDELs in *Gm10P* and *Gm20P*. One is a *gm20p* mutant, and the other is a *gm10p gm20p* double mutant. Seed protein content of the *gm10p gm20p* double mutant was significantly lower relative to the *gm20p* mutant and wildtype, with a concomitant increase in oil and starch content. A delayed maturity phenotype was observed in the single and dual edited lines under field settings across multiple environments. The delay in maturity phenotype was influenced by day length, wherein under a short-day environment, the delay mirrored what was observed under field conditions, while under a long-day environment, no delay in maturity was observed, but changes in growth were detected in the double mutant line. Transcriptomic analysis revealed repression of genes involved in light signaling, and enhanced expression of JAZs alleles that repress JA signaling, suppression of ABA-responsive genes, senescence-related genes, and elevation of genes that inhibit IAA signaling in the double mutant. The status of a wide range of downstream transporter genes for transport of hormones, metabolites, heavy metals, and essential nutrients, nitrogen (N) and phosphorus (P), was significantly reformed as a result of altered interactions of light-phytohormone signaling in the double mutant. Furthermore, the double mutant displayed higher sensitivity to salt and ABA during germination. These results suggest that both *Gm10P* and *Gm20P* alleles modulate light signaling, which in turn impact soybean development, maturity and seed reserve content.

Poster Presentation #114

Association Mapping and Genomic Prediction of Mid-Season Flood Tolerance in Soybean

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Flooding stress is an increasingly critical constraint to soybean (*Glycine max* [L.] Merr.) production due to the rising frequency and severity of extreme precipitation events. While previous studies have identified genomic regions associated with mid-season flood tolerance, a substantial portion of phenotypic variance remains unexplained. This study aimed to identify additional genomic regions contributing to flood tolerance and assess the utility of machine learning for genomic prediction. A panel of 281 soybean plant introductions (PIs) was evaluated over three growing seasons at two locations, using controlled flooding imposed during the early reproductive (R1–R3) growth stages for 8–10 days. Phenotypic response was quantified as foliar damage score (FDS) using a 0–9 scale, where 0 indicated minimal damage and 9 indicated severe necrosis or death. FDS evaluations were conducted seven days after floodwater removal. A genome-wide association study (GWAS) using the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) model identified significant loci on chromosomes 3 and 19 (LOD ≥ 6). SNP ss715585205 on chromosome 3 was newly identified, while ss715635284 on chromosome 19 had been previously associated with flood tolerance during germination. For genomic prediction, a Random Forest (RF) model was implemented using markers from the SoySNP50K iSelect BeadChip array. Predictors (markers), including the number of trees (ntree) and variables sampled per split (mtry), were optimized through cross-validation, and final predictions were derived by model averaging across iterations. After feature selection, the RF model achieved a prediction accuracy of 0.82 using 62 SNPs, effectively reducing multicollinearity and overfitting. These findings validate the efficacy of machine learning for predicting abiotic stress tolerance in soybean and demonstrate the potential of integrating genomic selection tools into breeding pipelines. This study provides a practical framework for accelerating the development of season-long, flood-tolerant soybean cultivars through data-driven breeding strategies.

Poster Presentation #115

Determine the cell-type-specific role of membrane nanodomain-associated proteins in the infection processes of nodule formation

Md Sabbir Hossain[1], Sandra Thibivilliers[1], Sahand Amini[1], Sergio A C Perez[2], Marc Libault[1]

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Legumes have the unique ability to establish symbiosis with rhizobia, enabling atmospheric nitrogen fixation within specialized root organs known as nodules. This interaction begins with rhizobial infection of root hair cells, which requires the formation of an infection thread. A critical early step in this process is the invagination of the plasma membrane that requires membrane nanodomain-associated proteins (MNAPs) such as FW2.2-LIKEs (FWLs), flotillins (FLOTs), prohibitins (PHBs), and remorins (REMs). Using state-of-the-art single-cell transcriptomics technology, we identified eight MNAPs (i.e. two GmFWLs, one GmFLOT, two GmPHBs, and three GmREMs) that are highly and specifically expressed in rhizobia-infected cells of soybean nodules. Spatial transcriptomics further validated the specific expression of *GmFLOT*, *GmPHB1*, and *GmREM1* in the rhizobia-infected cells of the nodule. Similarly, single-cell analysis in *Medicago truncatula* nodules also revealed the preferential expression of MNAP genes in a subpopulation of the infected cells of the Medicago nodule, suggesting a conserved role of MNAPs during legume nodulation. We hypothesize that multiple MNAPs redundantly work together to control legume-rhizobia interaction and that the co-expression of these MNAP genes is under the control of a few key transcription factors. To test these hypotheses, we are performing cell-type-specific functional genomic assays using CRISPR-Cas9, protein–protein interaction assays, and promoter motif analyses. This work provides mechanistic insights into how MNAPs contribute to membrane signaling and remodeling during legume–rhizobia symbiosis and opens new avenues to further understand the nodulation process and improve nitrogen fixation efficiency in legumes.

Poster Presentation #116

Integrative omics analysis elucidates the genetic basis underlying seed weight and oil content in soybean

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Synergistic optimization of key agronomic traits by traditional breeding has dramatically enhanced crop productivity in the past decades. However, the genetic basis underlying coordinated regulation of yield- and quality-related traits remains poorly understood. Here, we dissected the genetic architectures of seed weight and oil content by combining genome-wide association studies (GWAS) and transcriptome-wide association studies (TWAS) using 421 soybean (*Glycine max*) accessions. We identified 26 and 33 genetic loci significantly associated with seed weight and oil content by GWAS, respectively, and detected 5,276 expression quantitative trait loci (eQTLs) regulating expression of 3,347 genes based on population transcriptomes. Interestingly, a gene module (IC79), regulated by two eQTL hotspots, exhibited significant correlation with both seed weigh and oil content. Twenty-two candidate causal genes for seed traits were further prioritized by TWAS, including *Regulator of Weight and Oil of Seed 1* (*GmRWOS1*), which encodes a sodium pump protein. GmRWOS1 was verified to pleiotropically regulate seed weight and oil content by gene knockout and overexpression. Notably, allelic variations of *GmRWOS1* were strongly selected during domestication of soybean. This study uncovers the genetic basis and network underlying regulation of seed weight and oil content in soybean and provides a valuable resource for improving soybean yield and quality by molecular breeding.

Poster Presentation #117

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

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Aquaculture production depends heavily on marine-derived inputs such as eicosapentaenoic acid (EPA), a long-chain omega-3 polyunsaturated fatty acid (LC-PUFA), and the ketocarotenoid astaxanthin. These compounds are essential for fish growth, pigmentation, reproductive health, and nutritional value for human consumers. Marine fish and fish oil currently provide the main sources of LC-PUFA (EPA) 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 engineered soybean (Glycine max) seeds to biosynthesize EPA, astaxanthin, and tocotrienols through coordinated expression of heterologous genes from algae, moss, and flowering plants. The resulting transgenic soybean line (pPTN1331) accumulated up to 3.8% EPA of total seed fatty acids, 13 µg/g dry weight of astaxanthin, and 1,300 µg/g tocotrienols in multi-year field trials. Astaxanthin accumulation produced a distinctive pink seed phenotype, facilitating visual selection. Despite successful metabolite production, early-generation seeds displayed reduced oil content, abnormal wrinkled morphology, low germination rates, and markedly decreased abscisic acid (ABA) levels. Transcriptome profiling revealed downregulation of photosynthesis-related genes, suggesting that broad metabolic reprogramming contributed to these phenotypes. To improve EPA accumulation and seed quality, pPTN1331 was crossed with a high-α-linolenic acid (ALA) soybean. The resulting hybrid lines exhibited restored seed morphology, higher germination, and enhanced EPA content, reaching up to 14% of total fatty acids, while maintaining ketocarotenoid and tocotrienol accumulation. This work highlights the feasibility and complexity of engineering multiple high-value pathways into a major oilseed crop. The resulting soybean lines represent a sustainable, scalable terrestrial platform for producing omega-3 fatty acids and antioxidants, offering a promising alternative to marine-derived aquafeed components.

Poster Presentation #118

Development of a Reduced-Allergen Soybean Line and Preclinical Validation of Anti-Allergenicity

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Soybean(*Glycine Max* L.) is an important source of plant-based protein; however, its use in allergy-sensitive populations is limited by the presence of intrinsic allergenic proteins such as Kunitz Trypsin Inhibitor (KTI) and P34, while odor-associated enzymes like lipoxygenases contribute to undesirable flavor characteristics. In an effort to mitigate these challenges, a novel soybean line (SS line) was developed through targeted breeding aimed at eliminating both allergen- and odor-related components. Genetic and protein analyses confirmed reduced mRNA expression of allergen-associated genes and the absence of lipoxygenase and CG-1 proteins in two selected lines, validating their hypoallergenic and odor-reduced genotypes. To evaluate the preclinical anti-allergenic potential of the SS line, in vivo experiments were conducted using BALB/c mice sensitized with soybean proteins. Compared to wild-type controls, the SS protein group exhibited significantly lower levels of allergen-specific IgE and did not elicit allergic responses upon antigenic boosting. Moreover, antibody isotype profiling revealed a Th1-biased immune response (IgG2a > IgG1), indicating a reduced tendency toward Th2-mediated allergic inflammation. These results clearly demonstrate that the SS soybean line effectively suppresses allergic responses at both molecular and immunological levels. Supported by a project grant from "Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of soybean varieties adapted to the central and northern regions and customized to meet consumer needs(Step 2), Project No. PJ01722401)" Rural Development Administration, Republic of Korea.

Poster Presentation #119

Identifying regulatory elements that control *Rhg1* α-SNAP abundance in SCN resistance

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Soybean cyst nematode is by far the most economically damaging soybean pathogen causing the highest yield loss in the US. Genetic resistance conferred by the PI88788 type *rhg1-b* haplotype has provided multi-year control of SCN on millions of acres. However, co-evolving SCN populations are gradually overcoming *rhg1-b*, motivating scientists and plant breeders to develop improved SCN resistance. Our laboratory previously showed that α-$SNAP_{Rhg1}$ proteins (also known as GmSNAP18) encoded by *rhg1-b* contribute to resistance. We also found that α-$SNAP_{Rhg1}$ proteins specifically hyperaccumulate within syncytia, the plant cells altered by SCN effectors to become a multi-week feeding site for the nematode. The present study is identifying how the syncytial abundance α-$SNAP_{Rhg1}$ protein is upregulated. A primary current focus is on dissecting the promoter region of *Glyma.18G022500* to identify cis-regulatory elements relevant to syncytial expression. We have developed methods to assay the activity of modified promoter segments in specific soybean root cells. Leveraging confocal microscopy with two different fluorescent markers, this method is revealing both negative and positive regulatory phenomena. Understanding how SCN infection causes α-$SNAP_{Rhg1}$ protein abundance increases may reveal strategies for future improvement of soybean resistance to SCN.

Poster Presentation #120

Multi-Locus GWAS Mapping Kudzu Bug Resistance in Soybean (Glycine max L. Merr.)

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Soybean (*Glycine max* [L.] Merr.) is an important crop in the United States due to its high economic value. Primarily attributed to its rich protein and oil content, soybean is a vital source of nutrition for both human consumption and animal feed. However, soybean plants suffer significant damage from insect pests, leading to substantial yield losses. To investigate genetic resistance to kudzu bugs, an invasive pest in soybean, a multi-locus genome-wide association study (GWAS) was conducted using a mapping panel of 169 soybean accessions. Our study identified 17 quantitative trait nucleotides (QTNs) significantly associated with insect resistance, distributed across 13 chromosomes. These QTNs explained 0.7-34% of phenotypic variation, as determined using the R package mrMLM (v4.0). The QTNs on chromosomes 4, 10, 11, 12, 14, and 20 were consistently identified by multiple models using two years of assessment data. Notably, three QTNs associated with kudzu bug resistance were consistently identified using five models on chromosome 4, including mrMLM (2), FASTmrMLM (2), ISIS EM-BLASSO (2), pLARmEB (2), and FASTmrEMMA (1). These QTNs explained phenotypic variation up to 17.63% across both years using the multi-locus GWAS approach. Additionally, the QTN ss715612709 on chromosome 12 was associated with resistance and explained the phenotypic variation up to 33% in 2023 in three models. This QTN is co-located with a previously identified quantitative trait locus (QTL) anchored by SSR markers Sat_118, Satt442, Sat_175, and Satt334. Similarly, significant QTN on chromosome 18, ss715632177, was associated with kudzu bug resistance and shared the same genomic interval of a reported QTL flanked by SSR markers Satt472 and Satt191. In summary, this study provides valuable insights into the genetic basis of soybean resistance to kudzu bugs. The identified QTNs can be used developing new soybean varieties with improved pest resistance.

Poster Presentation #121

Screening of advanced high-yield MU-FDREEC soybean lines for resistance against Stink Bugs Complex

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The United States is the world's second-leading soybean producer and exporter. Several stink bug species can reduce soybean yield and seed quality. While insecticides are the most used control method, soybean resistant lines can be an alternative solution for managing stink bugs. This study aimed to screen advanced high-yield lines from the University of Missouri – Fisher Delta Research Extension and Education Center (FDREEC) under natural stink bug infestation and to identify potential lines with resistance to stink bugs for further molecular studies and cultivar development. Plots were established at Louisiana State University in the 2024 growing season. Thirty-one lines were selected for this study, representing the most advanced yield trials from the breeding program. The experiments were conducted in a 2-row plot with 30 inches between rows and four replications. Each line was harvested, and a random sample of pods was hand-shelled, and individual seeds were separated into one of four damage categories (seeds with no damage, one or more punctured but no shriveling, punctured seeds with some shriveling, and punctured with extensive shriveling of the seed coat). Then, a damage index was calculated for each line. All data were subjected to analysis of variance (ANOVA) using R software. The F-test was conducted, and significant means were separated into groups using Duncan's test (5%). The ANOVA analysis revealed significant differences in the Stink Bug Damage Index across the different lines. S22-24339, S21-11972 HP, S21-17588LL55, and S20-1541GT demonstrated potential tolerance or resistance to stink bug injury. On the other hand, lines S20-25654, S20-13179LL55, S20-14129GT, and S20-5669 appeared to be less resistant according to the damage index. Soybean lines with a low damage index will be used for crossing in the breeding program. Additionally, studies aiming to enhance our understanding of the interactions between soybeans and stink bugs will be performed.

Poster Presentation #122

Genetic Mapping of Novel QTL for Seed Protein Stability in Food Grade Soybean (*Glycine max*)

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Soybean seed protein content is a complex physiological trait under polygenic control and significant genotype by environment interaction. Protein content is largely influenced by ambient atmospheric temperature at pod-filling, with increased temperatures enhancing seed protein accumulation. The identification of genomic regions associated with protein content stability will facilitate an increased understanding of seed development physiology and assist in the development of more broadly adapted food-grade soybean cultivars. In this work, 210 recombinant inbred lines were derived from the intraspecific cross of the high protein accession BARC-6 (PI 555396), and the low protein MSU breeding accession E14077 for the investigation of quantitative trait loci associated with protein content and protein content stability across multiple years and test locations. Indices for static protein content stability were used to estimate genome by environment interactions across Northern and Southern soybean production regions. Composite interval mapping returned one stable major effect QTL associated with protein content on chromosome 20 explaining approximately 20.7% of phenotypic variation. Two novel QTL associated with absolute protein stability were detected on chromosomes 10 and 18, explaining approximately 8.6% and 7.6% of phenotypic variation, respectively. SNP-based haplotype analysis showed simultaneous favorable effects on protein content and stability when desirable alleles for these QTL were pyramided. These results serve as an initial proof-of-concept for the molecular breeding of broadly adapted food-grade soybean cultivars and provide QTLs for deployment within existing elite high-protein cultivars.

Poster Presentation #123

Assessing Fungal Synthetic Community Function in Soybean Growth and Drought Stress Adaptation

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Beneficial mycorrhizal fungi may improve crop stress tolerance through soil carbon storage, water and nutrient acquisition, and stress mitigation. The goal is to build a synthetic fungal community (FunSynCom) to improve growth and drought resilience in soybeans. In this study, we isolated, identified, and characterized a set of twenty fungal endophytes isolated from the rhizosphere of halophytic plants during low tidal summer with exponentially reduced soil moisture levels. Individual strains were screened via seed germination and plant growth assays. Phenotypic data were collected across two-week growth cycles, including biomass, shoot: root ratio, and photochemical measurements. Extremophile endophytic fungal strains such as Sclerostagonospora lathyri (F3) and Chaetosphaeronema sp. (F15) improved biomass and root development. Tremateia chromolaenae (F7) treated plants had significantly higher biomass under both normal and drought conditions ($P < 0.05$). In addition, we assessed these strains for organic acid analysis and extra-cellular enzyme assays (phosphatase and glucosidase). Based on the criteria of strains producing beneficial substances, improving plant biomass, and showing drought stress resistance (10% soil moisture), we designed FunSynCom. This comprised top-performing strains F3, F7, F15, F25, and F36 (Lophiostoma terricola), which were combined with prior fungal growth compatibility tests. Soybean plants were inoculated with FunSynCom and individual strains and placed under drought stress. The results showed that FunSynCom-treated plants maintained a significantly better growth trend and biomass than controls during drought, with F7-treated plants displaying the most significant drought resilience. Future work will incorporate transcriptomic and root exudate analyses to define host-microbe interactions and improve fungal synthetic community composition.

Poster Presentation #124

Pathogenicity Evaluation and High-Throughput Molecular Detection of Calonectria ilicicola in Soybean Using an HRM Marker

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Calonectria ilicicola, an emerging pathogen, causes red crown rot (RCR) in soybean and affects multiple crop species. However, the pathogenic potential of other Calonectria species in soybean remains poorly understood, and molecular diagnostic methods for this pathogen are limited. This study addresses both challenges by evaluating the pathogenicity of multiple Calonectria species and developing molecular tools for rapid C. ilicicola detection. Sixteen Calonectria isolates representing 11 species were phylogenetically analyzed and tested for their ability to cause disease symptoms on soybean seedlings using clamshell assays. Preliminary results identified five isolates as pathogenic to soybean. The pathogenicity of these five isolates was further evaluated using a growth chamber mycelial plug assay. Three Calonectria species induced disease symptoms similar to those caused by C. ilicicola, while two others produced mild RCR symptoms, underscoring the potential for misdiagnosis based on visual symptoms alone. A genetic analysis of over 200 Calonectria species and 250 C. ilicicola isolates in the NCBI database identified unique sequences in the TEF1-α gene. A high-resolution melting (HRM) marker targeting the TEF1-α region was developed and tested on multiple fungal species known to be pathogens of soybean and related plants. All C. ilicicola isolates showed strong amplification with distinct T_m values and melting profiles, whereas most non-ilicicola and unrelated fungi showed late or no amplification with divergent melt patterns, demonstrating the marker's specificity and diagnostic utility for C. ilicicola. To enable field diagnostics, multiple crude DNA extraction protocols are being evaluated to develop a method for direct DNA extraction from infected soybean tissues for molecular marker assays. These findings will support the development of high-throughput molecular detection methods for C. ilicicola from field samples, enabling rapid, culture-independent diagnostics.

Poster Presentation #125

Development of SNP markers for the identification of soybean varieties

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Soybean is an important crop used worldwide for food, feed, and industrial purposes, leading to the development and breeding of numerous cultivars. However, morphological characteristics alone are often insufficient to clearly distinguish between cultivars or determine their origins. Therefore, the use of molecular markers is essential for accurate identification. Among various types of molecular markers, single nucleotide polymorphisms (SNPs) are particularly efficient due to their abundance across the genome, stable inheritance across generations, and compatibility with high-throughput genotyping. In this study, we developed a set of SNP-based molecular markers capable of identifying soybean cultivars and their geographical origins using the Fluidigm platform. Based on genotypic data generated from the Axiom® SoyaSNP array, we selected approximately 1,000 linkage disequilibrium (LD) blocks spanning all 20 soybean chromosomes. From each LD block, one candidate SNP was randomly selected. These ~1,000 SNPs were able to differentiate 208 soybean accessions from five countries, including Korea. We then applied an R-based algorithm to derive a minimal set of markers, resulting in 14 marker sets composed of 18 SNPs. Finally, the discriminatory power of the selected marker sets was experimentally validated using unknown samples with the Fluidigm platform. This study presents an efficient and reliable approach for selecting molecular markers and demonstrates their utility as a practical tool for cultivar identification. These markers are expected to be applicable in future soybean breeding programs and cultivar registration processes.

Poster Presentation #126

Elevated seed oil phenotype associated with a single nucleotide polymorphism in GmNFR1α

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Soybean seed composition, particularly oil and protein content, has been a longstanding focus of research due to its economic and nutritional importance. Through forward genetic screening of an NMU-mutagenized population derived from the soybean cultivar Williams 82, a mutant line designated PID 17238 was identified for high seed oil content. This phenotype is associated with a decrease in levels of protein with respect to Williams 82. The phenotype was mapped to chromosome 2 to a region near Satt459. Fine mapping and whole-genome resequencing were used to identify the causative mutation. Analysis of the resequencing data within the candidate region uncovered 54 sequence variants. Among these, only one gene, Glyma.02G270800, contained a single nucleotide polymorphism (SNP) within coding sequence. This gene encodes a lysin motif (LysM) receptor-like kinase and has been previously reported in the literature as GmNFR1α. Importantly, this locus is allelic to the well-characterized rj1 locus, a recessive mutation known to cause a non-nodulating phenotype in soybean. Nodulation in soybean, which enables nitrogen fixation is crucial for protein synthesis in seeds and may explain the lower protein content in PID 17238.

Poster Presentation #127

Advancing Soybean Carbohydrate Profiles through Gene Editing and Next-Generation Mutation Breeding

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Soybean seed carbohydrates significantly impact its nutritional value, particularly for monogastric animals. While sucrose is readily digestible and beneficial, Raffinose Family Oligosaccharides (RFOs) including raffinose and stachyose, are antinutritional and poorly digested. Current commercial soybean cultivars lack ideal alleles for high-sucrose and low-RFO traits. The goal of this project is to develop soybean lines with >10% sucrose and reduced RFOs content to improve feed quality. We identified promising mutant lines with sucrose levels exceeding 8% in mid- to late-maturity groups. We are now stacking beneficial alleles and evaluating agronomic performance across environments. We have initiated gene-editing approaches targeting both novel and known genes to develop elite lines with superior carbohydrate profiles. The successful development of these lines will provide a nutritional and economic boost to the soybean value chain.

Poster Presentation #128

GS4PB: An R/Shiny Application to Facilitate a Genomic Selection Pipeline for Plant Breeding

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Data driven approaches will be necessary for plant breeders to drive the genetic gain required to produce more food using fewer resources, especially in the face of a changing climate. Despite the potential of genomics-assisted breeding, its application in most small- and medium-sized breeding programs is still incomplete. Several challenges and constraints, such as cost of genotyping, rapid turnaround time, and availability of trained personnel, impede adoption. One approach to facilitate the routine application of genomic selection among most such programs is the availability of a single integrated software tool capable of assisting breeders with the entire genomic prediction pipeline. To this end, we have implemented a streamlined genomic prediction and selection pipeline designed for plant breeding programs using open-source tools. The steps implemented in the pipeline include processing genotypic data (e.g., filtering and imputing genotypic data), merging genotypic and phenotypic data, collecting enviromics covariates, estimating environmental kinship, optimizing training sets, cross-validating genomic prediction models, and implementing genomic prediction for single and multiple traits across single or multiple environments. We also plan to integrate additional features for prediction of superior progeny means of all possible breeding crosses and ML/AI techniques for feature engineering of environmental covariates for improved prediction of genotype-by-environment interaction effects. This R Shiny application named “GS4PB” will help to lower entry barriers into advanced techniques of genomic prediction, enabling breeders to apply these technologies to improve genetic gain.

Poster Presentation #129

Decoding tripartite symbiotic relationships: Insights into AMF and Rhizobium Interactions for sustainable agriculture

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Increasing food demand and agriculture intensification have resulted in the increased use of chemical fertilizers, which is a leading cause of soil degradation. To meet global food demand without compromising environmental safety, it is essential to ensure sustainable agriculture. This can be achieved by leveraging the use of beneficial microorganisms. This study delves into the synergistic potential of Arbuscular mycorrhizal fungi (AMF), rhizobium, and soybean plant interactions. The mutualistic association of AMF and rhizobium with soybean roots facilitates nutrient uptake, especially Phosphorus, Nitrogen, and micronutrients, to promote plant growth under changing environmental conditions.

We have investigated the genetic basis of tripartite symbiosis using genome-wide association studies (GWAS), targeting traits related to root colonization, architecture, and biomass. GWAS was performed on over 200 diverse soybean germplasms to identify single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) associated with different traits. The GATK pipeline was utilized to assemble a comprehensive soybean genetic variation resource, leveraging whole genome sequencing data from over 500 soybean lines to identify more than 5,000,000 high-quality SNPs and indels. GWAS was conducted using phenotypic data from the studied soybean accessions and analyzed with GAPIT using the BLINK method. This analysis identified 9 significant SNP-trait associations.

Within the 200-kb flanking regions of these SNPs, 64 candidate genes were identified. Among them, two genes showed significant differential expression and are implicated in microbial colonization by sensing phosphate, cell-wall modification for AMF colonization and regulating cell division and differentiation in root cortex to facilitate rhizobium symbiosis. Additionally, two other genes are associated with carbohydrate metabolism and amino acid transporters, potentially involved in enhancing root traits that facilitate better colonization. Our findings reveal key genetic factors regulating root-microbe interactions and could lead to the development of soybean varieties with improved responses to microbial inoculation, enabling more resilient, sustainable agricultural to minimize chemical inputs.

Poster Presentation #130

Selecting Resilient Soybean Genotypes Using Multi-Trait Stability Index Under Drought Stress

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Drought stress significantly limits soybean seed yield and seed quality. This study evaluated 22 soybean genotypes including 17 breeding lines developed by the University of Missouri FDREEC and five commercial checks across three environments: Portageville, MO 2023, Clarkton, MO 2023, and Portageville, MO 2024. Trials were conducted under both rainfed and irrigated conditions using a randomized complete block design. Traits analyzed included seed yield, 100-seed weight, protein, oil, and fatty acid composition (oleic, linoleic, linolenic, palmitic, and stearic acids). A Multi-Trait Stability Index (MTSI) was used to identify genotypes with superior performance and stability across environments and treatments. Results showed significant genotype-by-environment interaction under drought conditions. MTSI analysis identified four breeding lines that ranked in the top 10% for yield stability and seed quality traits across both treatments. One line exceeded commercial checks by 12% in yield and had a 6% higher oleic acid concentration under rainfed conditions. Another line showed the most stable performance across all traits, with less than 5% variation across environments. This study demonstrates the utility of MTSI in selecting soybean genotypes that combine high yield potential and favorable seed composition under water-limited environments, contributing to the development of resilient soybean genotypes.

Poster Presentation #131

Are the Phytophthora resistance *Rps6* and *Rps13* genes allelic?

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Phytophthora root and stem rot, caused by *Phytophthora sojae*, is a serious disease that continues to threaten the soybean production system. Single *Rps* (resistance to *P. sojae*) genes provide race-specific *Phytophthora* resistance and has been successful in controlling the disease. As of today, 38 *Rps* genes have been mapped to nine chromosomes. The pathogen evolves rapidly. As a result, the life of an *Rps* gene has been estimated to be only 8-15 years. The *Rps4/6/13* region on Chromosome 18 carries six *Rps* genes. In this study, we are investigating if *Rps6* and *Rps13* are allelic or tightly linked genes. To accomplish this goal, we developed a segregating population from a cross between L89-1581 (*Rps6rps12rps13*) and PI 399036 (*rps6Rps12Rps13*). Analysis of the segregating population of 1,074 F_3 families yielded 358 informative recombinants with crossovers between two flanking indel markers spanning a region of 1,010 kb and 17.25 cM. Investigation of the recombinants should facilitate resolving tightly linked genes that are mapped at a genetic distance > 0.05 cM, assuming recombination in this region takes place randomly. Considering a 58.55 kb/cM relationship for this region, ≥ 0.05 cM is translated to ≥ 3 kb; and therefore, we should be able to test if *Rps6* and *Rps13* are allelic. We have developed a 'Soybean Disease SNP Panel' in collaboration with AgriPlex, Inc., for developing a high resolution and high-density SNP map of the *Rps6/12/13* region. The 358 recombinants are being phenotyped using three *P. sojae* isolates: Race 17 (virulent on *Rps6* and *Rps13* lines, avirulent on *Rps12* line), VI12.1a (virulent on *Rps6* lines), and V13 (virulent on *Rps12* lines). This phenotyping approach is expected to facilitate the development of a high-resolution genetic map for the *Rps6/12/13* region and clarify if *Rps6* and *Rps13* are allelic or tightly linked genes.

Poster Presentation #132

Mechanistic Insights into a Novel Transcriptional Regulator Conferring Broad-Spectrum Resistance to Soybean Cyst Nematode

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Soybean cyst nematode is a top pathogen of soybean with annual yield deficits exceeding US$1.5 billion in the United States. The prolonged reliance on the PI 88788 (rhg1) resistance source over the past three decades has caused “race shift” in SCN populations, leading to more virulent races. It is essential to characterize novel resistance sources, in addition to known major sources, to manage the ever-growing nematode infestation. We identified a qSCN10-resistant QTL that harbors the GmbZIP10 and GmSCT10 genes from the exotic line PI 567516C. Considerable significant allelic variations in GmbZIP10 and GmSCT10 strongly correlated with differential gene expression in response to SCN infection. The increased expression of GmbZIP10 and GmSCT10 in soybean composite transgenic hairy roots demonstrated enhanced SCN resistance compared to the susceptible allele. We generated knockout constructs for the GmbZIP10 and GmSCT10 genes utilizing CRISPR/Cas9 technology, employing a dual gRNA strategy. Transgenic composite roots were developed in resistant and nonresistant backgrounds. Protein localization and single-nuclei RNA sequencing provided the fundamental understanding of GmbZIP10 localization in the nucleus/cytoplasm and cellular heterogeneity in SCN-infected roots. To understand the genetic mechanism of SCN resistance, diverse approaches are employed, including engineering resistance through native and constitutive promoters in susceptible genotypes, as well as identifying genetic circuits and cell-specific responses through single-cell transcriptomics. The outcomes of our research hold the promise of not only enhancing our understanding of the molecular basis of SCN resistance but also paving the way for unconventional and sustainable strategies for durable SCN resistance in soybean.

Poster Presentation #133

Identification of novel genomic regions and candidate genes associated with soybean nodulation for future breeding programs.

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The soybean (Glycine max) is a crucial annual legume valued for its high protein and oil content, making it an important economic crop worldwide. Its high protein content necessitates substantial nitrogen, which is supplied through symbiotic nitrogen fixation (SNF) with Bradyrhizobia bacteria in root nodules. This biological process converts atmospheric nitrogen into ammonia, providing a sustainable alternative to chemical fertilizers, which are often costly and environmentally detrimental. Climate change has adversely affects soil conditions, root growth, water and nutrient absorption, and nodulation. Elucidating the genetic basis of soybean–rhizobia-mediated nitrogen fixation can enhance nitrogen availability in agricultural systems. Genome-wide association studies (GWAS) have emerged as an effective method for identifying genetic regions linked to phenotypic variation. In this study, nodulation traits were evaluated in 238 mid- to late-maturing soybean accessions. Soybean seeds were germinated on brown germination paper, and 7-day-old seedlings were inoculated with Bradyrhizobium japonicum before transferring to blue blotting paper. After 28 days, root images were captured, and 24 nodulation traits were analyzed using a newly developed YOLO-based pipeline. The YOLO model was trained and validated on the high-performance computing platform, run:ai (https://www.run.ai/), using two graphics processing units (NVIDIA A40 GPU) with a memory of 90 GB. Phenotypic data from the YOLO based pipeline and SNP markers from the Illumina Infinium SoySNP50K iSelect SNP BeadChip were subjected to GWAS using the TASSEL 5.0 mixed linear model and FarmCPU. This analysis identified 18 significant Single Nucleotide Polymorphims (SNPs) associated with nodulation traits and climate resistance, comprising 112 from TASSEL and 46 from FarmCPU, with the maximum SNPs on chromosome 14. The identified loci provide valuable genetic targets for enhancing nodulation and improving SNF efficiency. These findings can facilitate the development of soybean cultivars with improved symbiotic nitrogen fixation, resistance to adverse climatic conditions and promoting sustainable agricultural practices.

Poster Presentation #134

Characterization of genes involved in sitosterol to stigmasterol conversion in soybean

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Sterols are products of the mevalonate pathway with a role in the regulation of cell membrane fluidity and permeability. Unlike mammals and fungi, plants synthesize a diverse type of sterols, including sitosterol, stigmasterol, cholesterol and campesterol. Although campesterol is the precursor of brassinolide, a steroidal hormone essential for plant growth and development, the fluctuating levels of sitosterol and stigmasterol during development suggest that these sterols may also play a role in regulating plant growth and development. Consistent with this concept, sitosterol serves as a primer for cellulose biosynthesis and the exogenous application of stigmasterol leads to enhanced stress tolerance in plants. Further, sterols extracted from plants such as soybean serve important pharmaceutical applications. For example, stigmasterol serves as a precursor of progesterone and sitosterol as a dietary supplement to help reduce high cholesterol levels. To optimize the composition of such beneficial sterols requires knowledge of the genes involved in their biosynthesis. The objective of this study is to identify soybean genes involved in the conversion of sitosterol to stigmasterol. In silico analysis pointed to two genes with sequence similarity to known stigmasterol biosynthesis genes. Analysis of sterols and mRNA levels shows a correlation between candidate gene transcript abundance and stigmasterol content. Virus-induced gene silencing and heterologous expression of the candidate genes were used to confirm their role in stigmasterol biosynthesis. Our study is building a foundation for understanding the physiological function of stigmasterol in soybean growth and development.

Poster Presentation #135

Evaluation of Elite Soybean Lines from the MU-FDREEC Breeding Program for Resistance to Phytophthora spp.

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Different species of Phytophthora pose a serious risk to crops, leading to substantial losses and threatening the stability of global agriculture. In response to evolving pathogen pressures and environmental changes, the development of soybean cultivars with durable resistance, particularly to *Phytophthora* spp., has become a critical goal in modern breeding programs. This study evaluates the resistance of 187 elite soybean lines from the MU-FDREEC breeding program to two species of Phytophthora: *P. sojae* and *P. sansomeana* through disease screening. Ten seeds of each soybean line were planted and maintained in a greenhouse at 25°C with a 12-hour photoperiod for one week. Seedlings aged seven to ten days were then screened for resistance associated with *Rps* genes using two isolates: *P. sojae* (MPS17-90) and *P. sansomeana* (MPS17-22). The screening was conducted using the hypocotyl inoculation method described by Dorrance *et* al. (2004). A standard set of soybean differential lines was included in each replicate as a control. Following inoculation, plants were placed in a growth chamber at 25°C and covered for 24 hours to maintain high humidity. Disease assessments were performed seven days after inoculation, and plants were classified as resistant (<30% mortality) or susceptible (≥70% mortality). The experiment was repeated twice. Resistance responses varied notably between *P. sojae* and *P. sansomeana*. Among the soybean lines evaluated from the FDREEC breeding program, 67 exhibited resistance to *P. sansomeana*, while only two lines showed resistance to *P. sojae*. In contrast, 47 lines were susceptible to both species. Only two lines —S22-1294 and S22-3859— demonstrated resistance to both *Phytophthora* species, making them promising candidates for introgression of resistance into breeding programs aimed at improving resistance in soybean cultivars. Further research will be necessary to understand the mechanisms of resistance and the differences between species of *Phytophthora* spp.

Poster Presentation #136

Genome-Wide Association Study Reveals Novel Genetic Loci for Resistance to *Phytophthora sojae* in Soybean

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Phytophthora Root Rot (PRR), caused by Phytophthora sojae, is a major disease limiting soybean production worldwide, especially in areas with poorly drained soils and frequent rainfall. The pathogen can infect plants at any growth stage, causing seed rot, damping-off, root and stem lesions, and plant death, often resulting in significant stand losses. Due to the persistence of P. sojae in the soil and its adaptive potential, developing resistant soybean cultivars remains one of the most effective management strategies. This study aimed to perform a genome-wide association study (GWAS) to identify genomic regions associated with resistance to Phytophthora sojae in in a soybean diversity panel of 360 plant introduction lines. Ten seeds of each line were planted and grown in a greenhouse at 25°C with a 12-hour photoperiod. Seedlings aged seven to ten days were screened for Rps gene-mediated resistance using isolate MPS17-90 and the hypocotyl inoculation method. A standard set of differential lines was included as controls. Following inoculation, seedlings were incubated at 25°C under high humidity for 24 hours. Disease assessments were made seven days post-inoculation, classifying plants as resistant (<30% mortality) or susceptible (≥70% mortality). GWAS was performed using the statgenGWAS package in R, applying a linear mixed model to account for population structure and kinship. Significant associations were identified using a threshold of $-\log_{10}(p) > 3$ was used to highlight suggestive associations, balancing discovery potential and false-positive control. The analysis revealed several significant SNP associations with resistance to P. sojae, with notable peaks on chromosomes 2, 3, 5, 6, 8, 10, 11, 13, 14, 15, 16, and 18. Among them, 11 SNPs corresponded to previously reported loci, while 21 novel SNPs were identified. These novel regions represent valuable targets for further validation and marker-assisted selection, offering new opportunities to develop soybean cultivars with improved resistance to PRR.

Poster Presentation #137

Developing genotype-by-environment interaction (GxE) genomic prediction models for soybeans capable of predicting breeding values

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Genotype-by-environment interaction (GEI) effects are a major source of variation in crop variety development programs. Developing stable varieties capable of performing well across varying environments, even under changing climatic conditions, is the main goal of plant breeders. The objective of this project is to develop and validate genomic prediction models that capture GEI effects specific to observed environmental variables. If successful, these could be useful for predicting genotype performance in future environments under specific climate change scenarios. The SOYGEN (Science Optimized Yield Gains across Environments) initiative was started by Midwest public soybean breeders. The data to be used to address this objective is being generated from a large multi-institutional multi-environment trial, involving data collection on ~1200 soybean genotypes across >40 environments representing 10 states (MN, ND, NE, IA, IL, IN, MI, OH, KS, MO). These ~1200 lines are being evaluated for yield and seed composition traits in a replicated augmented alpha row-column design for two years. Phenotypic data from these trials will be combined with genomics and “enviromics” data to build and test such GEI genomic prediction models. The major outcome of this project will be a GxE genomic prediction model through which new genotypes can be predicted for traits of interest in untested environments. This will also result in a generation of new knowledge on the genotype-by-environment interactions in soybean.

Poster Presentation #138

From Sky to Canopy: Integrating UAV Imaging and Photosynthetic signatures for Soybean Drought-Resilience Breeding

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Soybean production is increasingly challenged by unpredictable rainfall and drought stress, necessitating the development of climate-resilient cultivars. Therefore, identifying high-throughput, reliable, and non-destructive traits such as canopy-level RGB indices and leaf-level photosynthetic traits is crucial for accelerating drought tolerance screening. This study evaluated the drought response of nine soybean genotypes through integrated analysis of seed yield, photosynthetic traits, morphological adaptations, and RGB imaging indices under irrigated and rainfed conditions. Field trials were conducted in a randomized complete block design (RCBD) with 5 replications across both water treatments. Results revealed a significant yield reduction under rainfed conditions, with HSW also declining moderately. Photosynthetic traits such as relative chlorophyll content and PSII max efficiency were slightly higher under irrigated conditions. Morphologically, drought induced a marked increase in leaf thickness and a reduction in leaf angle, highlighting adaptive changes to water stress. RGB-derived indices such as NGRDI, VARI and GLI captured canopy-level differences in drought response, correlating positively with photosynthetic performance under stress. These indices showed potential for non-invasive screening of drought resilience. This study highlights the utility of combining physiological traits with high-throughput phenotyping tools to identify promising genotypes for drought-prone environments. Future work will integrate UAV-based imaging and genomic tools to further enhance drought-resilient soybean breeding pipelines.

Poster Presentation #139

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

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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 (normal), oval, and lanceolate (narrow). Narrow leaflet soybean lines have a high NSPP with small seeds, while oval leaved lines produce low NSPP. Improving our understanding of genetic architecture underlying NSPP is promising for enhancing future yields. While the ln locus associated with narrow leaves and high NSPP has been mapped and characterized, little is known about lo, which is associated with oval leaflets and low NSPP. In this study, we are fine-mapping and characterizing the lo locus. Additionally, we are comparing the lo locus to the previously mapped ln locus. Fine mapping for the oval trait is being done by genotyping plants with KASP markers in a segregating backcross population. Our goal is to identify the gene/genetic element responsible for oval leaflet shape and low NSPP, in order to better understand genetic control of NSPP and ultimately improve yield.

Poster Presentation #140

From host plant immunity to agro-ecological IPM: a case study in *Phaseolus vulgaris*

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Plants deploy direct and indirect defenses in response to insect herbivory. The specific antiherbivore responses involve cell surface immune receptors that recognize herbivore associated molecular patterns (HAMPs), yet the ecological relevance of this molecular interplay remains unexplored. We demonstrate that the Inceptin Receptor (INR) encoded by common bean (Phaseolus vulgaris), upon recognition of inceptin, a HAMP in caterpillar oral secretions, orchestrates a defense response mediated by tritrophic interactions with predatory wasps. Laboratory and field experimentation in Mexico using a naturally occurring inceptin-insensitive mutant (BC6F2 inr-1) and its near-isogenic line (NIL) INR equivalent, revealed that inceptin recognition by INR activates an herbivore-specific immune pathway, and triggers the emission of a volatile blend of methyl salicylate and homoterpenes that recruits predatory wasps. In the field, we found that pairs of the responsive parent line PI 311785 and inr-1/inr-1 NIL plants treated with oral secretions derived from fall armyworm demonstrated 40% reduction of attack to sentinel caterpillars by predatory Polybia sp and Mischocyttaurus sp wasps to inr-1/inr-1 plants. Similarly in 2024, pairs of NILs treated with a physiologically relevant concentration or excess of In11 showed a 40% reduction of attack to inr-1/inr-1 across two independent experiments, while there was no difference in visitation for mock treated plants (w + H2O). Our findings establish a direct link between the molecular recognition of herbivores and ecologically relevant tritrophic outcomes, providing insight into the potential mechanistic basis for the effectiveness of traditional intercropping systems such as the Mesoamerican Milpa and push-pull agriculture. Findings from this Phaseolus vulgaris case study may also translate to other Integrative Pest Management (IPM) practices, e.g. companion planting and agroecosystems relevant to soybean agriculture in regions prone to outbreaks of lepidopteran pests.

Poster Presentation #141

Bioengineering of the Rice Pikm-1 NLR Protein for Creating an Artificial Effector Triggered Immunity System Against the Fungal Pathogen Fusarium virguliforme

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In rice, a pair of nucleotide-binding site leucine-rich repeat (NBS-LRR; NLR) receptor proteins, *Pikm-1* and *Pikm-2*, provide resistance against the fungal pathogen *Magnaporthe oryzae*, the causal agent of rice blast. *Pikm-1* recognizes the pathogen effector Avr-Pik through a unique integrated domain (ID) and activates *Pikm-2* to trigger effector-triggered immunity (ETI). Recently, modification of the ID of Pikm-1 has been shown to enable novel ETI responses against *Potato virus X*. In soybeans, the fungal pathogen *Fusarium virguliforme* causes sudden death syndrome (SDS). The SDS resistance is partial and conferred by over 100 quantitative trait loci, each conditioning a small effect. In nature, the single soybean NLR receptor conferring complete SDS resistance is unlikely to be present. To generate an NLR receptor conferring complete SDS resistance in soybean, we have initiated an investigation to determine if Pikm-1 can be modified to recognize the *F. virguliforme* 13.5 kDa FvTox1 protein that causes foliar SDS. We have engineered 11 versions of Pikm-1, each with a different ID replacement generated using four small peptides and two single chain variable fragments (SCVFs) known to interact with FvTox1. These modified receptors are co-expressed with *Pikm-2* gene and *FvTox1* in a transient *Nicotiana benthamiana* assay system to determine if FvTox1 can activate any of the 11 modified Pikm-1 proteins. Among the constructs tested, the modified *Pikm-1* genes containing Pep 5, Pep 6, Pep 8, Pep 9, SCVF-1, and SCVF-2 demonstrated low levels of self-activation but exhibited strong FvTox1-specific activation. These six candidates have been selected for stable soybean transformation to evaluate their potential to confer robust SDS resistance. In parallel to investigating the modified Pikm-1 proteins, AlphaFold-based structural modeling is applied in designing second-generation FvTox1-interacting peptides for a robust ETI, in case we fail to create robust SDS resistance with any of the 11 modified Pikm-1 proteins.

Poster Presentation #142

Discovery of Novel Resistance Resources Independent of *rhg1* and *Rhg4* for Broad-based SCN Resistance in Soybean

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Soybean cyst nematodes (SCN) cause severe yield loss (>$1.0 billion) throughout soybean production regions in the United States. Currently, about 98% of resistant varieties carry the resistance from PI 88788 and Peking (either through rhg1 or Rhg4). Continuous use of rhg1-derived resistance caused changes in the virulence of SCN populations (race shift) as well as due to narrow genetic diversity; currently, about 80% of SCN populations can overcome the host resistance offered by rhg1. Therefore, it is imperative to identify and develop additional SCN-resistance sources independent of rhg1 and Rhg4 to combat this ever-changing pathogen. The whole-genome resequencing-derived pangenome analysis of 1200 soybean lines demonstrated six unique new sources of SCN-resistant germplasms independent of rhg1 and Rhg4. Haplotype, gene expression, and SCN phenotyping analysis identified several Glycine max accessions, such as PI 567516C, PI 407729, and two wild G. soja accessions, which contain unique haplotypes and are not related to any known SCN resistant loci (rhg1, Rhg4, and Rhg2). These novel soybean lines are resistant to multiple SCN populations (HG types), providing new genetic resources for durable SCN resistance. Soybean exotic line PI567516C carries two novel loci (qSCN10 & qSCN18) for SCN resistance and displays resistance mechanisms different from those of known genes. qSCN10 and qSCN18 were fine-mapped to 142-kbp and 130-kpb regions containing 20 genes and 15 genes, respectively. Based on gene expression, gene ontology, in-silico and haplotyping analysis, two candidate genes from qSCN10 and four candidates from qSCN18 were selected for further gene functional characterization through overexpression, CRISPR knockout and single-nucleus RNA-seq. Overexpression of candidate genes in the SCN-susceptible Williams 82 composite transgenic roots showed cyst count reduction by 52 to 65% compared to wild type. These novel germplasms serve as crucial genetic material for broadening the genetic diversity and developing next-generation soybean varieties for the U.S. farmers.

Poster Presentation #143

A Comprehensive Evaluation of Spatial Adjustment and Genomic Prediction Models for Selecting Sudden Death Syndrome Field Resistance in Soybean (*Glycine max*)

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Sudden death syndrome (SDS) caused by Fusarium virguliforme, is a major soybean yield-limiting disease in the Northern United States. Genetic resistance remains the most effective control strategy; however, no fully resistant genotypes have been developed due to the partial and polygenic nature of resistance mechanisms. This study aims to evaluate the performance of genomic prediction models for SDS resistance by incorporating three spatial adjustment methods—two dimensional autoregressive (AR1×AR1), nearest neighbor, and P-spline—across five genomic prediction approaches: Bayes A, Bayes B, Bayesian Lasso, genomic best linear unbiased prediction (GBLUP), and ridge regression BLUP (rrBLUP). Historical phenotypic and genotypic data collected from 2012 to 2024 were curated, excluding years with low disease pressure. A total of 703 genotypes were used in the genomic prediction analyses on the three traits: disease severity (DS), disease incidence (DI), and disease index (DX). Ten-fold cross-validation revealed the highest prediction accuracy (0.52) for DS using the Bayes A model combined with a moving mean spatial adjustment. For DI and DX, the rrBLUP model coupled with spatial P-spline adjustment showed the highest accuracies, 0.54 and 0.60, respectively. These results highlight the importance of spatial adjustment in enhancing genomic prediction accuracy and demonstrate the utility of genomic selection for improving SDS resistance in soybean breeding programs.

Poster Presentation #144

An Effective Genomic Prediction Workflow to Optimize Early-stage Soybean Selection

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Genomic prediction has emerged as a powerful tool in plant breeding over the past two decades, widely applied to shorten breeding cycles and reduce costs selecting quantitative traits such as yield. This study evaluated the usefulness of a streamlined workflow for genomic prediction to advance lines in the preliminary yield trials (PYT) of the UMN Soybean Breeding Program. The workflow includes data organization in standardized formats and processing steps, including imputation of genotypic data from low-density (1K) to high-density markers (50K) for cost-effective genotyping of progenies. The lines in PYTs conducted at UMN are organized into tests according to target market and latitudinal zone (northern, central, and southern MN). Each test is evaluated in two locations with two replicates. Genotypic and phenotypic data of ~980 lines in 2023 PYTs were used to train a genomic prediction model incorporating genotype-by-environment interaction effects. Predictions were made using a training population of only half the lines to mimic a scenario where only 50% of the lines in PYTs are phenotyped, while others are advanced on the basis of genomic prediction. The top 20 highest yielding and bottom 20 lowest yielding lines selected using phenotypic selection and genomic selection from the 2023 PYT population were evaluated in central and southern Minnesota in 2024 across three locations in each zone. Results demonstrated that genomic selection performed as well as or better than phenotypic selection for yield in retaining top performing lines and discarding poorly performing lines. Additionally, training sets from prior years (2021 and 2022 PYTs) and historical data were evaluated to improve predictive ability of genomic prediction through cross validation. The genomic prediction workflow demonstrated to be a promising approach to conduct preliminary yield trials in a cost-effective manner with two-thirds or half the resources required in prior years, with potential for further improvement.

Poster Presentation #145

High-Throughput Gene Knockout in *Nicotiana benthamiana* via TRV-Delivered CRISPR and Agrobacterium Infiltration

Wai Tan Wong[1], *Matthew Hudson*[1]

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CRISPR-Cas9 genome editing is a powerful tool for functional genomics, but high-throughput gene knockout in plants remains limited by the need for tissue culture and stable transformation. Tobacco rattle virus (TRV2) offers a promising solution as a viral delivery system capable of systemic sgRNA transport in planta in a high-throughput context with the integration with iBIOFAB.

We aim to establish a high-throughput TRV2-based vector system that enables rapid and heritable gene knockouts in Cas9-overexpressing Nicotiana benthamiana lines without the requirement of tissue culture. This platform is intended to accelerate gene function studies, particularly for large gene families and stomatal genes.

Using a newly sequenced Cas9-expressing N. benthamiana line, we designed sgRNAs targeting genes associated with stomatal patterning via the CROPSR tool. Guides were cloned into TRV2 vectors using Golden Gate assembly, with high-throughput support from iBIOFAB. Agrobacterium-mediated infiltration was used to deliver the vectors into Cas9 plants. Editing efficiency was evaluated through genotyping and phenotyping. Proof-of-concept experiments targeting amCyan and PDS were used to visualize the virus propagation and editing events.

We successfully performed genome sequencing Cas9 N. benthamiana line with a N50 score of 94Mb to facilitate guide design using CROPSR. TRV2-mediated delivery of sgRNAs led to efficient genome editing, as demonstrated by visible phenotypes in amCyan- and PDS-targeted plants.

Our TRV2-sgRNA delivery platform enables scalable, rapid gene knockout in N. benthamiana without tissue culture, opening new possibilities for functional genomics in N. benthamiana and other Solanaceae species.

Poster Presentation #146

Optimizing root system performance in crops through functional-structural root modeling, target discovery and gene editing at Inari

Marlies Wouters[1], Joris Jourquin[1], Veerle De Schepper[1], Daniel Leitner[2], Tobias Selzner[2], Xavier Draye[4], Kerstin Nagel[3], Andrea Schnepf[2], Sonali Quantius[5]

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At Inari we strive to increase crop yields while reducing the need for water and fertilizer inputs. Amongst others, achieving this goal requires the development of crops with optimized root properties that can acquire these resources more efficiently. Identifying the most beneficial root system modifications can be challenging and is typically context dependent. Therefore, we employ functional-structural root models (FSRMs) that allow us to simulate resource uptake as a function of root architecture and anatomy in different environments. These FSRMs enable the identification of root system ideotypes for environments of interest and help to pinpoint root trait modifications with a strong impact on resource uptake capacity, but a limited or beneficial effect on the metabolic cost to build and maintain the root system. In addition, we are using high-throughput root phenotyping equipment to capture architectural and anatomical root traits from genetically diverse maize and soy populations, and make use of bioinformatics approaches (incl. GWAS, TWAS, eQTL mapping) for the discovery of target genes that influence each of these traits. The combination of FSRM-based trait selection with trait gene identification contributes to our Predictive Design work that informs strategies to rapidly improve root system performance with our gene editing toolbox.

Poster Presentation #147

Improved genomic prediction accuracy for complex seed composition traits using highly replicated, multi-environment phenotypic data in soybean (*Glycine max* L.)

Hee Jin You[1], *Professor Sungwoo Lee*[1]

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The accuracies of genomic selection (GS) are affected by factors such as genetic relatedness between the training and validation populations, marker density, and phenotypic data quality. In this study, several GS models were simulated using SoySNP50K chip data and highly replicated, large phenotypic data from 621 G. max accessions evaluated in multiple locations (Urbana, IL, Columbus and Wooster, OH, Plymouth, NC). Of 28 seed composition traits, protein, oil, methionine/protein ratio, oleic acid, and 100-seed weight were primarily used to investigate the effects of these factors on the prediction accuracies in the simulations based on the BayesB model. The primary research questions affecting prediction accuracy were as follows: i) How does genetic similarity between the training and validation populations impact prediction accuracy? ii) How do the number and significance of SNPs affect prediction? iii) How does multi-environment phenotypic evaluation contribute to prediction performance? The first key finding was that prediction accuracies significantly increased with higher genetic similarity between the training and validation populations. When using a training population composed of Korean accessions, the model showed a high accuracy of 0.67 for predicting Korean accessions, whereas the accuracy for predicting U.S. accessions with high genetic differentiation from Korean accessions (F_{ST} = 0.24) was 0.32. Second, the SNP markers with no-effect or trivial associations were not useful for improving prediction accuracy, thus recommending the exclusion of such neutral markers to reduce computational effort and minimize noise. Third, BLUP calculated from multi-environment phenotypic data could effectively capture environmental variation and estimate breeding values of each SNP, consequently leading to more precise predictions. When using BLUPs derived from multi-environment trials, the prediction accuracy was high (0.73), whereas accuracy was lower (0.56) with single-environment phenotypic data. These findings provide concrete guidelines for optimizing GS strategies in soybean breeding.

Poster Presentation #148

GA*A*NTRY launched gene editing T-DNA results in higher plant transformation efficiency relative to the binary plasmid

Guifen Li[1], Ed Williams[1], Nathalie Walter[1], Shawn Kaeppler[1], Ray Collier[1]

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While CRISPR/Cas9 mediated gene editing is an important approach currently used by many soybean researchers, curiously the experience of the WCIC since 2018 has been that successful production of transgenic soybeans/dicots with gene editing constructs has occurred at a demonstrably lower efficiency than what has been achieved for monocots. An experiment, originally designed to explore the impact on gene editing outcomes as a function of the strength of the polyadenylation sequence used for the guide RNA (gRNA) expression unit, additionally made a direct comparison between binary plasmid and ArPORT1 (the disarmed Ri plasmid of *Agrobacterium rhizogenes* strain NCPPB2659; the GA*A*NTRY system) launched gene editing T-DNA molecules. We targeted *GmJAGGED1* with two previously reported gRNA in a tRNA delimited polycistronic expression cassette, the expression of which was controlled with the Cestrum Yellow Leaf Curling Virus promoter (CmYLCVp), with either the *Agrobacterium tumefaciens Nopaline Synthase* (*NOS*), Cauliflower Mosaic Virus 35S (*35S*), *Nicotiana benthamiana Heat Shock Protein* (*NbHSP*) or *Nicotiana tabacum* intronless *Extensin* (*EU*) terminators for polyadenylation. To facilitate non-destructive identification and sorting of null segregant and transgenic T1 seeds, the *GmScream6* promoter driven *tdTomato* cassette was included in all constructs as a seed marker. All constructs carried the same plant selectable marker, and the same *Cas9* expression unit. The eight (4 binary, 4 GA*A*NTRY) constructs were transformed into Williams 82 cultivar soybean. Unexpectedly, all ArPORT1 (GA*A*NTRY system) constructs exhibited a remarkably higher (1.6 to 6 fold) plant transformation efficiency relative to the binary constructs assembled to contain exactly the same T-DNA cargo. Editing of the target *GmJAGGED1* loci was immediately apparent in many of the T0 individuals, with severe phenotypes recorded for several independent events, suggesting that the poor plant transformation efficiency observed with gene editing binary plasmids may not be related to *Cas9* in the construct.

Poster Presentation #149

Seed RUBYv1: Optimization of a tool facilitating non-destructive sorting of null segregant from transgenic soybeans.

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Gene editing is an important tool for developing genetic diversity and introducing desired traits in soybean. Gene editing typically requires multiple overexpressed transgenes which can negatively impact plant physiological performance and represent a regulatory roadblock to field testing. To accurately assess the impact on plant performance of any given edit, and reduce regulatory burdens associated with the transgenic editing machinery, the non-transgenic progeny from an edited T0 plant are the individuals which are typically advanced through the research pipeline. The polycistronic *RUBYv1* marker gene shows promise as an excellent visual seed marker; however initial experiments produced seed with high L-DOPA and betanin levels, which resulted in a severe germination defect. Many studies have demonstrated that different transcriptional terminator regions can fine-tune gene expression independent of a promoter's effect. Therefore, we aimed to modulate seed-specific *RUBYv1* expression driven by the *GmScream6* promoter by pairing it with five well-characterized terminators to identify those which result in easily identifiable, viable transgenic seed in the *Glycine max* cv. 'Williams 82' background. The seeds produced by twelve T0 plants per construct were visually assessed and separated by phenotype, counted, and weighed. Results indicated that the *Agrobacterium tumefaciens Nopaline synthase* (*NOS*) and *Nicotiana benthamiana Heat-shock protein* (*NbHSP*) terminators performed best, producing seeds with an average of only 12-15% exhibiting any type of phenotypic deviation compared to their null segregant siblings. The *GmScream6* terminator yielded the highest percentage of seed with deformities (55%), often with severely compromised germination, making it a poor candidate for control of seed *RUBYv1* but suggesting that it could serve as an excellent seed-specific terminator for other applications. The combination of the *GmScream6* promoter with either the *NOS* or *NbHSP* terminators to control expression of the *RUBYv1* are reliable seed marker options facilitating rapid, non-destructive sorting of null segregant from transgenic seeds.

Poster Presentation #150

The GA*A*NTRY System outperforms the binary plasmid in deliverable DNA cargo size, plant transformation efficiency, and transgenic plant quality

Ray Collier[1], Shawn Kaeppler[2,3], Roger Thilmony[3], James Thomson[3], Edward Williams[2], Guifen Li[2], Nathalie Walter[2]

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The plant transformation efficiency of the GAANTRY system, which uses recombinase mediated cassette exchange to assemble transfer DNA (T-DNA) molecules directly in the disarmed virulence plasmid of Agrobacterium rhizogenes strain NCPPB2659 (ArPORT1), has not been directly compared to the efficiency resulting from a binary plasmid containing exactly the same DNA cargo. To allow for full compatibility with the Wisconsin Crop Innovation Center Golden Gate gene parts repository the GAANTRY system was first improved by using HARBOR system BASE plasmids to make Golden Gate cloning compatible CONTAINER plasmids with DIRECTCLONE bacterially expressed scorable markers. Using these new GG-GAANTRY B and P CONTAINER plasmids, we designed, built, and tested matching T-DNA molecules in both GAANTRY and WCIC DICOT RK2 binary plasmid formats, and proceeded with soybean and tobacco transformation experiments. Remarkably, relative to the binary vector with matching T-DNA cargo, GAANTRY produced an average 3 fold increase in plant transformation efficiency. Additionally, we observed that plant transformation efficiency with a 40710 basepair long T-DNA, which could only be built with GAANTRY (it isn't possible to build a conventional binary construct of this size) exhibited a 1.5 fold higher plant transformation efficiency than that produced using the binary vector containing a T-DNA of just 16391 basepairs. We speculate that one possible explanation for this phenomenon is due to the inherent stability, relative to the binary plasmid, of the Agrobacterium virulence plasmid (pRiNCPPB2659) which is not lost from Agrobacterium during the non-selective conditions of the co-cultivation of Agrobacterium cells with plant explants. These results clearly demonstrate that the GAANTRY system, which allows for the construction of much larger T-DNA than has ever been possible in a binary plasmid, provides plant biology researchers the ability to efficiently deliver entire metabolic pathways into plants, greatly expanding the possibilities for plant performance enhancement.

Poster Presentation #151

Genetic improvement of soybean for increased oil production

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With its high economic value and multiple uses, soybean (Glycine max (L.) Merr.) is an oilseed crop that plays an important role in addressing increasing global demand in the food, feed, fuel, and chemical sectors. However, as we enter an era in which global food production is likely to double due to an increasing human population, utilizing prime agricultural lands and resources for food, feed, and energy production, all within the context of global ecological change, becomes an even greater challenge. Advances in traditional breeding and agronomic practices have steadily increased soybean seed yields, as well as tolerances to biotic and abiotic stresses, which have significantly enhanced seed composition. However, due to the relatively limited genetic diversity of soybeans, especially when compared to crops such as maize, further gains from soybean breeding may be incremental. This study investigates the ectopic expression of the WRINKLED1 (WRI1) transcription factor, both individually and in combination with terminal enzymes involved in oil biosynthesis, to evaluate its effects on seed storage compounds, including oils and proteins. Transgenic T_0 lines were generated via Agrobacterium-mediated transformation. Subsequent molecular and biochemical analyses are currently underway to assess transgene integration, expression levels, and alterations in seed composition

Poster Presentation #152

CRISPR/Cas9-Mediated Genome Editing to Enhance Soybean Mosaic Virus (SMV) Resistance in USDA and African Originated Soybean (*Glycine max*) Lines

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Soybean, a prominent leguminous crop, is a significant reservoir of protein and oil, rendering it indispensable for poultry and livestock. Soybean cultivation confronts multifarious biotic adversities, with the soybean mosaic virus posing a formidable challenge. Its deleterious impact culminates in a yield reduction of ~35%, with severe manifestations capable of decimating fields by ~94%. The existing reservoir of germplasm encompasses a limited defense mechanism, encouraging researchers to explore innovative avenues. Recent advancements, particularly CRISPR/Cas9-mediated genome editing, have emerged as a beacon of promise for enhancing crop genetics. Yet, its efficacy and replicability pivot significantly on the optimization of the soybean regeneration protocol. To achieve this, a meticulous screening process of diverse American and African genetic lineages was carried out to identify ten highly susceptible lines to SMV strains at all developmental stages. In the subsequent investigation, seven distinct PGR combinations (BAP/NAA/IBA/IAA/GA3) were tested to delineate line-specific combinations conducive to robust shoot and root regeneration. Notably, only two protocols proved effective for four genotypes of African provenance, demonstrating the highest regeneration efficiencies, ranging from 90-95%. Four negative regulators (A/B/C/D) and their homologs that promote SMV replication were identified. Guide RNAs and vectors in various combinations of target genes were constructed. The gRNAs+Cas9 complex was transformed using Agrobacterium-mediated transformation. a, b, and ab-edited lines exhibited SMV patterns reduced by 40-60%, and the cd-edited line demonstrated 70-80% resistance. T0 plants exhibited broad-spectrum widespread SMV resistance, and these plants are under various molecular, biochemical, and field tests. This investigation furnishes both a foundational framework and profound implications for prospective breeding initiatives aimed at fortifying soybeans.

Poster Presentation #153

Pangenome analysis of nine Soybean Cyst Nematode genomes reveal hidden variation contributing to diversity and adaptation

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Soybean cyst nematode (SCN) poses a persistent challenge to soybean production, driven by its capacity to overcome resistant cultivars resulting in crop yield loss. Currently available SCN reference genomes fall short in representing intraspecific diversity, impeding our understanding of virulence evolution and host adaptation. Here, we leverage high-fidelity long-read sequencing and comparative analysis to generate a pangenome from nine SCN populations, or subspecies differing in their pathotypes.

We identified over 19,000 orthologous gene families, with nearly 35% comprising the conserved core genome. Accessory genes—often associated with environmental sensing and host manipulation—comprised nearly 50% of the total gene space. Surprisingly, a large portion (40%) of the core genome exhibited signatures of more rapid evolution in a positive selection analysis, particularly in domains related to host interaction and immune evasion. Structural variants in genomic regions under selection suggest population-specific haplotypes that may underlie differential virulence. Furthermore, the secretome, comprising ~1,400 genes per genome, revealed dynamic effector content across accessions.

Our study highlights the power of pangenomics in revealing hidden genetic diversity in SCN. The dynamic nature of both core and accessory genomes, shaped by selection and structural rearrangements, illustrates the genomic evolutionary arms race between SCN and soybean. These insights provide a foundational resource for resistance breeding and pathogen surveillance, with broader implications for managing rapidly evolving crop pathogens.

Poster Presentation #154

Research Status of Soybean Red Crown Rot in Taiwan

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Soybean red crown rot, caused by the soilborne fungal pathogen Calonectria ilicicola, has emerged as a growing global threat to soybean production, leading to root rot and crown lesion that ultimately reduce seed quality and yield. Since its initial detection in southern Taiwan in 2017, the disease has advanced northward, raising concern due to the pathogen's broad host range and the long-term survival of its microsclerotia in soil. Current management approaches—including seed fungicide treatments, crop rotation, soil sterilization, and delayed planting—offer only limited and inconsistent efficacy, and no standardized control strategy has yet been established in Taiwan. Recent studies have suggested that seed-associated microbiota may contribute to plant defense. In this study, we demonstrate that seed-associated bacteria play a role in conferring resistance to seed rot caused by C. ilicicola, although this protection does not extend to root rot. Through PacBio full-length 16S rRNA gene sequencing, we identified 14 amplicon sequence variants (ASVs) enriched in resistant soybean varieties, including two associated with Bacillus altitudinis. Isolates of B. altitudinis exhibited antagonistic activity against six soilborne fungal pathogens. When introduced to soybean seeds, these strains restored seed rot resistance in varieties that supported higher levels of bacterial colonization. Quantitative PCR analysis confirmed that bacterial persistence varied between resistant and susceptible varieties, with prolonged colonization observed on both apical shoots and roots in a resistant cultivar. These findings underscore the role of B. altitudinis in enhancing resistance to seed rot and highlight the importance of microbial compatibility and colonization dynamics in the development of biological control strategies. Collectively, current understanding presents an integrated strategy of fungicide seed treatments, beneficial microbial inoculants, resistance breeding, and agronomic practices as a comprehensive approach to managing red crown rot and sustaining soybean production in Taiwan.

Poster Presentation #155

Early Identification of High-Yielding and Stable Soybean Genotypes

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Improving grain yield is the primary goal of most soybean breeding programs. During early-stage yield trials, breeders often evaluate thousands of genotypes; however, limited seed availability constrains the number of tested environments and replications, which reduces selection accuracy. Thus, developing genomic prediction models to identify high-yielding and stable genotypes early in the breeding cycle is critical. In this study, 1,382 genotypes ranging from maturity groups III to V were evaluated for grain yield across six environments in 2023 and 2024. Best Linear Unbiased Estimators (BLUEs) were calculated for each year-location combination. A Modified Selection Index (MSI) was calculated as the average yield deviation from the check mean across tested environments, with zero serving as the baseline. Genotypes with MSI ≥ -5 were classified as high-yielding, while those with MSI < -5 were classified as low-yielding. Two classification-based genomic prediction models, Random Forest (RF) and XGBoost, were developed using the SoySNP3K BeadChip markers as predictors and yield classes as the response variables. The data were split into an 80:20 ratio for training and testing, and the model's performance was assessed using repeated 5-fold cross-validation (5 repetitions) with the R package 'caret'. XGBoost outperformed RF in terms of precision (0.78 vs. 0.73), sensitivity (0.84 vs. 0.81), and F1-score (0.81 vs. 0.77), demonstrating an overall balanced classification across yield classes. These results suggest that combining MSI with classification-based genomic prediction may allow an accurate identification of either high- or low-yielding genotypes, which can have a substantial impact on resource allocation in the early stages of soybean breeding. For instance, one may confidently discard low-yielding genotypes, thus prioritizing the allocation of resources towards promising ones.

Poster Presentation #156

Identification of causal R genes involved in soybean response to *Phytophthora sojae* infection

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Phytophthora sojae is a hemibiotrophic oomycete pathogen of soybean that causes various diseases throughout the plant's growth stages including damping off of seedlings and Phytophthora root and stem rot. Due to the hardy oospores that can survive several years in soils, outbreaks can occur throughout the growing season. Control for this pathogen is largely reliant on the presence of resistance to *P. sojae* (*Rps*) genes, but there are often virulent *P. sojae* populations of the genes currently available in commercial lines, reducing their effectiveness. This work focuses on the yet-unidentified *Rps2*, located on chromosome 16 in a complex locus near characterized resistance genes for rhizobium and powdery mildew. We compared the response of a resistant soybean line containing *Rps2* and a susceptible line to *P. sojae* infection. Virus-induced gene silencing with bean pod mottle virus (BPMV) was conducted, targeting several putative genes in the *Rps2* locus of the resistant line. RNA-sequencing of leaf, stem, and root tissue at multiple timepoints after inoculation was conducted to better understand timing of response to the pathogen throughout the plant, as well as provide support for genes or pathways involved in the response. Utilizing target genes identified in the RNA-seq results, future VIGS experiments can be conducted to further elucidate the identity of *Rps2* and other factors and pathways involved in its function.

Poster Presentation #157

Identification of QTLs and Candidate Genes Associated with Yield and Yield Stability in Soybean

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Yield and yield stability are crucial for release and commercial growing of a crop cultivar. Soybean is a major crop grown worldwide and an important supply of plant protein and vegetable oil for human consumption and animal feeds. Identification of QTLs and candidate genes related to yield and yield stability will help promote soybean yield improvement and enhance breeding efficiency, but related work has rarely been reported. This study evaluated seed yield of 196 soybean varieties and lines over eight years of replicated trials and assessed their stability using seven indices. GWAS was performed based on phenotypic data and genotypic data of BACKSoy6KSNP assay. The combined analysis of yield overall years indicated that the broad-sense heritability was 83.71%, whereas for low-productivity years (2016-2019) and normal-to-high-productivity years (2020-2023), the broad-sense heritability was estimated to be 66.63% and 76.29%, respectively. Estimates of heritability for stability were mostly low but were 50.78% for S_j^2 and 61.23% for CV_j. Four QTLs associated with yield were identified on chromosomes 6, 8, 13 and 19 based on the combined analysis overall years and Bonferroni corrections, and of them, two were confirmed by the analysis of data from low productivity or normal-to-high productivity years. The analysis for normal-to-high productivity years identified eight additional QTLs related to yield. For yield stability, 39 QTLs were identified in total, and six were repeatedly detected using different stability indices. Relatively, the number of QTLs identified for S_j^2 and CV_j was greater than that of other stability measures. The QTL with ss715599704 on chromosome 8 was associated with both yield and yield stability. For each identified QTL, multiple candidate genes of known function were annotated. The position of genomic loci and information of candidate genes revealed in this study will be helpful for soybean yield improvement and related research.

Poster Presentation #158

Epigenetic Regulation of Soybean Defense Responses During *Phytophthora sojae* Infection

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Plants have evolved with complex mechanisms to combat pathogen infection. During an infection, the plant resources are shifted towards protective responses - the genes that control housekeeping functions such as metabolism, growth, and reproduction are suppressed, shifting the balance towards activating immune response genes. This switch is partly controlled by epigenetic mechanisms like DNA methylation, where methyl groups attach to cytosine bases. This change leads to altered gene expression without changing the DNA sequence, hence activating or suppressing genes. Although several components of soybean immune responses have been identified, a comprehensive understanding of how molecular pathways and epigenetic regulators coordinate this response to pathogens is still lacking.

To further investigate the role of DNA methylation in pathogen response, we performed high-throughput sequencing to detect genome-wide methylation on soybean samples infected with *Phytophthora sojae*, a major oomycete pathogen responsible for significant yield losses in soybean, along with mock-treated controls. At 12 hours post-infection, *P. sojae* triggered widespread promoter methylation changes in soybean. A total of 3,821 sites were hypermethylated and 2,071 were hypomethylated across all methylation contexts. Functional categorization using Gene Ontology analysis indicated that hypermethylated genes were primarily associated with primary metabolic processes, whereas hypomethylated genes were linked to immune response and signaling. To gain a temporal perspective, we collected DNA samples at 0-hour and 24-hour post-infection and examined four differentially methylated defense-associated genes, along with the previously analyzed 12-hour time point, to track changes in their methylation profiles over time using McrBC-based quantitative-PCR. This targeted approach will help us understand how defense-related genes are epigenetically regulated during the early stages of *P. sojae* infection and this can guide towards the development of disease-resistant soybean varieties with more robust and timely defense responses. Furthermore, this foundational knowledge can be applied to other crops and pathogens, helping long-term sustainability in agriculture.

Poster Presentation #159

Pleiotropic functions of a gibberellin receptor gene in the regulation of plant architecture, yield and nitrogen fixation in soybean

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The yield of soybean is far below that of major cereal crops. The green revolution increased the yield of cereal crops partially through high-density planting of lodging-resistant semi-dwarf varieties, but required more nitrogen fertilizers, posing an environmental threat. Genes that can improve nitrogen use efficiency need to be integrated into semi-dwarf varieties to avoid the overuse of fertilizers without the loss of dwarfism. Unlike cereal crops, soybean can assimilate atmospheric nitrogen through symbiotic bacteria. Here, we created new alleles of *GmGID1-2* (*Glycine max* GIBBERELLIN INSENSITIVE DWARF 1) using clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated nuclease 9 (Cas9) editing, which improved soybean architecture, yield, seed oil content, and nitrogen fixation, by regulation of important pathways and known genes related to branching, lipid metabolism, and nodule symbiosis. *GmGID1-2* knockout reduced plant height, and increased stem diameter and strength, number of branches, nodes on the primary stem, pods, and seeds per plant, leading to an increase in seed weight per plant and yield in soybean. The nodule number, nodule weight, nitrogenase activity, and nitrogen content were also improved in *GmGID1-2* knockout soybean lines, which is novel compared with the semi-dwarf genes in cereal crops. No loss-of-function allele for *GmGID1-2* was identified in soybean germplasm and the edited *GmGID1-2s* are superior to the natural alleles, suggesting the *GmGID1-2* knockout mutants generated in this study are valuable genetic resources to further improve soybean yield and seed oil content in future breeding programs. This study illustrates the pleiotropic functions of the *GID1* knockout alleles with positive effects on plant architecture, yield, and nitrogen fixation in soybean, which provides a promising strategy toward sustainable agriculture.

Poster Presentation #160

Searching for alternative hypotheses: Investigating sequence diversity in novel genes differentially expressed between lines varying for resistance to *Phytophthora sojae* conferred by QDRL-18 in soybean

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Phytophthora root and stem rot, caused by the oomycete *Phytophthora sojae*. It is a major disease of soybean that can lead to significant yield losses, which are estimated at up to 1–2 billion U.S. dollars annually worldwide. To provide effective resistance against the multiple races of *P. sojae*, integration of race-specific *Rps* genes with non-race specific quantitative disease resistance (*QDR*) alleles is needed. Quantitative disease resistance locus-18 (*QDRL-18*) enhanced resistance across all tested races of *P. sojae* in laboratory and greenhouse tests. Our previous work identified *Glyma.18G026900*, predicted to encode a serine-kinase, as a candidate for *QDRL-18*. It was the only co-localized gene in which expression increased after *P. sojae* inoculation in susceptible (S) near isogenic lines (NILs) compared to resistant (R) NILs. Additionally, we identified 267 novel “genes” based on a transcriptome assembly which were also differentially expressed between R and S NILs post-inoculation with *P. sojae*. Based on sequence similarity to genes involved in defense, we selected 11 of the 267 novel “genes” to evaluate for sequence differences. Nine of the 11 novel “genes”, were able to be sequenced but possessed no nucleotide differences between R and S parents, removing them from our list of possible candidate genes. While *Glyma.18g026900* remains our primary candidate gene for *QDRL-18*, it remains possible that resistance is controlled by a non-differentially expressed gene within the *QDRL-18* region or a novel "gene" which we have not yet studied. Thus, we continue development of transgenic lines to study *Glyma.18g026900*, while in parallel, we develop lines with recombination within the *QDRL-18* region to narrow the genetic region and facilitate candidate gene identification should future studies eliminate *Glyma.18g026900* as a *QDRL-18* candidate.

Poster Presentation #161

Analysis of Genetic Diversity, Population Structure, and Virulence of *Cercospora sojina* Isolates Causing Frogeye Leaf Spot Disease of Soybeans Over 42 Years

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Cercospora sojina is the fungal pathogen responsible for frogeye leaf spot (FLS) of soybeans, a significant foliar disease especially in the southern United States due to warm, humid weather. FLS accounts for annual yield losses ranging from 10 to 60% in key production regions such as the United States, Brazil, and China. Management of FLS predominantly relies on chemical fungicides and use of resistant cultivars. Although preventative fungicide applications are beneficial, planting resistant varieties is the most effective and cost-efficient management strategy. Three genetic loci, *Rcs1*, Rcs2, and *Rcs3* have been identified that provide resistance to FLS in soybean. Among these, *Rcs3* confers broad resistance to all known U.S. *C. sojina* races. However, the increasing genetic diversity of *C. sojina* has led to the emergence of new virulent isolates capable of overcoming host resistance. In this study, the genetic diversity among 178 *C. sojina* isolates collected across 11 U.S. states, Brazil, China, and Nigeria over 42 years was analyzed using 686 SNPs. Moderate to high genetic differentiation (G_{ST}=0.1–0.4) was observed within *C. sojina* populations, highlighting the pathogen's potential adaptability to selection pressures and the ongoing threat to soybean host plant resistance. Population structure analysis using DAPC and STRUCTURE identified 14 *C. sojina* populations that were grouped primarily by collection year, not geography. Ongoing virulence assays aim to correlate *C. sojina* genetic diversity and virulence phenotypes with soybean resistance and will be used to facilitate the development of genetic markers for early detection of *C. sojina* to help identify durable resistance genes that are effective against evolving *C. sojina* populations.

Poster Presentation #162

Application of Crop Pest Classification System based on Deep Learning Vision Algorithms in Soybean (*Glycine max* (L.) Merr.)

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Soybean (*Glycine max* [L.] Merr.) is a globally important crop due to its high protein and oil content, responding to growing bio-industrial demands. However, biotic stressors (e.g., *Heterodera glycines*, *Riptortus pedestris*) disrupt host metabolism and reduce crop vigor. Traditional detection methods, though accurate, are labor-intensive and lack scalability. Therefore, artificial intelligence (AI) and deep learning technologies have been integrated to enable early detection, precision management, and sustainable cultivation under unstable environmental conditions.

First, the push-pull strategy was adopted using volatile chemical treatment with camera traps (GoPro camera) mounted at 10 cm from the soybean plant unit to record the behavioral patterns of *Riptortus pedestris*. Second, soil samples were collected from the rhizosphere of infected plants, with 1 kg of soil gathered per spot. The *Heterodera glycines* was isolated using oostenbrink dishes and quantified under the stereo microscope. High-resolution RGB images were annotated using Smart-Polygon, then preprocessed and split into training, validation, and test sets (7:2:1 ratio). Deep learning models (YOLOv5/8/11, Detectron2) were benchmarked using precision, recall, F1-score, and mAP@50 metrics.

Initially, the YOLOv11e-seg model was calculated as the best detection performance, with precision, recall, and mAP@50 values of 0.978, 0.980, and 0.988, respectively. Secondly, the root mean squared error (RMSE) calculated 0.837 between the AI-detected and manually-counted number of 1527 *Riptortus pedestris* across all deep learning-based camera traps. Finally, the Pearson correlation was $r=0.999$ ($p<0.001$), the Wilcoxon signed-rank test was $p=0.058$, and the Bland-Altman mean difference was –0.04 (95% limits –2.04 to +1.96) between AI-based and manual number of *Heterodera glycines*. Therefore, reducing the discrepancy between AI-generated predictions and field-derived observations is significant for implementing a reliable AI phenotyping system. Consequently, this study has the potential to serve as an effective phenotyping tool for identifying resistant germplasm and uncovering genes associated with pest resistance in soybean breeding programs.

Poster Presentation #163

Detection of Symptoms of Bacterial Disease and Development of Damage Assessment Algorithms based on Deep Learning Vision Technology in Soybean (*Glycine max* (L.) Merr.)

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Global agricultural systems face several biotic and abiotic pressures from both internal and external causes including pathogens, pests, and environmental stresses. Therefore, it is crucial to provide accurate information and farm management guidance. This study utilized an artificial intelligence (AI) technique to categorize phenotypic characteristics by segmenting target features of bacterial pustules caused by *Xanthomonas axonopodis* pv. *glycines* in soybean (*Glycine max* [L.] Merr.).

Bacterial pustule was cultured on TSA media, the two parental lines and F8 recombinant inbred lines (RILs) were inoculated at the V3 stage. Phenotypic features data were captured via a 4K stereo camera and benchmarked based on state-of-the-art models (e.g., YOLOv8/9/11, Detectron2, MRCNN). Performance metrics included precision, recall, loss, and mAP@50. Manual counts, ImageJ sampling, and macro-based algorithms were applied to obtain phenotypic data for quantitative trait loci (QTL) mapping.

The bacterial pustule and chlorosis features were localized from initial infection sites to entire soybean leaf areas, with polygonal boundaries, outlining each symptom across recombinant inbred lines RILs. The deep learning model was trained over 150 iterations as trained 75.48 sec/iteration, calculated in a maximum loss of 1.625, a minimum loss of 0.2571, and mAP@50 scores of 0.972, 0.954, and 0.819, respectively. Hex codes and pixel volumes were extracted to classify symptoms into six defined infection stages (e.g., 0, 1, 3, 5, 7, 9) based on disease spread volumes. Therefore, the QTL was identified on chromosome 5, between SNPs Chr05:1,124,800 and 1,453,684 (~0.33 Mb interval), with LOD scores and PVE (%) as 47.35 and 7.747, respectively (i.e., identified QTLs as related research). It is anticipated that the integration of AI-based image analysis with genetic mapping will accelerate precision phenotyping and provide an effective tool for identifying genes of interest.

Poster Presentation #164

Can We Breed Soybeans For Intercropping: Characterizing Interactions and Ideotypes for a Pennycress Relay System

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As the US agriculture industry changes to meet future demands, soybeans will have to adapt to new environments. One growing demand is for sustainable aviation fuel from winter oilseeds, such as winter camelina and pennycress. While large advances in production have been made, there are still barriers in how these winter annuals fit into today's cropping landscape. One solution is relay-cropping these winter annuals with soybeans. Towards this approach, we screened 40 soybean genotypes for specific adaptation to pennycress intercropping across five environments in Minnesota. Our objectives include quantifying genotype-by-cropping system interactions and characterizing soybean traits relevant to winter oilseed intercropping. Competition for resources is high during this intercropping period and some environments saw drastic yield reductions. Significant genotype-by-treatment interactions were found which suggest future breeding efforts should be successful. Interestingly, some soybean architectural changes under intercropping were unexpected and may explain why some varieties saw minimal yield decreases while others saw 50 percent decreases. This characterization of soybean traits when intercropped with pennycress has led us to develop a trait-informed framework to select new varieties adapted to a winter oilseed intercropping system. These results indicate the need for a separate breeding pipeline with intercropping objectives. As soybean breeders develop new varieties for future environments, they should consider breeding for new systems, beyond the expected corn and soybean rotation.

Poster Presentation #165

Identification and Molecular Mapping of a Major Gene Conferring Resistance to *Phytophthora sansomeana* in Soybean 'Colfax'

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Phytophthora root rot (PRR) is one of the most important diseases in soybean *(Glycine max)*. PRR is well known to be caused by *Phytophthora sojae*, but new studies showed that *Phytophthora sansomeana* also causes extensive root rot of soybean. Depending upon the isolate, it might produce aggressive symptoms, especially in seeds and seedlings. Unlike *P. sojae* which can be effectively managed by *Rps* genes, no known major resistance genes have yet been reported for *P. sansomeana*. Our previous study screened 470 soybean germplasm lines for resistance to *P. sansomeana* and found that soybean 'Colfax' (PI 573008) carries major resistance to the pathogen. In this study, we crossed 'Colfax' with a susceptible parent, 'Senaki', and developed three mapping populations with a total of 234 F2:3 families. Inheritance pattern analysis indicated a 1:2:1 ratio for resistant: segregating: susceptible lines among all the three populations, indicating a single dominant gene conferring the resistance in 'Colfax' (designated as *Rpsan1*). Linkage analysis using extreme phenotypes anchored Rpsan1 to a 30 Mb region on chromosome 3. By selecting nine polymorphic SNP markers within the region, *Rpsan1* was genetically delimited into a 21.3 cM region between Gm03_4487138_A_C and Gm03_5451606_A_C, which corresponds to a 1.06 Mb genomic region containing nine NBS-LRR genes based on Gmax2.0 assembly. The mapping results were then validated using two breeding populations derived from 'E12076T-03' × 'Colfax' and 'E16099' × 'Colfax'. Marker-assisted resistance spectrum analyses with nine additional isolates of *P. sansomeana* indicated that *Rpsan1* may be effective towards a broader range of *P. sansomeana* isolates and has strong merit in protecting soybeans from this pathogen in the future.

Poster Presentation #166

Characterization of *Glyma.18G270900* and a Related Kinase Gene Family as Candidates for *Phytophthora sojae* Resistance in Soybean

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Glyma.18G270900, which encodes a malectin/receptor-like protein kinase, emerges as a compelling candidate for *Phytophthora sojae* resistance. The gene co-localizes with the resistance-associated phQTL_18b and is differentially expressed between the resistant and susceptible parents, cultivars Conrad and Sloan, respectively. This differential expression is cis-regulated. Notably, *Glyma.18G270900* shares homology with *FERONIA* in *Arabidopsis thaliana*, a well-characterized receptor-like kinase involved in modulating the ethylene response, reactive oxygen species–mediated root hair development, and suppression of jasmonic acid signaling.

Glyma.18G270900 belongs to a gene family within the protein kinase superfamily, with several members co-localized with phQTL_18b and exhibiting high sequence similarity to the target gene. To explore this further, long-range PCR was used to amplify two neighboring homologs, *Glyma.18G271000* and *Glyma.18G271100*. Sequence alignment to the Williams 82 reference genome revealed that *Glyma.18G271100* in Sloan harbors numerous SNPs, warranting further investigation.

To assess the functional impact of these polymorphisms, the resulting amino acid sequences will be analyzed to predict potential effects on protein function. Additionally, a gene tree constructed using kinase family members from *Glycine max* and related species will provide insight into their evolutionary relationships and homology. The dN/dS ratios will be calculated using whole-genome resequencing data from SoyKB accessions to evaluate the selective pressures acting on this gene family. Furthermore, expression profiling under *P. sojae* inoculation, particularly focusing on salicylic acid-responsive expression, will help elucidate their roles in defense signaling. These combined approaches will not only clarify the function of this gene family but may also identify potential targets for gene editing to enhance disease resistance in soybean.

Poster Presentation #167

Deciphering Core and Context-Specific Gene Regulatory Networks in Soybean Through Multi-Study Transcriptomic Analysis

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Soybean (Glycine max), the second-largest cash crop in the United States, is increasingly threatened by a range of soilborne pathogens such as Fusarium virguliforme, Phytophthora sansomeana, Phytophthora sojae, and Heterodera glycines. These pathogens cause significant economic losses (164.9 million bushels) and continue to challenge crop resilience. Traditional management strategies primarily rely on race-specific resistance genes, which offer limited durability due to rapid pathogen evolution. This evolutionary pressure underscores the need to move beyond single-gene solutions and instead focus on the broader transcriptional regulatory networks that orchestrate soybean immune responses.
To this end, we developed a comprehensive systems biology approach to uncover conserved and context-specific transcriptional regulatory mechanisms across six independent soybean RNA-seq studies involving responses to the aforementioned pathogens. Using DESeq2, we identified differentially expressed genes (DEGs) within each dataset. The DEG profiles were clustered using k-means to reveal co-expressed gene modules. These modules were functionally annotated and integrated with transcription factor (TF) data from PlantTFDB and public TF binding information to reconstruct gene regulatory networks (GRNs).
The analysis revealed two distinct regulatory classes: (1) broadly conserved TFs that maintain essential immune functions, and (2) specialized TFs that are selectively activated in response to specific pathogens. This study represents the first cross-experimental GRN synthesis in soybean pathogen interaction, offering new insights into transcriptional control and highlighting critical regulatory nodes with potential for crop improvement.

Poster Presentation #168

Assessing inoculation methods for screening soybean varieties against white mold under greenhouse conditions

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White mold can cause marked yield losses in soybean. Effective inoculation methods to screen for resistance to white mold are essential for soybean breeding. We assessed three inoculation methods under greenhouse conditions using a set of 30 elite varieties and plant introduction (PI) lines. We affixed agar gel pieces (0.5 cm x 0.5 cm) with fungal hyphae to the topmost fully grown bud of the 19-day plants in the first two methods. In one method, we covered the gel piece with a sticky transparent sealing tape; in the other, we wrapped the gel piece with a strap of moist paper towel. Thirdly, we employed the cut-petiole method (inserting filter tip with hyphae containing a gel piece to the cut end of the same aged petiole). We maintained the plants under low light conditions in growth chambers with misting programmed for 30 secs every 10 mins. After 11 days of inoculation, we measured the length of the lesion. The paper towel method, although easier than employing sealing tape, provides inconsistent disease development. Sealing tape and cut-petiole methods provide consistent disease development. The cut petiole method required three labor hours to inoculate 90 pots with each pot containing five plants. The sealing tape method was more tedious and required nine labor hours to inoculate a similar set of plants. By observing the mean lesion lengths of checks, we considered 7 mm long lesion from both sealing tape and the cut petiole method as the cut-off to identify partially resistant genotypes. We found eight partially resistant genotypes. The sealing tape method is not feasible for screening a large number of plants. As the cut-petiole method does not account for epidermal resistance, we currently assess the effectiveness of spraying mycelia using a sprayer and dropping mycelia using a squeeze bottle as alternative inoculation methods.

Poster Presentation #169

Rhizosphere Microbiome Dynamics in Soybean Under Fusarium virguliforme Stress

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Sudden death syndrome (SDS), caused by a soil-borne fungus, Fusarium virguliforme, is one of the major diseases of soybean with limited disease management options. Previous studies have revealed that the rhizosphere microbiome is a complex and dynamic ecosystem that significantly influences soybean health by providing a defense barrier against soil-borne pathogens. Therefore, understanding the interactions between soybeans, F. virguliforme, and rhizosphere microorganisms can offer microbial candidates for SDS management. We utilized a novel long-read amplicon sequencing platform from Oxford Nanopore Technologies to profile bacterial and fungal communities of two commercial soybean cultivars, with and without F. virguliforme inoculation. The results indicated a significant decline in the abundance of the nitrogen-fixing bacterial genus Bradyrhizobium following pathogen inoculation. Further analysis using a customized RNA extraction and library preparation workflow confirmed that inoculation with F. virguliforme also suppressed Bradyrhizobium activity. To explore potential interactions between F. virguliforme and Bradyrhizobium, we performed an in vitro antagonism assay, which revealed no direct interaction between the two microbes. This result suggests that F. virguliforme likely impairs root nodulation in the plant, indirectly limiting the recruitment of Bradyrhizobium. The observed disruption of beneficial bacteria like Bradyrhizobium by F. virguliforme highlights the importance of maintaining a healthy microbial community for plant resilience. Further investigation into soybean root response to F. virguliforme stress could provide insights into the underlying mechanism involved in the host-mediated suppression of beneficial bacteria. Such research could advance our understanding of the molecular approaches to microbial interventions for SDS management.

Poster Presentation #170

Genomic Characterization and Phylogenetic Analysis of *Calonectria ilicicola* isolates from Illinois Soybean Fields

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Soybean production is increasingly affected by fungal pathogens such as *Calonectria ilicicola*, the causal agent of red crown rot which has progressively affected the soybean fields across Midwest since its first detection in Illinois in 2018. Despite its emerging significance, population structure and genomic data on *C. ilicicola* populations in the U.S. remains limited. Our initial observations of isolates collected across Illinois (manuscript in preparation) revealed minimal sequence divergence of seven PCR amplicons commonly used for phylogenic analyses of *Calonectria sp*. Therefore, to gather and assay more genetic data, we conducted whole genome DNA sequencing using Illumina short read technology and identified sequence variations for phylogenic analysis. Variant calling was conducted using the Sentieon pipeline, followed by phylogenetic inference with IQ-TREE. Two related fungal species, *C. pseudonaviculata* and *Fusarium virguliforme* were used as outgroups for tree rooting. Variant calling identified 757,939 variants which were filtered down to 407,720 high confidence polymorphic SNPs. Although the average missingness was 87%, the dataset retained strong natural signal for genomic variation and was not imputed. Chromosome 6 exhibited the highest SNP density, while Chromosome 8 had the least. Transition and transversion counts were 367,209 and 132,885, respectively, yielding a Ti/Tv ratio of 2.76 which is indicative of a high-quality SNP dataset. The preliminary phylogenetic reconstruction suggests possibly six major clades, with isolate groupings showing decent alignment to geographic origin. Having produced 10,000X more sequence data, this work uncovers genome-wide variations not previously detected using the PCR amplicon data. However, the results are still consistent with the overall conclusion that genomic divergence among the US field isolates is low. This study provides a genomic framework for *C. ilicicola* in the U.S., highlights the local population structure, emphasizes the need for a MidWest-relevant reference genome, and promises to serve as a tool for surveillance and breeding.

Poster Presentation #171

Not Assigned

Poster Presentation #172

Molecular, genetic, and evolutionary study into enhancing seed quality traits and yields of US soybean

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Seed protein content, oil content, and yield are three of the most important output traits in soybean improvement, significantly impacting soybean economic value, US farmer's profitability, and the competitiveness of U.S. soybeans in the global market. However, each trait is regulated by multiple interacting QTLs, associated with each other and the other important agronomic traits. To date, over 300 quantitative trait loci (QTLs) associated with seed oil and protein content have been reported and located all over the soybean genome, underscoring the complex genetic regulation and coordinated activity of numerous genes and biological pathways governing seed protein and oil. It is often observed that the strong negative correlation of seed protein content with oil content, and yield present a major challenge for breeding programs aiming to optimize these economically critical traits. There is a strong and urgent need to understand molecular basis of those QTL and gene network controlling the major traits for determining feasibility of using the QTLs, how to use it and design effective breeding and editing strategies and tools for soybean seed quality and yield improvement.

Poster Presentation #173

Cell-type-resolved transcriptomics sheds light on SCN-induced remodeling and resistance in soybean roots

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Soybean cyst nematode (SCN) (Heterodera glycines) is a small plant-parasitic roundworm that feeds on soybean roots and is the most damaging soybean pathogen in the United States, causing approximately $1.5 billion in yield losses annually. SCN enters the root and induces the formation of a multinucleated feeding site called a syncytium by reprogramming host cellular processes. Traditional bulk transcriptomics lack the resolution to uncover cellular heterogeneity. Here, we present a study utilizing single-nucleus RNA-sequencing (snRNA-seq) to dissect early cellular responses in soybean roots during SCN infection. We identified major root cell types and found distinct transcriptional perturbations across various cell types. The most extensive changes were observed in vascular tissues, including xylem, phloem, and vascular cambium. SCN modulated cell type-specific expression of immune receptor genes NBS-LRR (CC, TIR, and RPW8-type classes) and hormone pathways (jasmonic acid, salicylic acid, and ethylene). We observed cell-type-specific expression of cell cycle genes, indicating a coordinated manipulation of nuclear proliferation in syncytium formation. Additionally, SCN enhanced induction of genes involved in vesicle trafficking, exocytosis, endocytosis, autophagy and vacuolar transport in cell specific manner, suggesting SCN facilitates massive remodeling to allocate resources for syncytium development. We also assessed cell-type-specific expression patterns of key genes from major SCN resistance loci such as Rhg1, Rhg2, Rhg4 and qSCN10. By employing confocal microscopy (using promoter-GFP) we have validated SCN infection and cell type-specific induction of marker genes identified in our SnRNA-seq dataset. Furthermore, by using CRISPR-Cas9-mediated gene targeting, we functionally characterize the role of novel SCN-induced genes. Our study provides vital insights into the cellular regulatory networks influenced by SCN infection in soybean roots at the single cell level, thus laying the foundation for developing a novel and durable source of SCN resistance.

Poster Presentation #174

Supercharging Soybean Development: How Comprehensive Genomic Resources Accelerate Trait Discovery and Breeding

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Biotic and abiotic stresses cause devastating yield losses in soybean, with diseases and environmental constraints reducing global productivity significantly. To address these challenges, we developed a comprehensive suite of genomic resources following a strategic progression from capturing broad genomic diversity to targeted breeding applications. Initially, we constructed a high-resolution first-generation haplotype map by mapping germplasm-scale sequencing data from diverse cultivars, landraces and wild relatives to the improved Williams 82 reference genome, capturing fine-scale recombination patterns and population structure. Further, we captured the genus-wide allelic diversity by sequencing and de novo genome assembly of 119 phylogenetically and geographically representative soybean lines using PacBio HiFi long-read sequencing technology. The assemblies exhibited high contiguity with an average contig N50 of ~18Mb and BUSCO completeness scores of ~99.2%. Subsequently, we developed a graph pangenome revealing extensive structural variations and presence-absence variants previously undetectable through single-reference approaches. This graph pangenome enabled us to enhance our original haplotype map by incorporating structural variants, creating a comprehensive variation landscape that captures both SNPs and complex genomic rearrangements. Leveraging this enriched haplotype framework, we are conducting genome-wide association studies that are expected to identify novel quantitative trait loci and structural variants controlling resistance to major pathogens as well as tolerance to drought and heat stress. Finally, we developed a cost-effective 6K AgriSeq SNP panel by systematically selecting most polymorphic markers from the first-generation variation map, prioritizing SNPs with optimal minor allele frequencies and genome-wide distribution to maximize breeding utility. This integrated genomic toolkit helps accelerate breeding for stress resistance, enabling precise selection for complex traits while reducing variety development time from decades to years, supporting sustainable soybean production under increasing environmental pressures. This comprehensive genome resource provides insights into the genus' genomic architecture and evolutionary history, serving as a valuable resource for future breeding programs and functional studies.

Poster Presentation #175

Advancing Spatial Transcriptomics for Soybean Research: High-Resolution, Cost-Effective Spatial Profiling

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Understanding spatial-temporal gene regulation is essential for studying complex traits in plants, such as seed development. Our recent work has used spatial transcriptomics to transform this understanding by creating detailed spatial transcriptome atlases. This foundational research uncovered shared gene programs that underlie multicellularity across different plant lineages, providing unprecedented cell-type-resolved insights into key processes like endosperm development and embryonic fate determination. Building on this, we have adapted and optimized OpenST, an open-source, low-cost spatial transcriptomics platform, specifically for developing soybean seeds. We successfully generated high-quality spatial libraries for soybean seeds, uncovering unique transcriptional profiles at a resolution much higher than 10X Visium. Our adapted OpenST significantly lowers the cost per library, making high-resolution spatial transcriptomics more accessible and widely available.

Poster Presentation #176

Climate-adaptive soybean: root architecture and plasticity tuning for nutrient capture and stress resilience

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U.S. soybean production is challenged by the need to improve yield under adverse climatic conditions. Roots are the key organ that enables plant soil anchorage, water and nutrient absorption, biotic and abiotic stress signaling, and therefore one of the major factors that can improve yield in stress environments. Adapting soybeans for sustainable production environments requires expedient tapping of genetic resources to capture effective root system architectural (RSA) and plasticity traits that provide multiple stress resilience. Prior root phenotyping of soybean germplasm is largely limited to single-time-point measurement of root morphology, missing root developmental and plasticity dynamics. Here, we report a high-throughput root phenotyping system with support from the United Soybean Board (USB). This system integrates Internet of Things (IoT) sensors and RGB imaging sensors for time-dependent monitoring of soil water stress and its impact on root and shoot growth dynamics. Standard RSA traits like root length, average diameter, surface area and root volume were non-destructively estimated. Several key root developmental traits (root tip count, angle, elongation rate, age-dependent radial root growth, root length per volume & rooting depth) were quantified to study root plastic variations on a spatial-temporal scale. The imaging sensor-based direct estimation of these root traits showed a high level of accuracy ($R2 > 0.94$ at p value < 0.001) with ground truth throughout the growth stages of soybean. Among the root traits, a novel stress adaptive trait (dark lesions) was found to be highly plastic in flooding-tolerant and sensitive genotypes under varied water stress levels. The genetic analysis of these RSA traits showed a significant genetic variation in carbon allocation patterns among soybean genotypes. The novel germplasms identified with contrasting RSA traits can be potentially utilized for molecular-assisted breeding or gene editing to develop smart soybean ideotypes for higher yield in unfavorable farming situations.

Poster Presentation #177

Dual stress response of near-isogenic soybean lines to *Fusarium graminearum* and iron deficiency

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Iron deficiency and infection by the fungus Fusarium graminearum (Fg) are major abiotic and biotic stresses that limit soybean productivity in the upper Midwest of the US. These two stresses are frequently present in the same fields, yet their combined effects on soybean plants remain poorly understood. In this study, we used RNA-Seq to analyze transcriptome changes that occurred in soybean near-isogenic lines Clark (PI548553, iron efficient) and IsoClark (PI547430, iron inefficient) in response to F. graminearum infection and iron deficiency. Clark and Isoclark were grown in hydroponic system for two weeks in one of the four treatments: i) iron sufficient conditions without infection (control), ii) iron sufficient condition and Fg infection, iii) iron deficient conditions without infection and iv) iron deficient conditions and Fg infection. Phenotypic evaluation included growth parameters, chlorophyll content and root morphology. RNA sequencing generated 1.9 billion reads, 1.7 billion of which (~84%) mapped uniquely to the soybean genome. A total of 33,659 genes in leaves and 35,028 genes expressed in roots were expressed. Differentially expressed genes were clustered across genotypes responding to single stresses and combinatorial stresses in both roots and leaves. The genes identified by this research provide valuable insights into the understanding of iron-Fusarium interactions that could be useful in the development new approaches to broadening resistance of soybean.

Poster Presentation #178

Branching out: How genotype and row spacing shape soybean branch angle

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Soybean shoot architecture plays a critical role in light interception, resource allocation, and yield potential, yet remains underexplored compared to major crops such as wheat and rice. Branch angle is an important trait influencing canopy coverage and structure. This project investigates the impact of row spacing and genotype on soybean shoot morphology. A total of 10 genotypes chosen for diverse branching habits were grown using two row spacings – 30 inches and 15 inches. We assessed the interaction between genotype and row spacing on branch angle. Using an analysis of variance on data collected in 2022 and 2023, we found that both genotype and row spacing had a strong effect on branch angle ($p < 0.0001$). Specifically, plants grown in wider rows had a significantly wider mean branch angle (40.2°) compared to those in narrow rows (35.7°). Genotype also had a strong effect, with branch angles ranging from 29.2° (PI404188A) to 45.6° (PI612717) across the 10 genotypes. Additionally, year effects were significant ($p < 0.01$), as the magnitude of the row spacing effect differed between years. The interaction between genotype and row spacing, however, was not significant. Together, these complementary studies advance our understanding of how agronomic practices and genetic factors shape soybean shoot architecture. The findings will support soybean breeding and management strategies aimed at optimizing plant structure for maximum yield.

Poster Presentation #179

Genetic and Epigenetic Responses Insight into Soybean Resistance and Susceptibility to *Phytophthora Sansomeana*

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Phytophthora root and stem rot (PRSR) is one of the most destructive diseases in soybean (*Glycine max*), causing substantial global yield losses. While considerable progress has been made in understanding and managing PRSR associated with *Phytophthora sojae*, relatively little is known about the newly recognized pathogen, *P. sansomeana*. While resistance genes have proven effective against *P. sojae*, no resistance genes have been identified in soybean for *P. sansomeana*. Through the screening of 470 soybean lines, we identified 16 promising soybean lines, particularly the 'Colfax' variety, conferring resistance to *P. sansomeana*. Our QTL mapping results indicated that a single dominant gene plays a pivotal role in conferring resistance to *P. sansomeana* in Colfax. Further understanding the molecular responses, we conducted a comprehensive analysis of transcriptomic and epigenetic responses of two resistant (Colfax and NE2701) and two susceptible (Williams 82 and Senaki) soybean lines at four time points after *P. sansomeana* inoculation, and identified a great number of differentially expressed genes (DEGs), long non-coding RNAs (lncRNAs), and transposable elements in the resistant lines. These DEGs were associated with multiple phytohormones, including ethylene, salicylic acid, and jasmonic acid, along with various transcription factors and signaling cascade proteins. In combination with our QTL mapping results, we identified two *LURP-one-related* genes and a lncRNA within the mapped region that exhibited significant differential expression exclusively in the resistant lines after inoculation, suggesting their potential roles in regulating the responses to the pathogen. Furthermore, our methylome analysis also demonstrated dynamic local DNA methylation changes, particularly alterations in CHH (H = A, T, or C) methylation, in soybean in response to *P. sansomeana*. These findings collectively contribute valuable insights into the intricate genetic and epigenetic mechanisms governing soybean resistance and susceptibility to *P. sansomeana*.

Poster Presentation #180

Decoding soybean cyst nematode infection using single-cell technologies

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The soybean cyst nematode (SCN) is the most damaging pathogen affecting U.S. soybean production. SCN forms a specialized feeding site, the syncytium, by reprogramming root cells within the vascular cylinder. Although previous studies have characterized the morphological and physiological changes during syncytium formation, its precise cellular origin and developmental trajectory remain unresolved. To address this, we applied an integrated single-cell approach—including single-nucleus RNA-seq (snRNA-seq), single-cell ATAC-seq, and spatial transcriptomics—to mock and SCN-infected soybean roots at 5 days post-inoculation (DPI). By integrating our snRNA-seq data with a legacy laser-capture microdissection (LCM) transcriptome dataset, we found that syncytia at 2, 5, and 10 DPI most closely resemble procambium cells, not pericycle cells as previously thought, suggesting a procambial origin. Our data further reveal three transcriptionally distinct stages of syncytium development: 1. Early Stage – marked by upregulation of genes involved in cell wall remodeling; 2. Endoreduplication Phase – characterized by elevated S-phase gene expression and G2/M inhibition, indicating DNA replication without cell division; 3. Established Syncytium – defined by G1/S gene expression and suppression of defense-related pathways, consistent with a stable feeding site. To identify candidate resistance genes, we intersected cell-type-resolved expression data with known SCN resistance QTLs. Within qSCN3-1, GmWRKY28, a defense-related transcription factor, was specifically downregulated in syncytium cells. Functional validation using a hairy root assay demonstrated that GmWRKY28 overexpression significantly reduced SCN infection, nominating it as a strong resistance candidate. Together, these findings redefine the cellular origin of SCN-induced syncytia, uncover transcriptional programs driving their development, and provide molecular targets for improving SCN resistance in soybean.

Poster Presentation #181

SCN resistance protein WI12$_{Rhg1}$ modulates core defense and GA signaling, and may be 55 amino acids longer than annotated

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Rhg1 continues to be a primary means of control of soybean cyst nematode. *Rhg1* is a multi-gene locus that encodes three proteins that contribute to SCN resistance. WI12$_{Rhg1}$, the least-characterized *Rhg1*-encoded protein, carries a 'wound-inducible 12' domain. Plant WI12 proteins have no known function but have been implicated in reproductive development and responses to various biotic and abiotic stresses. This work investigated the SCN resistance function and evolutionary context of WI12$_{Rhg1}$. Silencing *rhg1-b* WI12$_{Rhg1}$ in transgenic plants compromised SCN resistance. WI12$_{Rhg1}$ contributed significantly to resistance against an HG 2.5.7 SCN population that partially overcomes *rhg1-b* and reduces modern soybean production. Transcriptomic and metabolomic dissection revealed that silencing WI12$_{Rhg1}$ causes multiple changes to established defense pathways, including strongly reduced accumulation of salicylic acid, inhibited induction of some defense signaling and lignification pathways, reduced expression of many receptor-like cytoplasmic kinases, and dysregulation of gibberellic acid (GA) signaling. This work also clarified conflicting reports regarding GA impacts on SCN resistance, as exogenous application of GA increased SCN resistance. WI12$_{Rhg1}$ may disrupt the capacity of SCN to manipulate GA pathways. Exploration of *WI12$_{Rhg1}$* gene structure and phylogeny revealed a non-canonical in-frame CTG translation start site upstream of the annotated ATG start site and found that this *WI12* CTG and resulting 55 amino acid N-terminal extension are highly conserved across land plant lineages, with *Medicago truncatula* ribo-seq data indicating translation from the CTG. We are presently working to confirm use in soybean of the WI12$_{Rhg1}$ CTG start site. Immunoblotting revealed that WI12$_{Rhg1}$ forms stable higher molecular weight complexes, potentially due to heterologous protein-protein interactions. Together, these findings establish WI12$_{Rhg1}$ as a broad modulator of plant defense and hormone signaling, highlight non-canonical translation initiation in the *WI12* gene family, and suggest that the correct WI12$_{Rhg1}$ may be a longer 17.7 kDa protein.

Index

A

B

C

D

E

F

G

H

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

MEMORIAL UNION BUILDING MAP

MEMORIAL UNION