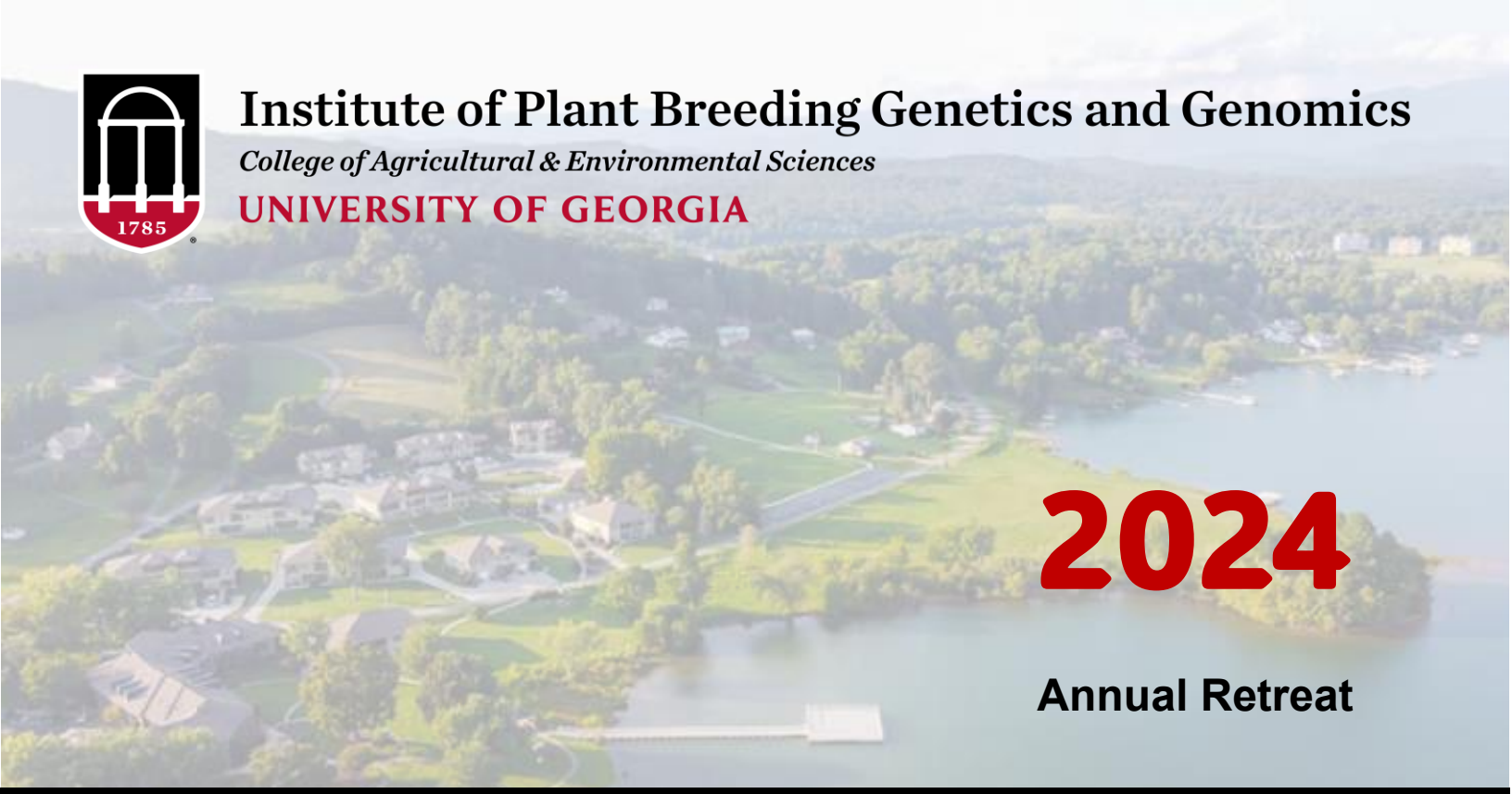

Institute of Plant Breeding Genetics and Genomics

College of Agricultural & Environmental Sciences

UNIVERSITY OF GEORGIA

2024

Annual Retreat

Abstract booklet

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MS Category

1. Amelia Boettcher

MS student

Screening for Resistance to Gummy Stem Blight in an Interspecific F2 Population

University of Georgia Institute of Plant Breeding, Genetics, and Genomics

Co-Authors: N/A

Gummy stem blight (GSB) is a fungal disease caused by three genetically distinct *Stagonosporopsis* spp. that infects watermelon and other cucurbits, causing large losses in yield. *Stagonosporopsis citrulli* has the largest geographical distribution and is widespread across the southeastern United States. Currently, there are no commercial cultivars with GSB resistance that can hold their genetic resistance in the field. It is important to search for new sources of resistance for future breeding programs. The objective of this screen was to collect phenotypic and genotypic data on an F2 population derived from an interspecific cross between the elite cultivar 'Sugar Baby' (*C. lanatus*) and the resistant PI 482379 (*C. amarus*). The screening was conducted in a humidity tunnel in the greenhouse for three days, with symptoms recorded seven days post-inoculation (7dpi) after spraying the plants with the aggressive *S. citrulli* isolate 12178A. There were 537 plants across 11 trays, with each tray containing 2 of each control and 33 F2s, along with two treatments across two repetitions. At the same time, there

were 170 plants across 4 trays with two treatments that were used for gene expression studies. Samples were collected before inoculation, one-, and two-days post-inoculation (1dpi, 2dpi). Phenotypic data was collected on true leaves and stems, and scored on a scale of 0 to 10, with 0 = no infection and 10 = dead plant. The results show that there is a wide range of resistance across the F2 population, including a few genotypes with promising disease resistance. Three of the best genotypes were selected to continue in the breeding program. The results of this research will aid in the selection of GSB-resistant watermelon for future breeding programs.

2. Stephanie Botton

MS student

Exploring Peanut Root-Knot Nematode Resistance in *Arachis cardenasii* Introgressed Cultivated Peanut

Co-Authors: Walid Korani (3); Josh Clevenger (3); Patricia Timper (4); Ye Chu (1,2); Corley Holbrook(4); Peggy Ozias-Akins (1,2)

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Peanut root-knot nematode (Meloidogyne arenaria; PRKN) is a microscopic roundworm that preys on the roots of many crops, including cultivated peanut (*Arachis hypogaea*). Without mitigation, these roundworms can lead to major yield losses for growers. In 2020, PRKN was responsible for a 3% reduction in the peanut crop value in the state of Georgia. To combat this pest, strong genetic resistance from a wild relative (*A. cardenasii*) was introgressed into peanut in the 1990's. Genetic studies revealed that this introgression covers ~92% of chromosome A09 in cultivated peanut. It was also revealed that the upper portion of the introgression was responsible for strong resistance while the lower portion yielded a moderate resistance. Beyond this analysis, little is known about the exact locations of the responsible resistance genes. The objective of this study was to perform PRKN greenhouse assays on recombinant peanut lines. Results from these trials hopefully will bring further understanding of this introgression that will help breeders develop improved cultivars that have a stable and strong resistance.

3. Caitlin McCann

MS student

Data mining of the Cooperative Regional Moderate Chill Peach Variety Development Project UGA-UF-USDA Pedigree Database and Historical Phenotypic Evaluations

Co-Authors: McCann, C.1, P. Conner2,3, J.X. Chaparro4, T.G. Beckman5, and D.J. Chavez1,6. 1Institute of Plant Breeding Genetics, and Genomics. University of Georgia, Griffin, GA 30223. 2Institute of Plant Breeding Genetics, and Genomics. University of Georgia, Tifton, GA 31793. 3Department of Horticulture. University of Georgia, Tifton, GA 31793. 4Horticultural Sciences Department. University of Florida, Gainesville, FL 32611. 5Southeastern Fruit and Tree Nut Research Laboratory. USDA-ARS, Byron, GA 31008. 6Department of Horticulture. University of Georgia, Griffin, GA 30223.

UGA IPBGG, University of Florida, USDA

The Cooperative Regional Moderate Chill Peach Variety Development project commenced in 1986 as a cooperative regional effort involving the USDA-ARS (Byron, GA), the University of Georgia (Tifton, GA) and the University of Florida (Gainesville, FL). Originally, the project was sited near Quitman, Georgia but moved in 1991 to its current location at the University of Georgia Research and Education Center outside of Attapulgus in the SW corner of Georgia. Its goal was and still is to develop new peach and nectarine varieties adapted to the lower coastal plain shipping industry of the southeastern United States. Since its establishment, pedigree records and phenotypic data have been recorded. However, this data has never been consolidated and mined to aid with the breeding process. The purpose of this project is to use this data to better understand our breeding germplasm through pedigree visualization and data analyses. Different pedigree analyses software will be tested. These analyses will allow the breeding program to make prudence decisions regarding the improvement of the peach germplasm.

4. Mylee Mobley

MS student

Evaluation of Cowpea, *Vigna unguiculata* L., Resistance to the Cowpea Curculio

Co-Authors: Peggy Ozias-Akins, David G. Riley, Shyam Tallury & Amanda Brooks

UGA IPBGG graduate student & USDA-ARS-PGRCU

The cowpea curculio, *Chalcodermus aeneus* Boheman, is the key pest of cowpeas in the southeastern United States. Even with moderate levels of infestation, over 50% of losses have occurred due to insect damage. The cowpea curculio causes damage to both the pods and the peas inside, through feeding and oviposition. Control of the cowpea curculio is difficult, the adults feed upon the pods and hide within the foliage, and the eggs and larvae are protected within the pod where the larvae cause the most damage. Currently, control tactics are entirely foliar insecticide applications that target the above-ground adult life phase and are failing to provide adequate control. The objective of this research is to evaluate cowpea germplasm for resistance and identify curculio resistant genotypes. Fourteen genotypes and a susceptible check were evaluated in four replicated, open-choice field experiments over three years. Insect damage was recorded on sub-samples of mature fresh green pods, measuring the feeding and oviposition marks on pods and peas and the number of larvae remaining in pods. Results confirmed the potential presence of resistance mechanisms. Based on these results, PI 533009, PI 533010, PI 582349, PI 582468, PI 663152, and a susceptible check were selected for a no-choice greenhouse experiment to further evaluate genotypic differences for pod and pea damage. While there was no significant difference in number of stings on pods, there was a significant difference in the number of damaged peas across genotypes with the susceptible check having the greatest damage. An additional replicated open-choice field experiment of the selected genotypes will be conducted to verify results. Research results will assist in future development of resistant varieties to the cowpea curculio.

5. Gema Nugraha

MS student

Finding Resistance: Exploring Wild Peanut Species to Combat Tomato Spotted Wilt Virus

Co-Authors: Chandler Levinson1, Ye Chu1, Walid Korani3, Josh Clevenger2,3, Soraya Leal-Bertioli2, David Bertioli2, Corley Holbrook4, and Peggy Ozias-Akins1

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Tomato spotted wilt virus (TSWV) significantly impacts the peanut industry. Despite its detection in the US over 50 years ago, no cultivated peanut variety has yet demonstrated high resistance to TSWV. Given the limited genetic diversity among cultivated peanuts, their wild relatives (*Arachis* spp.) offer a promising alternative for introducing beneficial alleles, including those for disease resistance. Our first objective was to evaluate the TSWV resistance of an uncharacterized collection of wild peanut species collected in the 1940s from South America and to generate interspecific hybrids that are cross-compatible with cultivated peanuts. These accessions were genotyped using the Axiom_Arachis2 48K SNP array and subsequently through targeted sequencing. All accessions were confirmed as A-genome species. Two years of field studies indicated that these accessions possess strong resistance against TSWV. These accessions were crossed with B-genome species and then treated with colchicine to generate allotetraploids that are cross-compatible with cultivated peanuts. Additionally, this study aimed to investigate the source of TSWV resistance QTLs in IpaCor (*Arachis ipaensis* x *A. correntina*)-derived populations. Prior analyses suggested that the resistance QTLs might originate from *A. correntina*. Two IpaCor4x hybrids were sequenced using HiFi technology; the assembled genome sequences were aligned with *A. ipaensis* to construct putative *A. correntina* genome sequences, resulting in genome sequences of approximately 1.4 Gb in size. These genomes provided valuable information for developing molecular markers in future breeding programs. This research offers novel disease-resistant resources from wild peanuts, potentially leading to the development of more resilient peanut cultivars.

6. Aasish Pokharel

MS student

Mapping seed size and shelling quality traits in an elite peanut population

Co-Authors: Aasish Pokharel, Zack Myers, Walid Korani, Josh Clevenger, Nino Brown

Institute of Plant Breeding Genetics and Genomics, University of Georgia Tifton Hudson Alpha Institute for Biotechnology, Huntsville, Alabama Institute of Plant Breeding Genetics and Genomics, University of Georgia Tifton

In the peanut industry, seed size is an important factor that determines the intended use of the product. It also drives consumer preference, processing efficiency, seed quality and yield potential. Maintaining diversity in seed size among breeding lines along with yield improvement is important to peanut breeding programs. An F2 mapping

population was developed by crossing peanut cultivars, 'Georgia-11J' and 'Tifguard' to map quantitative trait loci (QTL) regulating seed size. Phenotypic data was collected on pod samples from F2 individual plants. Leaf tissues were collected from these individual F2 plants and DNA was sequenced at HudsonAlpha. The initial mapping analysis indicated statistically significant, large-effect QTL for pod and seed size on chromosome 16 (B06); testa color on Chr. 6 (A06); and potential small-effect QTL for individual plant pod weight, seed weight, and sound mature kernel weight on several chromosomes. Individual plants were selected from segregating F3 lines to validate the markers and QTL regions identified. Heterozygous individuals from these segregating F3 lines were identified for potential fine mapping of the QTL of interest. The effect of these QTLs will be quantified in replicated trials in the coming seasons. Successfully mapping genes controlling seed and shelling characteristics will be of great benefit for incorporation into marker-assisted breeding programs.

7. Rachel Rackers

MS student

Understanding the role of miRNAs in AMF symbiosis to increase sorghum biofuel production

Co-Authors: Thomas H. Pendergast IV, Ching-Ting Huang, and Katrien M. Devos

The Institute of Plant Breeding Genetics and Genomics

Increasing biomass yields in biofuel crops, such as sorghum, while minimizing inputs and land use requirements is an important aspect in making bioenergy production more sustainable. To this end, understanding the genetics involved in nutrient acquisition and biomass accumulation can be valuable. In terms of nutrient uptake, arbuscular mycorrhizal fungi (AMF) are important microbial symbionts present in plant roots that aid the plant in obtaining essential nutrients like phosphorus. Some genes linked to AMF symbiosis have been found to be post-transcriptionally regulated by miRNAs, which are small non-coding RNAs that target mRNA sequences for degradation or inhibit translation to downregulate protein levels. My research seeks to identify miRNAs from a bioenergy sorghum panel of 337 accessions. Analysis focuses on determining potential roles miRNAs may have in mediating AMF symbiosis and biomass accumulation. Additionally, an expression GWAS will be conducted to identify expression QTL that control miRNA expression level variation across accessions. Better understanding how miRNAs contribute to higher biomass yields can be useful for selecting high performing biofuel sorghum lines and aid in breeding better lines for the future.

8. Skye Remko

MS student

Enhancing Switchgrass Transformation and Regeneration Through the Use of Morphogenic Regulators

Co-Authors: Pete LaFayette and Wayne Parrott

Institute of Plant Breeding, Genetics and Genomics

As switchgrass is developed as a biofuel, there is a need to use genetic transformation to confer traits for biofuel production that are not feasible to incorporate via traditional breeding techniques. Currently most switchgrass transformation uses tissue culture, but many genotypes are recalcitrant to this process. Agrobacterium-mediated switchgrass transformation currently requires mature genotypes to produce embryogenic callus to be used as an explant for transformation. However, specific genotypes of biofuel interest may not possess this ability. To allow for genotype-flexible switchgrass transformation, this project seeks to evaluate multiple morphogenic genes that have been used previously to improve transformation efficiency, increase somatic embryo production and improve plant regeneration rates after transformation. The primary objective of this study is to evaluate which combination of morphogenic regulators will allow plant regeneration from young, meristematic leaf tissue explants. The first construct replicates a methodology demonstrated in maize . It uses the overexpression of maize-derived morphogenic regulators, Babyboom and Wuschel2. This approach enables transformation process of immature leaf tissue, which is much easier to obtain than the conventional immature embryos. The second construct retains the use of the same morphogenic regulators but incorporates an additional chimeric protein, Growth Regulating Factor 4 – GRF Integrating Factor 1. This addition is anticipated to enhance plant regeneration. Each of these constructs also incorporates a Cre-loxP recombinase system to excise the morphogenic regulators prior to plant regeneration. The subsequent two constructs incorporate the switchgrass morphogenic regulator PvWox2a: Wuschel-like homeobox 2a, whose maize homolog has been shown to induce regenerable somatic embryos.

9. Sydney Webb

MS student

Breeding for improved disease resistance in *Arachis hypogaea*: A focus on Tomato Spotted Wilt Virus

Co-Authors: Ye Chu, C. Corley Holbrook, Josh Clevenger, Walid Korani, Baozhu Guo, Albert Culbreath, Peggy Ozias-Akins

Institute of Plant Breeding, Genetics and Genomics, UGA Tifton

Arachis hypogaea (cultivated peanut) is an important crop in the U.S. Tomato spotted wilt virus (TSWV) can be highly detrimental to susceptible peanut varieties, specifically in yield loss. PI 576638 (also known as SSD6) has been identified as a source of resistance to TSWV in peanuts. NC94022, a resistant offspring of SSD6, was used to identify a quantitative trait locus (QTL) on chromosome A01 that is linked to TSWV resistance. Through a recombinant inbred line (RIL) progeny of SSD6 x Tifrunner, this QTL has been incorporated into eight populations within our breeding program for further evaluation of TSWV resistance. Field evaluation of these populations has identified improved TSWV resistance when compared to known susceptible varieties and favorable agronomic traits when compared to Georgia-06G. These populations however are not as resistant as their RIL parent or NC94022, indicating that additional resistance loci need to be discovered from this source. DNA of advanced lines in these populations was also screened for other traits of resistance and oleic acid content from their female parents. Progenies were identified with diverse oleic acid profiles and multiple resistant loci against late leaf spot and nematode, along with the known TSWV resistance. Further evaluation and advancement of these populations to select the best lines will provide growers with improved resistance to multiple diseases and favorable agronomic traits for a successful crop each season.

10. Dalton West

MS student

LINKAGE DRAG ASSOCIATED WITH INTROGRESSION OF MELOIDOGYNE INCOGNITA RESISTANT GENES FROM WILD RELATIVES INTO UPLAND COTTON

Co-Authors: Lubbers, E., Wan, S., Khanal, S., Davis, R., Jones, D., Kumar, P., Singh, R., Suassuna, N., Paterson, A., Chee, P.

Crop and Soil Sciences Department

The Southern root-knot nematode (*Meloidogyne incognita*) is the most economically important pathogen to cotton (*Gossypium hirsutum* L.) production in the United States. Commercial cultivars that possess high levels of resistance to root-knot nematode (RKN) have been available for some time but they are not popular among producers, possibly due to their lower yield potential compared to susceptible varieties. The RKN resistance genes, originating from an obsolete cultivar and wild accession, may be associated with deleterious effects when incorporated into elite material. The objective of this study was to determine if RKN resistance QTLs qMi-C11 and qMi-C14 are associated with linkage drag on agronomic and fiber quality traits. Eight resistant experimental lines and two near-isogenic lines possessing resistance QTLs were compared to their susceptible parents, and the results showed no substantial evidence of linkage drag for lint yield and fiber quality traits. However, the results suggest that the resistance QTLs may be causing a negative effect on lint percentage, which can be overcome through breeding efforts. Overall, the experimental lines expressed high levels of resistance to RKN in field and greenhouse screenings, and have excellent lint yield and fiber quality packages. The top performing RKN resistant experimental lines with high yield potential and excellent fiber quality packages will be publicly released to aid in future breeding efforts.

PHD Category

11. John Bagwell

PHD student

Identification of quantitative trait loci for field resistance to Hessian fly in the Southeastern US using two wheat recombinant inbred line populations

Co-Authors: Mohamed Mergoum (1,2), Madhav Subedi(1), Bikash Ghimire(1,3), Luis Rivera-Burgos(4), Daniela Miller(5), Benjamin Lopez(2), Mohammed Guedira(5), G. David Buntin(6), Gina Brown-Guedira(4,5), Bochra A. Bahri (1,3)

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Hessian fly (HF) is an insect that can cost wheat farmers millions of dollars by substantially reducing yield and quality. HF research has been estimated at a 300:1 benefit-cost ratio, and genetic resistance can provide a cost-effective, eco-friendly control solution. Emerging HF biotypes are defeating the few effective HF R genes in the US southeast, therefore novel resistance is warranted. To elucidate the HF field resistance of GA06493-13LE6, it was crossed with MPV57 and Hilliard to develop 186 and 202 recombinant inbred lines (RIL), respectively. Populations were evaluated in Plains and Williamson, GA from 2021-2023. Traits measured included visual scoring for infestation, percent infested tillers, and number of pupae/larvae per tiller. Thirteen significant quantitative trait loci (QTL) were detected via inclusive composite interval mapping. Six QTL were major, explaining up to 35.16% phenotypic variation (PV). Two major QTL flanked each other on chromosome 6A: QHf.ux1992.6A.1 snapping a 2.93 cM region (609-611Mb region in Chinese Spring v1.0; up to 11.39% PV) and QHf.ux2029.6A, snapping a 12.73 cM region (608-609Mb; up to 31.75% PV). Five H7 KASP markers underlaid QHf.ux1992.6A.1. H7 KASP marker KASP2639 underlaid QHf.ux2029.6A. R protein RPM1 was found 0.24 Mb from QHf.ux1992.6A.1. One disease resistance protein was found 0.317 Mb from the QHf.ux2029.6A peak. QHf.ux1992.1A.1 snapped a 6.8 cM region (8-14Mb, up to 25.91% PV) on chromosome 1A. SSR marker Xbarc263 and h4 KASP marker KASP974 were underlying QHf.ux1992.1A.1. GA06493-13LE6 provided most of the HF resistant alleles for significant QTL. 22 candidate genes discovered from chromosome 6A exome capture data were involved with disease resistance and may increase HF resistance via marker-assisted selection.

12. Emile Barnes

PHD student

Wild Species *Arachis stenosperma* Provides a Novel Source of Root-Knot Nematode Resistance in High-Yielding Backcross Lines of Cultivated Peanut

Co-Authors: Tim Brenneman, Nino Brown, Soraya Leal-Bertioli, David Bertioli

Institute of Plant Breeding, Genetics & Genomics (EB, SL-B, DB), Department of Plant Pathology (TB, SL-B), Department of Crop & Soil Sciences (NB, DB), University of Georgia, Athens GA 30602

Peanut root-knot nematode (PRKN, *Meloidogyne arenaria*) is a major pathogen of peanut that can incur yield losses in excess of 50% when not adequately managed. Currently, all PRKN-resistant peanut cultivars derive their resistance from an introgression from the wild peanut relative *Arachis cardenasii*. While this source of resistance has served growers well for nearly 30 years, the proactive introduction of new sources of resistance is crucial to preventing resistance breakdown. Previously, our lab has demonstrated a novel source of near-total RKN resistance from the wild species *A. stenosperma* in advanced backcross lines of cultivated peanut through controlled inoculation and in vitro bioassays. Presently, we sought to measure PRKN resistance in these backcross

lines in field conditions while also testing lines for yield and agronomic performance. Through trials at three field locations in Georgia during summer 2023, we found that (1) the previously identified locus on chromosome A02 provides PRKN resistance as strong as that found in resistant cultivars available to growers today; (2) backcross lines significantly outperform common cultivars under PRKN infestation; (3) lines with the introduced locus exhibited yields that were comparable to the commercial cultivars tested in the absence of PRKN pressure; and (4) backcross lines were comparable to, and in some cases outperformed, commercial cultivars in agronomic traits including seedling vigor, stand rating, 100-seed weight, pod constriction, and canopy closure. The lines presented here are being used in the development of peanut cultivars that are stably high-yielding and agronomically acceptable with strong genetic protection against PRKN.

13. Nathaniel Burner

PHD student

Improving soybean drought tolerance through genomics and phenotyping

Co-Authors: Nathaniel Burner, Price Akiina, Guoyu Lu, Donna K. Harris, Zenglu Li

Univ of Georgia, Univ of Wyoming

Drought is the most damaging abiotic stress for soybean, reducing yields by approximately 40%. This is a particular problem for soybean as only a fraction of harvested acres is irrigated. The severity of drought and damages is unpredictable across years. Therefore, it is necessary to develop drought tolerant soybean cultivars to reduce yield losses in unfavorable climate conditions yet are competitive in yield in favorable years. Drought tolerance is conferred by numerous physiological mechanisms governed by many genetic loci. Soybean breeders are challenged in identifying drought tolerance QTLs and introgressing them into elite backgrounds. Additionally, drought tolerance phenotyping is traditionally time consuming and subjective. A high-throughput phenotyping (HTP) platform would be a valuable tool for breeders for more efficient and objective data collection, providing additional metrics for genetic studies. The objectives of this research are to identify genetic variability underlying PI 603535 drought tolerant phenotype, develop an HTP methodology to associate spectral metrics with drought tolerance and to characterize QTLs derived from drought tolerant PI 416937. A NIL population was developed by introgressing the PI 416937 QTL into two elite breeding lines. These lines were evaluated under a rainout shelter and rainfed conditions. Preliminary results indicate that QTLs on Chrs 4 and 5 confer substantial reductions in canopy wilting. Thirteen QTL for canopy wilting and spectral metrics were identified across single environment analyses in a RIL population derived Benning × PI 603535. Additionally, using deep learning to estimate drought stress is being explored. Future work will focus on further characterization of NIL introgressions and confirmation of newly identified QTLs from PI 603535 by a high-throughput phenotyping platform.

14. Kamalpreet Kaur Dhillon

PHD student

Cloning and Characterization of Zoysiagrass Gene controlling Male Sterility

Co-Authors: Joann A. Conner1,2, Sameer Khanal1, Carlos Cardon1 and Brian M. Schwartz1,3

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Zoysiagrasses (*Zoysia* spp. Willd.) are allotetraploid (2n=4x=40) perennial warm season grasses that require little maintenance and tolerate drought, low soil fertility, and saline environments. However, the presence of fertile inflorescences in zoysiagrass can lead to unwanted seedling establishment and cross pollination with other zoysiagrasses. To address this issue, we explored reverse genetics approach to induce sterility in zoysiagrass. All land plants possess a tough sporopollenin wall that encloses and protects reproductive propagules from desiccation and UV-B radiation. Mutation in male sterility 2 (MS2) gene confers male sterility in grass species such as *Arabidopsis*, maize, wheat, barley and *Brachypodium*. Knocking out of a putative moss homologue of AtMS2 gene led to spores with highly defective walls and extremely compromised germination. Conversely, the moss PpMS2 gene could not rescue the Arabidopsis ms2 mutant pollen wall formation. Phylogenetic analysis of different land-plant orthologs of AtMS2 protein demonstrated high sequence similarity between AtMS2 and zoysiagrass ZjMS2 proteins and conservation of essential domains. Our gene expression analysis indicated tight spatio-temporal regulation of MS2 gene expression in zoysiagrass. The coding sequence of ZjMS2 gene was cloned and sequence verified by Sanger sequencing from 'Meyer' zoysiagrass flowers. A construct has been developed in the background of pMDC32 binary vector to determine if ZjMS2 complements Arabidopsis ms2 mutants. In the future, we aim to generate Zoysia knock-out mutants by CRISPR-Cas9 mediated genome editing. The impact of these pioneering efforts for the Zoysia breeding community could include increased vigor, better adaptation to the Southeastern United States, and eventual plant sterility for maintaining purity and uniformity in commercial cultivars. Key words: Arabidopsis thaliana MS2 (AtMS2), Physcomitrella patens MS2 (PpMS2), Zoysia japonica (ZjMS2).

15. Danielle Essandoh

PHD student

A new source of multiple resistances from amphidiploid, MagDio incorporated into cultivated peanut (*Arachis hypogaea*)

Co-Authors: Soraya Leal-Bertioli, David Bertioli

Center for Applied Genetic Technologies, University of Georgia Department of Plant Pathology, University of Georgia Department of Crop and Soil Sciences, University of Georgia

Globally, diseases significantly lower peanut yields, with late leaf spot (LLS) and groundnut rosette virus (GRD) being particularly devastating in East Africa. Groundnut rosette disease caused by a complex of three viruses is the most prevalent causing a yield loss of 100% annually. Resistance limited resistance exists in the pure pedigree of cultivated peanut lines and novel sources of resistance are required to develop new varieties. Efforts to diversify gene pool has resulted in the development of amphidiploid magdio generated from Arachis magna (accession K30097) and *Arachis diogoi* (accession V10602) which presumably may have strong resistance to leafspot, GRD and other important traits of interest in Uganda. Previous work by ICRISAT to identify alternative sources of

resistance to GRD revealed that resistance to all three components of GRD was found in the present *Arachis diogoi* accession used in this work. Similarly, studies on resistance to foliar diseases in peanuts have also demonstrated the role of Arachis magna in improving resistance to leaf spots and rust. In this work, 150 F2's generated from crosses between IAC 321, a leaf spot resistant cultivar and magdio was generated. The F2's have been phenotyped for pollen viability and also the number of flowers produced for each plant. Significant differences were observed amongst the genotypes for their pollen viability with most lines showing high values for viability; an indication that the wild parent, magdio has very little influence on the fertility of the lines. Following the initial assessment, F2 genotypes exhibiting high pollen viability were selected as male parents in crosses with TifNV-HG, GA-12Y, and Serenut 6T to confirm the presence of QTLs. Currently, the F2's are being advanced to F4 as part of the breeding effort to generate recombinant inbred lines.

16. Qian Feng

PHD student

Identification and Characterization of an E8 Paralog in Flavor Aroma Volatile Production in Tomato Fruits

Co-Authors: Yasin Topcu, Manoj Sapkota, Esther van der Knaap

Institute of Plant Breeding, Genetics, And Genomics, University of Georgia, Athens, United States.

Tomato is one of the highest valued fruit crops worldwide and an important part of a healthy diet in the United States of America. Flavor has become an important trait for targeted crop improvement. Because of the historical emphasis on yield and other agronomically important traits, many modern tomato varieties have lost their rich flavor, causing consumer dissatisfaction. The complexity of the biochemical networks that contribute to aroma, and the requirement for specialized equipment to evaluate each volatile make the study and the improvement of fruit aroma a challenging task. A GWAS of a diverse tomato panel companied with biparental population mapping with GBS was performed to discover novel loci that control volatile production in tomato fruits. A paralog of a known ethylene- and fruit ripening-induced gene tomato E8 was identified to affect the volatile production of various pathways. CRISPR-Cas9 was used to create single and double KOs of E8 and its paralog to validate and characterize the gene function in tomato volatile biochemical pathways. Haplotype study was also conducted to mine potential beneficial alleles for introgression into modern cultivars.

17. Anita Giabardo

PHD student

The High-Resolution Transcriptional Landscape of Xylan Biosynthesis: Insights from Single Cell RNA-seq

Co-Authors: Anita Giabardo, Amanda Woodstock, Deepti M. Nambiar, Brieanne Vaillancourt, Joshua C. Wood, Breeanna R. Urbanowicz, C. Robin Buell

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Poplar (*Populus* spp.) has emerged as a model organism for the study of tree physiology and as a potential renewable source of biomass for bioproducts and biomaterials production, such as biofuels and bioplastics. Xylan is the main hemicellulosic component of the secondary cell walls of woody dicots, representing around 20% of poplar woodchips dry weight. Despite their abundance, plant xylans are often underutilized or discarded due to their structural complexity and diversity. Through the targeted engineering of xylan structure, it is possible to increase the quality of hemicellulose in planta, making it more suitable for post-harvest processing and valorization. Hybrid aspen Populus tremula x P. alba INRA 717-1B4 (poplar 717) is a particularly suitable system for production of bioproducts and biomaterials due to its amenability to transformation and gene editing, fast growth, and rich genomic resources. To investigate xylan biosynthesis at single-cell resolution, we analyzed three publicly available single cell gene expression datasets derived from poplar stem tissues with the haplotype-resolved poplar 717 genome. These datasets encompass three different growth stages, from primary growth to transitional zone to secondary growth. We used a curated list of genes known to be involved in xylan biosynthesis to identify gene co-expression modules. We identified gene modules involved in the different steps of heterogenous xylan biosynthesis, including reducing end sequence biosynthesis, backbone elongation and decoration. The gene expression profiles show clear cell cluster specificity, suggesting that different biosynthetic steps may be partitioned into different cell clusters. This analysis will serve as foundation in the identification of targets for cell type-specific xylan engineering.

18. Kelly Goode

PHD student

Nematode Wars: Identifying Soybean's Genetic Arsenal Against Root-knot Nemeses

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Root-knot nematodes (RKN) are a major cause of yield loss for soybean production in the southeast US, costing an estimated $178 million in yield losses in 2022. *Meloidogyne incognita* is the most prevalent RKN species in the region. The wide host range of RKN species makes crop rotation a difficult strategy to implement for controlling RKN. Available nematicides are highly toxic, posing a threat to the environment and to those applying them. These conditions make genetic resistance the superior choice for managing yield loss due to RKN. Previous work has mapped a single additive gene for resistance to *M. incognita* (*Rmi-1*) to a region on soybean chromosome 10 containing no canonical resistance genes coding for receptor-like proteins. A larger mapping population of 883 F5 lines was created from a cross between the susceptible cultivar Bossier and the resistant cultivar Forrest and used to further refine the interval of candidate genes for Rmi-1 resistance. Promoter-GUS analyses revealed the temporal and spatial expression of these genes in response to RKN infection. Results of bioassays to test these candidates for a functional role in resistance using CRISPR-Cas9 gene editing and overexpression in transgenic hairy roots of soybean composite plants are underway. Identification of the causal gene(s) will allow for more targeted breeding against RKN, as well as guided implementation of the resistance to prevent RKN field populations from overcoming the Rmi-1 resistance.

19. Austin Hart

PHD student

FINE-MAPPING OF A NOVEL LOCUS REGULATING BCAA DERIVED VOLATILES IN TOMATO (*Solanum lycopersicum*)

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A major focus in plant breeding has been the improvement of crops through various traits that affect disease resistance and yield. However, the focus on productivity has led to an inattentiveness to other traits that specifically affect produce quality. An example of a critical fruit quality trait is its flavor, contributing to our perception of aromatic volatiles. Even at nanomolar concentrations, aromatic volatiles can be perceived by the olfactory system and influence the liking of the fruit. The focus of this study was to investigate the genetic aspect of the branched-chain amino acid (BCAA) volatiles, derived from L-valine, L-isoleucine and L-leucine, in tomato fruits. It is generally considered that these BCAA-derived volatiles contribute positively to overall liking, possibly because these are essential amino acids required by the human diet. To identify quantitative trait loci (QTLs) affecting the biosynthetic pathway for 11 BCAA-derived volatiles, I investigated a bi-parental population derived from two semi-domesticated tomatoes, BGV006232 and BGV007931. A genetic map was constructed from 148 markers across the genome using genotyping by sequencing. Linkage analysis was conducted using compositive interval mapping, in which a QTL on chromosome 2 was identified. To validate the QTL, progeny testing was conducted in the F3 population. This further resolved the QTL region to <800kb. Recombinant screening will be conducted in the Spring 2024, to further fine-map and evaluate the effect of the QTL on chromosome 2 for BCAA-derived volatiles. This study has been funded by NSF IOS 1564366.

20. Rachel Hill

PHD student

Identification and Distribution of *Ramulariopsis* Species in Georgia

Co-Authors: Peng Chee, Benjamin Culver, Edward Lubbers, Jennifer McBlanchett, Nelson Saussuna, Alejandra Jimenez Madrid, Ian Small, and Kaitlyn Bissonnette

IPBGG, EMBRAPA Brazil, UGA, UF, and Cotton Incorporated

Cotton, *Gossypium hirsutum*, is an important fiber crop in the southern United States and ranks as Georgia's number one row crop commodity. Ramularia Leaf Spot (RLS), also known as Areolate Mildew, is a late-season foliar disease characterized by necrotic lesions, white mildew, and defoliation. RLS in cotton can stem from two different species, *Ramulariopsis gossypii* or *Ramulariopsis pseudoglycines*. Historically, *R. gossypii* was presumed to be the primary causal agent in the U.S. However, a recent study in Brazil, where the disease causes 14% to 31%

losses in yield, revealed *R. pseudoglycines* as the causal agent for 94.4% of the natural infection. As the presence of Ramularia in Georgia cotton fields has increased, the identity and distribution of the causal species were brought into question. Building upon the methodologies established in the Brazilian study, PCR primers with the ability to distinguish between the two causal species were utilized in this study. The species specificity of these primers was confirmed via PCR of cultured isolates of each species, followed by Sanger Sequencing. During the 2023 growing season, 170 leaf samples presenting RLS symptoms were collected from cotton fields throughout the state. A method to extract the fungal DNA directly from the leaf without culturing was developed to expedite the process. Preliminary results identify *R. pseudoglycines* as the primary species present on leaf samples collected in Georgia in the 2023 season. These samples will be cultured, sequenced, and mapped to study the genetic diversity of the pathogen.

21. Anne Frances Jarrell

PHD student

Mining the wild species *Solanum microdontum* for the improvement of cultivated potato

Co-Authors: Joshua Wood (2), Jessica Norling (3), Joseph Coombs (3), David Douches (3), C. Robin Buell (1, 2, 4)

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Solanum microdontum is a diploid wild Andean relative of potato that has shaped the domestication and adaptation of modern cultivated potato to diverse environments. It has the potential to provide a wealth of untapped genetic material for use in addressing current challenges in potato breeding. This project contains two objectives, which together will provide breeders with genetic, molecular, and germplasm resources to be used within the context of newly developed diploid potato breeding programs. The first objective is to identify accessions of *S. microdontum* with characteristics that make them favorable for crossing with cultivated potato (S. tuberosum). Traits of interest include resistance to the late blight pathogen (*Phytophthora infestans*) and tolerance to heat stress. Toward this goal, a diversity panel of 117 *S. microdontum* lines has been phenotyped for late blight resistance, and six accessions have been identified as resistant to all four late blight isolates tested. The second objective is to is to generate a high-quality reference genome sequence for *S. microdontum* and to characterize genetic diversity within the species. The resulting genome assembly has a BUSCO score of 99.1%, indicating a high level of completeness. Illumina short-read sequencing data has been generated and quality control has been performed for each accession in the diversity panel, which will be utilized for the analysis of genetic diversity between publicly available *S. microdontum* accessions. The project will contribute to much-needed publicly available potato genome resources and permit robust data mining of *S. microdontum* trait loci.

22. Samuel Josiah

PHD student

Novel ClSUN25-26-27a Alleles and their Association with Ovary and Fruit Shape in Watermelon

Co-Authors: Cecilia McGregor, Douglas Vines

University of Georgia Department of Horticulture

The genetic mechanisms controlling fruit morphology in watermelon is not fully understood. Only one candidate gene, ClSUN25-26-27a (Cla011257), has been identified as a significant contributor to the ovary and fruit shape. ClSUN25-26-27a is a member of the SUN gene family, which has been extensively studied in relation to fruit morphology in tomatoes. In tomato, the effect on fruit shape facilitated by this gene is detectable during the early stages of fruit development, with the cell patterns established pre-anthesis. In watermelon, three alleles of ClSUN25-26-27a are known to be associated with ovary and fruit shape variation: the wild-type, a SNP, and a 159bp deletion in the 3rd exon. This study aims to determine the effect of the novel allelic variation for ClSUN25-26-27a on ovary and fruit shape. Four novel alleles have been identified and sequenced in the coding region of ClSUN25-26-27a across three Citrullus species. Marker assisted backcrossing was used to introgress the different alleles into a common genetic background. The NILs were phenotyped in the field to determine the effect of the novel alleles on ovary and fruit shape. Ovary length and width were measured four days pre-enthesis, at anthesis, and four days post-anthesis and used to calculate the ovary shape index (OSI). Mature fruit was also phenotyped using Tomato Analyzer. One of the novel alleles was significantly associated with ovary and fruit shape. These findings contribute to the understanding of the important SUN gene family and the genetic mechanisms contributing to watermelon fruit shape. Future research will determine the gene expression patterns of the different alleles and the effect of the alleles on the cell number or cell size in watermelon.

23. Prasanna Kharel

PHD student

Genetic Engineering of Antioxidant Genes for Aflatoxin Resistance in Peanut

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Reactive oxygen species (ROS) produced during heat and drought stress are linked with aflatoxin production in peanut. Therefore, developing peanut with high antioxidant enzymes may reduce aflatoxin contamination. Developing peanuts with high antioxidant activity through traditional breeding methods remains slow and partial due to high G*E interactions. As such, genetic engineering may provide this avenue. The objective of this research was to develop genetically engineered peanuts with different antioxidant capacities and link the antioxidant capacity of the host plant to its resistance against aflatoxin contamination. Gene encoding antioxidant enzyme catalase1 (CAT1) was overexpressed using overexpression cassette containing kanamycin and hygromycin selection markers, eGFP, actin2 promoter, complete gene encoding enzyme, and transcription terminator. In addition, CAT1 gene was mutated using CRISPR/Cas9 to disrupt its function. The constructs were delivered using biolistic method. Successful transformants (T0) were identified on a hygromycin selection medium and planted in the greenhouse to produce T1 seeds. GFP-positive T1 seeds from overexpression lines were selfed to produce T2 seeds. A high level of antioxidant enzyme activity and reduced aflatoxin contamination is expected for overexpression lines while the reverse is expected for CRISPR/Cas9 edited lines. So far, difference in catalase

concentration has not been observed in the seeds and leaves of the overexpression lines. Once the functional insertion lines are confirmed, their seeds will be used to further characterize their response to Aspergillus flavus infection. Successful completion of this experiment will provide evidence that enhancing antioxidant capacity is a valid approach to improving aflatoxin resistance.

24. Jordan Knapp-Wilson

PHD student

High-throughput Phenotypic Analysis of Peach Tree Architecture of a Diverse Panel of Rootstock and Scions with Varying Degrees of Vigor Utilizing 3D Modelling

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Grafting and the use of size-controlling rootstocks for commercial temperate fruit tree production has been of significant agronomic importance for centuries. Today, the ability to control and to manipulate scion vigor has become even more important. The advent of high-density orchard models to increase production efficiency and facilitate automation can be seen in different fruit systems. However, little has been reported on the effects on tree architecture that a diverse panel of rootstocks have on scions, both with varying degrees of vigor. In this study, we examine the differences in tree architectural traits (e.g., total number of branches, avg. branch length, tree height, canopy volume, etc.). Three peach scion commercial cultivars were used in this study: Elberta (medium-vigor), Flavorich (high-vigor) and Julyprince (high-vigor). Four rootstocks were used: MP-29 (low-vigor, dwarfing), Guardian (high-vigor), P-22 (medium to high-vigor) and P-47 (high-vigor). Trees were planted in 2016. Terrestrial laser scanning (TLS) and 3D modelling were used to create digital twins of these trees in the orchard for in-silico data collection. A total of 72 trees were modelled (3 scion cultivar × 4 rootstocks × 6 tree replicates). The effects of rootstock, scion, and rootstock × scion interaction on tree architectural traits were analyzed using two-way ANOVA in RStudio. Our results show that both the rootstock and scion factors explain a significant amount of variation for all tree architectural traits (p values ≤ 0.01). Differences between high/low-vigor rootstocks and scions were apparent during modelling, with the collected architectural trait data likewise supporting this. Julyprince for example, had an average difference of approx. 300 branches depending on if the rootstock used was either MP-29 or Guardian. A similar result was seen when Julyprince and Elberta on Guardian rootstocks were compared, with the average difference in branch number being approx. 250 branches.

25. Dallas Kreisa

PHD student

Locating the major resistance genes to Frogeye Leaf Spot in Soybean

Co-Authors: Sam McDonald(1), Shavannor Smith(2), James Buck(2), and Zenglu Li(1)

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Frogeye leaf spot (FLS), caused by *Cercospora sojina* K. Hara, is a fungal disease that causes leaf lesions, reduces photosynthesis and lowers yields in soybeans worldwide. Rcs1 was initially found in cultivar Lincoln in 1952 and provides resistance to some races of *C. sojina,* while Rcs3, discovered in cultivar Davis in 1982, is resistant to all known races in the US and Brazil. However, the genetic location of Rcs1 has not been identified. Our previous research indicates that the Rcs3 resistance originated in Davis or its parent N45-1497, since its grandparents Arksoy, Ralsoy, and Ogden are susceptible. We mapped the Rcs3 locus to a 1.15 Mb region containing over 100 genes on chromosome 16. This region could not be resolved further due to structural variation between these cultivars and the Williams82.a2 reference, and gaps in the reference genome. Our objectives are to map the Rcs1 locus using 182 F5-derived recombinant inbred lines (RILs) from a biparental cross of Forrest × Lincoln and to fine map the Rcs3 locus further to pinpoint candidate genes associated with FLS resistance. All plants were phenotyped for FLS disease response in a greenhouse. Bulked segregant analysis will be used to identify potential genes at the Rcs1 locus. To pinpoint Rcs3 candidate genes, DNA from cultivars Davis, Arksoy, Ralsoy, and Ogden is being sequenced using PacBio's Revio system. Sequencing data analysis is underway. Additionally, three near-isogenic lines (NILs) containing the Rcs3 resistance allele from Davis and their recurrent parents Gordon, Thomas, and Wright were genotyped using five markers flanking the Rcs3 region. The haplotype comparison between NILs and their recurrent parents confirmed the introgression of the Rcs3 allele and revealed the location of the Davis haplotype in the recurrent parent background. This research will lead to the identification of candidate genes and functional markers to be integrated into soybean breeding programs for breeding FLS resistance.

26. Samuele Lamon

PHD student

Stronger selection response in polyploid peanut than in wild diploid ancestors

Co-Authors: Samuele Lamon 1, Soraya Leal-Bertioli 1 & 2 & David Bertioli 1 & 3.'

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Peanut (*Arachis hypogaea* L.; 2n = 4x = 40; AABB), originated from the spontaneous hybridization of two diploid wild Arachis species*, A. duranensis* (2n = 2x = 20, AA) and *A. ipaënsis* (2n = 2x = 20, BB), followed by a whole genome duplication. The polyploidization process led to reduced genetic diversity in peanut, which also found itself isolated from the other diploid Arachis species due to ploidy inequalities. However, the merging of different genomes during peanut origin caused in peanut a genetic shock, which in return produced different types of genetic instabilities in the peanut genome. In this study, we hypothesized that early polyploid peanuts exhibited an increased response to artificial selection (domestication) compared to their wild diploid ancestors, displaying enhanced phenotypic plasticity. To test this hypothesis, we subjected colchicine-induced neopolyploids [*Arachis ipaënsis x Arachis duranensis*]4x (IpaDur1) and their wild diploid ancestors to selection for contrasting phenotypes over three generations, focusing on divergent traits such as heavy and light seed weight. IpaDur1s showed increased variability for several agronomic traits, e.g. flower color, chlorophyll content, seed weight, and pod shape. They exhibited increased allelic variability and displayed a superior response to artificial selection, along with enhanced phenotypic plasticity compared to their wild diploid ancestors. These findings support the hypothesis that genetic instability increased peanut's response to selection and conferred upon it the phenotypic plasticity and adaptability that favored its domestication and adaptation to different agroenvironments.

27. Namrata Maharjan

PHD student

Survey and deep sequence characterization of peanut viruses in Georgia, USA

Co-Authors: (Namrata Maharjan)1, (David J. Bertioli)1,3, and (Soraya C.M. Leal-Bertioli)1,2

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Tomato spotted wilt virus (TSWV) is a viral disease of peanuts, causing severe stunting and yield reduction. It has a tripartite genome consisting of L, M, and S segments, a host range of over 1000 species, and is found worldwide. This virus evolves rapidly resulting in novel isolates. With the introduction of wild-derived allotetraploid in the breeding schemes, TSWV encounters novel peanut hosts that are phylogenetically distant from cultivated peanuts. In this study, we aimed to conduct a survey and deep sequence characterization of TSWV isolates affecting wild-derived allotetraploids ([*A. gregoryi x A. stenosperma*]4x and [*A. vallsii x A. williamsii*]4x) and cultivated peanuts. In 2022, TSWV-infected leaves were collected from both induced allotetraploids and cultivated peanuts from Midville, GA. Total RNA was extracted and sequenced after rRNA depletion using Illumina NovoSeq4. De novo sequencing of TSWV isolates revealed high coverage (98-100%). Phylogenetic analysis demonstrated variation among the isolates from both sample types and with reference genomes, particularly in the M and S segments which exhibited lower pairwise identity with the reference genome from 1991. Furthermore, phylogenetic analysis was conducted using complete segments of TSWV isolates available in NCBI, revealing the clustering of four isolates from our study. We also observed different clustering patterns of other isolates from the USA: generally, isolates clustered predominantly by location, with multiple clustering observed for isolates from the same location. Interestingly, no significant pattern of clustering based on collection year or host was observed, suggesting the unpredicted yet consistent evolution of the virus. Additionally, peanut mottle virus (PMV) was detected in all samples sequenced in this work. These findings contribute to the understanding of TSWV evolution across diverse peanut hosts, laying the groundwork for future management strategies for this virus.

28. Daniel Matusinec

PHD student

Using Dual Mapping Populations to Identify Stem Rot Resistance in a Wild Peanut Species

Co-Authors: Mounirou H. Alyr1, Yun-Ching Tsai2, Mark S. Hopkins1, Soraya C.M. Leal-Bertioli1,2, David J. Bertioli1,3

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Peanuts are an important agricultural commodity in the American Southeast, particularly in Georgia, which produces most of the peanuts in the United States. However, cultivated peanut (*Arachis hypogaea* L.) is an

allotetraploid species with a narrow genetic base to select for disease resistance from. Stem rot, caused by the fungus *Athelia rolfsii*, is currently the disease that costs peanut growers in Georgia the most each year, upwards of $80 million between the cost of damage and control. Related diploid wild species provide the potential for introducing disease resistance traits to the cultivated peanut germplasm. The objective of this research is to identify stem rot resistance QTL in one of these wild species, *A. microsperma,* which has previously shown resistance to stem rot. Two diploid species, *A. microsperma* and *A. valida*, were used to develop a novel allotetraploid that was then crossed with cultivated peanut and used to produce F2 mapping populations. One population was screened for stem rot resistance using a greenhouse bioassay method, while another population was screened for resistance in the field. A genetic map has been developed for the bioassay population, and some putative minor quantitative trait loci (QTL) have been identified for both resistance and susceptibility. The analysis of the field population will be used to further investigate these and other potential QTL. The results of this research will be used to identify segments from *A. microsperma* to introgress into cultivated peanut, leading to the development of peanut cultivars that are more resistant to stem rot.

29. Saptarshi Mondal

PHD student

Assessment of salinity tolerance of finger millet accessions through metabolomics

Co-Authors: David Jespersen

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Crop cultivation is presently facing many challenges due to soil salinization. Crop plants encounter various physiological and biochemical alterations under salinity stress, which greatly reduces yield and productivity. Finger millet (Eleusine coracana; 2n=4x=36, AABBDD) is a highly nutritious climate-resilient crop that is suitable for marginal lands. The existence of genotypic variation for salt tolerance in Finger millet suggests the genetic control of the character and possible crop improvement via plant breeding. Identification of genotypes with greater stress tolerance is essential for understanding tolerance mechanisms and the development of elite cultivars. Based on the consensus of several phenotypic data of a prior experiment with six finger millet accessions at the seedling stage, we selected two accessions (I.E. 518 and I.E. 405) for further evaluation with the morphophysiological parameters and metabolomics. Significant phenotypic separation of I.E. 518 and I.E. 405 for salt tolerance was reflected through differences in plant height (PH), maximum quantum yield of photosystem II (FV/FM), electrolyte leakage (EL), net photosynthesis rate (Pn), shoot Na+ ion accumulation, biomass, and malondialdehyde (MDA) content. However, both accessions showed retention of K+ ions in their shoot tissues which might be a conserved mechanism for salt tolerance in Finger millet. Strong positive correlations among PH, Pn, FV/FM and strong negative correlations of the aforementioned parameters with EL and MDA content were observed. Metabolomic analysis with Liquid Chromatography and Mass Spectrometry (LC-MS/MS) identified differential metabolite accumulation patterns in these two accessions under salt stress. A higher accumulation of osmoprotectants like gamma-aminobutyric acid (GABA) and proline were detected in I.E. 518 than I.E. 405 and pathways associated with respiration, amino acid biosynthesis, and fatty acid biosynthesis were upregulated in I.E. 518.

30. Sindoora Nalajala

PHD student

Improving the efficiency of utilization of interspecific hybrids through flow cytometry and genome sequencing

Co-Authors: Kendall Lee2, Josh Clevenger2, Paul M. Lyrene3, Peggy Ozias-Akins1,4, Ye Chu1,4

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Blueberry plants are perennial and highly heterozygous. Breeding for improved cultivars involves crossing wild diploid (e.g. *Vaccinium elliottii* and *V. fuscatum*), wild tetraploid (e.g. *V. angustifolium* and *V. corymbosum*) cultivated tetraploid (highbush) and hexaploid (rabbiteye- *V. virgatum*) species. In this study, various interspecific crosses were made involving highbush blueberry cultivars (4x) × V. elliottii (2x), highbush cultivars (4x) × *V. fuscatum* (2x) and highbush cultivars (4x) × *V. virgatum* (6x). The success rates of these interspecific crosses were lower than that of homoploid crosses. The lowest success rate of F1 hybrid formation was found in the combinations with *V. elliottii* as parents. Seed formation and seedling establishment was observed in crosses involving southern highbush with *V. fuscatum* and *V. virgatum*. The F1 seedlings were tested with flow cytometry to determine the ploidy level. The analysis of the progeny from tetraploid highbush cultivars × *V. elliottii* cross revealed five tetraploids (4x) and three triploids (3x). Further determination of the hybrid nature of the tetraploid progenies will be performed by SNP genotyping from sequencing analysis. Confirming the hybrid progenies from these interspecific crosses will allow them to be utilized efficiently in the breeding programs and will reduce the time required for the introgression of beneficial alleles for blueberry cultivar development.

31. Swikriti Pandey

PHD student

Exploring diversity and observing genetic variation of mutagenized peanut populations

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Genetic and phenotypic variation in peanuts is limited owing to a domestication bottleneck. Induced mutations through mutagenesis can be one approach to expanding diversity in the primary gene pool of the peanut. TILLING (Targeting Induced Local Lesions in Genomes) is one reverse genetic approach that identifies nucleotide changes in a chemically mutagenized population. In an effort to identify novel genetic variations, mutagenesis of peanut seeds was conducted about a decade ago. Different concentrations of EMS (Ethyl methanesulfonate), DES (Diethyl sulfate) and NMU (N-Nitroso-N-methylurea) chemical mutagens were used in a breeding line C34-24 (Tifrunner).

Among three mutagen treatments, prior study conducted on a subset of this mutagenized population found highest mutation frequency in EMS treated samples. The study was also able to identify allergen mutants indicating the success of chemical mutagens in creating mutations. In our current study we phenotyped a subset of 384 M3 lines and monitored for segregation of various pre-harvest traits such as plant architecture, growth habit and flowering time. We also phenotyped post-harvest traits such as 100 pod and seed weight, pod shape and seed color. An additional 384 lines will be phenotyped this summer. In the future, we aim to sequence DNA using exon capture probes combined with low-pass (Riptide) whole genome sequencing to characterize variation and catalog mutations.

32. Sameer Pokhrel

PHD student

Unleashing the power of pangenome in MAGICal peanuts

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A pangenome can represent the full spectrum of variations present in a population and can substantially improve genotyping accuracy. No pangenome has been published for cultivated peanuts (*Arachis hypogaea*). Genome assembly is challenging for tetraploids like peanut due to rampant subgenomic similarity. For this study, 18 divergent peanut genomes were sequenced and successfully assembled with high contiguity, completeness, and reduced sub-genomic collapse. The assembled genomes were scaffolded to the progenitor genomes of peanut and were aligned to develop a pangenomic graph. The graph was used to visualize larger structural events at known QTL and to characterize cultivated peanut variation, much of which has not been evident in the single reference approach. The pangenome will be evaluated in the 16-way MAGIC population we have developed with the same 18 founder lines used to generate the pangenome. This pangenome is also unique in that it incorporates all parental genotypes of the MAGIC populations. The parents of this MAGIC population are widely grown cultivars or landraces with desirable traits such as resistance to diseases, drought tolerance, higher yield, and desirable agronomical traits, and low aflatoxin levels. This population offers advantages in precision QTL mapping and the development of elite material for use as breeding lines or release as a variety. We have collected phenotypic data for drought tolerance and stem rot resistance in our MAGIC peanut population. Our future work is to identify QTL associated with those complex traits using the “PanMAGIC” approach.

33. Emily Powell

PHD student

Validating the Epistatic QTL Interaction Responsible for Resistance to SCN Races 2 and 4 in Soybean

Co-Authors: Alexandra Ostezan(1), Melissa G Mitchum(2) and Zenglu Li(1)

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Soybean cyst nematode (SCN) is the most yield-limiting pest affecting soybean production in the US. Development and use of SCN-resistant cultivars is the most effective means to control SCN damage in soybean production. The most widely utilized SCN resistance sources are Peking and PI 88788, however, these lines lack efficacy against SCN races 2 and 4. Previously, our research discovered the epistatic Interaction of two QTLs, rhg1 on Chr 18 and QTL on Chr 11 provided resistance to both SCN races 2 (HG type 1.2.5.7) and 4 (HG type 1.2.3.5.6.7). The objective of this study is to validate the epistatic QTL interaction in a different genetic background, providing further evidence that this QTL will be useful in cultivar development for SCN resistance. For this confirmation, F5-derived recombinant inbred lines (RIL) derived from 'Woodruff' × G20-52-33SCN were developed. Woodruff is an elite soybean cultivar that carries Peking-type resistance (rhg1-a and Rhg4), while a breeding line G20-52-33SCN is a highly resistant RIL derived from a cross between Bossier and PI 437654 that possessed the rhg1-a and chr 11 QTL. A total of 208 RILs were genotyped for the chr 11 QTL, rhg1, and Rhg4 using the SNP markers discovered. Based on the genotyping result, RILs were subsequently divided into four genotypic categories:1) rhg1-a; 2) rhg1-a and Rhg4; 3) rhg1-a and chr 11 QTL; and 4) rhg1-a, Rhg4, and chr 11 QTL. Twenty-six RILs from each genotypic group, except group 4, were selected for evaluation of resistance to SCN race 2. The results confirm that combining rhg1-a and chr 11 QTL confers resistance to race 2 and that Rhg4 does not play a role in race 2 resistance. The Chr 11 QTL is an incredibly valuable source of SCN resistance and could be incorporated into elite cultivars to improve SCN resistance.

34. Holly Wright Presley

PHD student

DArTag sequencing reveals significant SNPs associated with low pH and aluminum tolerance in alfalfa

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Alfalfa is an economically valuable crop whose production in the southeastern USA is significantly limited by low pH and aluminum intolerance. This trait has proven difficult to improve through lab and greenhouse assays. Field screening and identification of genetic markers significantly associated with low pH and aluminum tolerance will facilitate marker assisted selection (MAS) and speed the breeding process for this elusive trait. The objective of the present study is to identify germplasm and molecular markers significantly associated with low pH and aluminum field tolerance. 139 half-sib families originating from a diverse panel of plant introductions have been evaluated for forage dry matter yield (DMY) in a field with an adjusted pH=6.37 and a field with a low pH=4.90 and exchangeable Al3+ concentration of 10.41 mg/kg in two replications at two locations throughout 2020-2023. An Acid Soil Adaptability Index (ASAI) score was assigned to each half-sib family to identify families with superior yields across both low and adjusted conditions. DaRTag sequencing was done on bulked tissue from each family, targeting 3,000 well-characterized SNPs in predominately genic regions. GWAS analysis was conducted to find SNPs associated

with the ASAI phenotype using GWASpoly2 and five models. ASAI scores ranged from 0-4.406, and identified unadapted (<1), well-adapted (>1), and very-well-adapted genotypes (>2). The GWAS analysis revealed 4 SNPs significantly associated with the ASAI trait at LOD>4.68. Of these, 2 SNPs (chr6.1_56760518 and chr8.1_75631452) were identified by three different models and were associated with an average increase of ASAI of 0.478 and 0.393, respectively. The results of this study identified two SNP markers associated with large effect sizes on ASAI that may be of interest in a MAS program to improve low pH and aluminum tolerance and contribute to the development and release of a new alfalfa cultivar adapted to the southeastern USA.

35. Samikshya Rijal

PHD student

Effect of 3-ketoacyl-CoA synthase 5 (KCS-5), a wax candidate gene, on resistance to *Puccinia emaculata*, a causal agent of rust on switchgrass Q7 –

Co-Authors: Rahele Panahabadi1, Thomas H. Pendergast IV, Eudald Illa-Berenguer, Wayne A. Parrott, Arthur J. Ragauskas2, Laura Bartley1, Katrien M. Devos, James Buck, Bochra A. Bahri

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Switchgrass (*Panicum virgatum* L.) is a grass native to North America with biofuel production potential. In switchgrass, the waxy trait known as glaucousness typically presents as a bluish-white color on leaves and internodes. Cuticular wax in plants influences stress tolerance. Our objective was to assess the effect of CRISPR/Cas9 knockout of a 3-ketoacyl-CoA synthase 5 (KCS-5), responsible for the synthesis of β-diketones and formation of tubular wax crystals, on resistance to *Puccinia emaculata*, a pathogen causing rust in switchgrass. We also investigated the effect of rust infection on fifteen cell wall components. *P. emaculata*-inoculated and non-inoculated KCS-5 knockout lines and non-edited control were assessed under growth chamber settings. In the first experiment, a non-edited line had a significantly lower rust severity than one of the knockout lines. A second experiment confirmed the significantly lower disease severity in three non-edited lines compared to three knockout lines. Significant effects of rust inoculation and accession and their interaction were observed for up to six cell wall components. Overall, rust infection significantly decreased the content of arabinose, rhamnose, galactose, and glucose in the leaves of all accessions. Under rust infection, a decrease in xylose, glucose, and para-coumaric acid pellet was observed for one or both knockout lines compared to the non-edited control. Understanding the role of wax in disease response is crucial for developing disease-resistant cultivars, contributing to sustainable production practices.

36. Harshita Saxena

PHD student

Exploring Host Range and Genomic Diversity of *Magnaporthe oryzae* Isolates from Oats and Turfgrass in the United States

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Magnaporthe oryzae is a pathogenic fungus affecting numerous Poaceae species, including crops like rice, wheat, barley, millets and oats, leading to blast or gray leaf spot diseases. Previous studies have revealed several distinct genetic lineages within M. oryzae, each predominantly associated with a specific host genus. This suggests ongoing speciation processes following host shifts or expansions in the host range. In this study, we aimed to increase our understanding of the genetic diversity, evolutionary relationships, and host interactions of ten *M. oryzae* isolates obtained from oats, tall fescue, and other turfgrasses. The host range of two isolates, Oat-GLS from oats and GLS-TF from tall fescue, were evaluated across 5 and 8 different cereal and turfgrass species, respectively. Both isolates induced symptoms of gray leaf spot disease on oat, tall fescue, and annual and perennial ryegrass. These findings indicate that both the Oat-GLS and GLS-TF isolates belong to the Lolium lineage. Additionally, whole-genome sequencing was conducted using the Illumina® Sequencing platform for all ten isolates to explore the genetic makeup. Potential evolutionary relationships of these *M. oryzae* isolates relative to 45 other previously sequenced *M. oryzae* isolates from 12 grass genera were assessed. Our findings offer valuable insights into the eco-evolutionary mechanisms driving the diversification of *M. oryzae* and to the general knowledge of fungal pathogenicity and host specificity.

37. Iago Schardong

PHD student

Evaluation of fiber fineness in two Upland Cotton (*G. hirsutum*) populations with introgressed genomic regions from *G. barbadense*

Co-Authors: 1.Samantha Jo Wan, 1.Nino Brown, 1.Pawan Kumar, 1.Sameer Khanal, 2.Don Jones, 2.Neha Kothari, 1.Andrew H. Patterson1 & 1.Peng W. Chee

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The cultivated form of *Gossypium hirsutum*, Upland Cotton, has a very narrow gene pool due to its evolutionary and domestication histories. The practice of crossing closely related genotypes has negatively affected the genetic diversity in modern cotton germplasm, resulting in a high degree of relatedness among cultivated germplasms. Other species from the Gossypium genus, such as G. barbadense are known to have longer, stronger, and finer fiber than Upland Cotton elite cultivars. Previously, we have determined that an obsolete Upland germplasm line, Sealand 883, contains introgressions of *G. barbadense* in at least five chromosome regions, and harbors three QTLs for improved fiber fineness. The main objective of this study is to evaluate the phenotypic effects of the introgressed genomic regions from *G.barbadense* into Upland Cotton. Near-isogenic lines were leveraged from two different elite cultivar backgrounds: Deltapine 50 and Paymaster HS26, which both were crossed with SL883. The genetic populations were planted in 2020 and 2022 at the Gibbs Farm in Tifton, Georgia. Results show the progeny

genotypes had significantly improved fiber quality throughout both trials. These indicate that the G. barbadense introgression in SL883 that confer better quality fiber traits were segregating in the progeny. In future work, we will investigate further if the QTLs are present in the progenies through Linkage Map and QTL analysis. Further, we will use transcriptome profiling to verify putative regions and identify genes that confer better fiber quality.

38. Gurjot Singh Sidhu

PHD student

Knocking Out a Candidate Gene for Wax Production in Switchgrass Yields an Unexpected Pleiotropic Phenotype.

Co-Authors: Gurjot S. Sidhu (1), Eudald Illa-Berenguer (1,2), Bochra A. Bahri (1,3), Wayne Parrott (1,2), Thomas H. Pendergast IV (1,2,4), Katrien M. Devos (1,2,4)

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Switchgrass, *Panicum virgatum*, a grass native to the US is of intense interest as a dedicated feedstock for the production of sustainable aviation fuel. Switchgrass ecotypes differ by a number of characteristics, including the presence of wax on leaves and stem. Lowland ecotypes generally contain high levels of C33 β-diketones and hydroxy-β-diketones, which are associated with the formation of crystalline wax tubes on the abaxial leaf side and a blueish plant color1,2. In contrast, β-diketones are largely lacking from upland accessions, which have glossy green leaves. We previously identified a cluster of genes as strong candidates for the quantitative trait locus that was identified for wax variation on the abaxial leaf side in an F2 population generated from a cross between the lowland genotype AP13 and the upland genotype VS163. One of the candidate genes, a likely 3-ketoacyl-CoA synthase 5 (KCS-5), was knocked out in Performer, a transformable lowland accession, using CRISPR/Cas9. Interestingly, while edited plants had the expected glossy green color, they were also shorter in stature and had more tillers compared to the controls. To determine the effect of KSC-5 knockout on transcription, an RNA-Seq analysis was conducted on two independent KSC-5 knockout plants and two non-edited control plants. A total of 415 and 1781 genes were differentially expressed (DE) in stems and leaves, respectively, between the KSC-5 knockout lines and non-edited controls (p-value ≤ 0.05, log2fold difference ≥ 1). Sixty four percent of the genes DE in stems were also DE in leaves. Work to determine the affected pathways as well as the effect of the KSC-5 knockout on sustainability is ongoing.

39. Madhav Subedi

PHD student

NOVEL MAJOR-EFFECT QTLS CONTROLLING END-USE QUALITY TRAITS IDENTIFIED IN SOFT RED WINTER WHEAT

Co-Authors: John W. Bagwell, Benjamin Lopez, Byung-Kee Baik1, Md. Ali Babar2, Mohamed Mergoum

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Wheat is used as the primary ingredient to make many food products, owing to its diverse and complex quality attributes among different wheat classes. Laboratory analyses of end-use quality traits are costly and time-consuming, so genetic dissection of these traits is challenging. The objective of this research was to identify major and/or novel QTLs associated with important end-use quality traits. A diversity panel of 266 soft red winter wheat lines (SRWW) grown in the US southeast region was utilized for genome-wide association study (GWAS) of 10 end-use quality traits, including kernel protein, flour protein, flour yield, softness equivalence, four solvents (lactic acid, sodium carbonate, sucrose, and water) retention capacity, cookie diameter and top-grain. The genotypes were evaluated in two locations in Georgia, Griffin and Plains, over two years (2020-2022). A total of 27,466 single nucleotide markers were used in the GWAS, and a total of 80 significant MTAs were identified at a false discovery rate ≤ 0.10. Out of 53 quantitative trait loci (QTLs), 13 explained > 10% phenotypic variance (PV) for the traits and were considered major-effect QTLs. Among these, five were stable, expressed across multiple environments, and four showed pleiotropic effects. All major-effect QTLs were considered as putative novel loci, not overlapping with previously reported MTA/QTLs. Candidate genes were identified for eight of these major-effect QTLs, including genes associated with starch biosynthesis and nutritional homeostasis in plants. These findings increase genetic comprehension of the end-use quality traits and could potentially be used to improve the quality of SRWW in the US southeast.

40. Bukhtaawer Talat

PHD student

Genetic mapping to identify diagnostic markers and identifying major genomic regions containing potential QTLs for resistance gene (s) to Cotton Leafroll Dwarf Virus

Co-Authors: Divya Bhanu Sharma1, Sameer Khanal1, Andrew Paterson1, Patrick Conner 1, Nino Brown1, Josh Clevenger2, Jenny Koebernick3, Sudeep Bag1, and Peng Chee1

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Cotton leafroll dwarf virus (CLRDV) is a single-stranded non-enveloped RNA virus from the genus Polerovirus and family Solemoviridae that is transmitted through aphids (Aphis gossypii Glover). Virus infection can be asymptomatic but susceptible genotypes can produce symptoms such as stunted growth, reddening of leaves, petioles and stems, v-shaped curling, drooping, and disappearing of wilting symptoms in the non-peak heating hours. The objectives of this research are to detect QTL and candidate gene for the resistance gene(s). In 2019, a total of 136 F2s were planted and out of these, 76 F2 populations were segregating for symptoms for CLRDV. Three F2 populations were selected as genetic mapping populations based on the pedigrees of the segregating populations as well as for having the highest disease frequency. DNA samples were sent to Texas A&M for genotyping using the Illumina 64k cotton SNP array. The preliminary results suggest that a major resistance QTL may be located on telomeric region of Chr10. Additional work is now in progress to validate the QTL using different genetic populations and mapping approaches such as QTLSeq.

41. Brandon Tonnis

PHD student

Identification and Confirmation of High Oil Germplasm in the USDA Peanut Collection for Creating High Oil Breeding Populations

Co-Authors: Ming Li Wang, David Bertioli, Soraya Leal-Bertioli, Shyam Tallury, Mylee Mobley

USDA-PGRCU, UGA-PBGG

Demand for peanut oil is projected to increase in many countries such as China and India in the future. The oil is used for frying, as a food ingredient, and as a renewable source of biodiesel for powering buses and farm tractors. To meet the expected increased market demand, American farmers may opt to refocus their efforts towards high oil cultivars for maximizing oil production. To that end, we screened the USDA National Plant Germplasm System (NPGS) peanut collection housed in Griffin, GA to identify high oil accessions that could potentially be used in breeding programs for generating high oil cultivars. Based on preliminary oil measurements of stored inventories, we selected one hundred, ninety accessions to grow in 2022 and 2023 for confirming the oil phenotype. Significant correlations between the oil content of stored and fresh seeds ($r = 0.70$, $p < 0.001$) and the oil content over two years ($r = 0.74$, $p < 0.001$) suggested a strong genetic effect. Based on these results, we identified two high oil accessions, PI 370331 and PI 365554, that also showed good agronomic performance and yield in 2023. Preliminary yield trials of these two accessions will be conducted at two locations in 2024, and they with be crossed with disease resistant breeding lines to produce new breeding populations for future research studies.

42. Katie Toomey

PHD student

Recombinase-Based Strategy for Targeted Gene Stacking in Plants

Co-Authors: Pete LaFayette, Wayne Parrott

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Poplar's rapid growth and carbon capture potential, along with its status as a model organism for woody perennials, make it an ideal candidate for genetic modification. The BioPoplar project aims to take advantage of these traits by changing and introducing new metabolic pathways, enabling the development of poplar varieties with diverse chemical and physical traits. Accomplishing this requires a “genomic landing pad” that facilitates targeted and repetitive insertion of specific elements into a plant without disturbing its other traits, known as gene stacking. My primary objective is to develop a genomic landing pad to enable the insertion of multiple desired genes in a stepwise order and precise location in planta. I am developing custom recombinase sites (RS) based on the Cre-loxP system from bacteria. These bespoke RS pairs can be recombined with minimal crosstalk between the pairs to create a system for iterative insertions genes into *Populus tremula x Populus* alba INRA 717-1B4 (717). In the right manner, these can be used to sequentially add transcriptional units in a unidirectional path into a

precise location of the genome. The utilization of the modified recombinase sites offers a promising solution for targeted gene stacking in plants. This approach eliminates the need for stacking in bacteria before transformation and would be beneficial in regulating transgene number, expression, location, and heritability.

43. Samantha Jo Wan

PHD student

Validation and End Use Evaluation of qFL-Chr.25, a Gossypium barbadense-sourced fiber length QTL in Four Diverse Upland cotton (*G. hirsutum*) backgrounds

Co-Authors: Sameer Khanal1, Nino Brown1, Pawan Kumar2, Dalton West1, Neha Kothari3, Donald Jones3, Lori Hinze4, Josh Udall4, Chris Delhom5, Andrew Paterson6, and Peng Chee1

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Limited genetic diversity within the cultivated cotton germplasm has hindered the long-term improvement in fiber quality of Upland cotton (*Gossypium hirsutum* L.). A closely related species, G. barbadense, known for having superior fiber quality has been used with some success to transfer favorable fiber quality alleles into Upland cotton. An obsolete Upland line with introgressions from *G. barbadense*, Sealand 883, was shown to carry a quantitative trait locus (QTL) for fiber length (qFL-Chr.25). This QTL was later transferred into four diverse genetic backgrounds (Acala SJ-4, Deltapine 50, GA 2004089, and Paymaster HS-26) that represented four major cotton-growing regions of the United States Cotton Belt. To more precisely determine the effect of the QTL, it necessitated the development of near-isogenic lines (NILs). Previously, we have validated the effects of qFL-Chr.25 with the use of High Volume Instrument (HVI) testing as well the dissection of individual genotypes fiber profiles with the use of Advanced Fiber Information System (AFIS) analysis. In the current study, we evaluated the down-stream impact of this QTL by using a Miniature-Spin test to determine if the NILs containing the QTL had improved yarn characteristics over the NILs without the fiber length QTL. A four year, multilocational study was conducted to test the deployment of qFL-Chr.25 into the four different backgrounds and preliminary Miniature-Spin data suggests the QTL has the potential for positive effects on yarn characteristics.

44. M Habib Widyawan

PHD student

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Identification of the Rdm1 Resistance Allele from Soybean Line D85-10404 for Resistance to Southern Stem Canker

Co-Authors: James W. Buck, Zenglu Li

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Southern stem canker (SSC), caused by *D. aspalathi* fungus, significantly impacts soybean production in the southeastern United States (US). Five loci (Rdm1 to Rdm5) conferring resistance to SSC have been named based on classical segregation analysis. However, SSC resistance in the elite soybean germplasm is known to be provided by a single locus with narrow allelic variation, namely the Rdm3 locus. The Rdm3 gene is the only resistance source mapped on the soybean genome and is used extensively in soybean breeding. The Rdm1 alleles from the SSC-resistant soybean line D85-10404 possesses unique resistant phenotype responses to various *D. aspalathi* strains compared to the Rdm3. It has the potential as an alternative source of SSC resistance or complement the Rdm3 in the breeding program. The objective of this research is to map the genomic location of the Rdm1 locus from soybean line D85-1040 for SSC resistance. Using a modified toothpick method, a recombinant inbred line (RIL) population (N = 182) derived from G81-2057 (susceptible) D85-10404 (Rdm1) was evaluated for SSC resistance. QTL-sequencing, composite interval mapping (CIM), and recombination breakpoint analyses revealed the Rdm1 is located on a 164 Kb interval on chromosome 2, which accounted for 70% of phenotypic variation. Two KASP SNP assays GSM1249 and GSM869, the most significant markers from CIM, could well discriminate lines based on their resistance provided by the Rdm1 allele. The results of this research will enable an efficient utilization of the Rdm1 resistance allele in soybean breeding through marker-assisted selection.

Staff/Postdoc Category

45. Sailaja Bhogireddy

Postdoc

Engineering Apomixis: A Comparative Exploration in Monocots and Dicots

Co-Authors: Sailaja Bhogireddy, Joann A. Conner, Peggy Ozias-Akins*

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Apomixis, a remarkable phenomenon in plant reproduction, diverges from the traditional processes of fertilization and meiosis. Unlike sexual reproduction, apomixis enables the development of embryos and seeds within the ovule independently of these mechanisms, resulting in the generation of clonal seeds identical to the maternal parent. This trait holds immense potential for breeding desired plant traits, to stabilize heterosis, preserve valuable traits, thereby perpetuating elite genotypes. However, apomixis remains rare among major food crops. To address this, researchers aim to artificially induce apomixis in major food crops through engineering 'synthetic apomixis'. This innovative approach involves targeting modifications in both meiosis and fertilization processes to produce clonal seeds. In rice, for instance, the introduction of MiMe (mitosis instead of meiosis) alongside the activation of the parthenogenesis gene, BabyBoom 1 (BBM1), within the egg cell can trigger the production of clonal offspring in hybrid rice while maintaining genome-parental heterozygosity. Moreover, the combination of MiMe with the MATRILINEAL (MTL) gene facilitates the production of clonal seeds. Despite these advancements, challenges

persist, such as lower fertility observed in apomictic lines, possibly due to parthenogenesis frequency or endosperm development. In dicots, achieving synthetic apomixis has been less successful. Investigations into the molecular mechanisms of apomixis in dicots have revealed both shared and distinct pathways compared to monocots. In tomato, for example, efforts to harness synthetic apomixis for asexual propagation have been hindered by difficulties in inducing embryo formation from egg cells, a process that remains poorly understood in dicot plants. BBM genes in tomato have been found to be insufficient in inducing embryo formation at noticeable rates. Overall, the advancement of apomixis research stands at the forefront of agricultural science.

46. Carlos Cardon

Postdoc

Gene expression in peanut seed that could contribute towards reduced aflatoxin contamination in peanuts.

Co-Authors: Chandler Sprueill1, Carolina Chavarro1, Scott Jackson1, & Peggy Ozias-Akins1

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Aflatoxin contamination in peanuts/groundnuts is one of the biggest problems worldwide. *A. flavus* and *A. parasiticus* are the two main strains that produce aflatoxin. Peanut is contaminated through pod/seed colonization during maturation in the field or during post-harvest storage. The seed coat and pericarp are important barriers to protecting the seed from diverse external factors. The objective of this study was to analyze expressed genes in different parts of the seed and help find genes that can act against Aspergillus flavus infection and aflatoxin contamination and provide a foundation for metabolic engineering. Our study analyzed the embryo, seed coat, and pericarp RNA sequences from one *Arachis hypogaea* ssp. hypogaea cultivar (Tifrunner) and one mixed subspecies cultivar (NC 3033) from seeds at R4-5, R6, and R7 stages of development. Among the 5% most highly expressed genes (2271 genes across both genotypes), we found different genes related to response to stress in Tifrunner and NC3033, 30 and 26 genes in embryo, 27 and 29 genes in the seed coat, 19 and 20 genes in the pericarp, respectively. GO enrichment analysis identified terms associated with plant response to biotic stress for highly expressed genes unique to the pericarp tissues. Comparing genotypes, NC3033 showed more terms for response to fungus and up-regulated genes associated with anthocyanin biosynthesis in the seed coat than Tifrunner. In addition, looking for specific promoters for gene engineering in localized tissue types, we found 19, 54, and 49 genes expressed uniquely in embryo, pericarp, and seed coat, respectively. With these results, seed tissue-specific promoters and pathways that could be enhanced or diverted to alter the metabolome being explored.

47. Renato Augusto Correa dos Santos

Postdoc

Correlation analysis of paired transcriptomic and metataxonomic datasets of leaves across diverse maize lines

Co-Authors: Renato Augusto Correa dos Santos1,2,3; Diego Mauricio Riaño-Pachón1,2; Jason G. Wallace3

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Maize is a genetically diverse crop of global importance. Our research group previously integrated maize genetics and meta-taxonomic data (microbiome) across approximately 300 diverse lines to understand how microbial composition and potential functions are affected by plant genetics. In this work, we integrated 3'-mRNA expression sequencing across the same leaf samples to identify possible associations between gene expression and the abundance of bacterial taxa. Both RNA expression and bacterial taxonomic counts were re-analyzed from the original sequencing data following standard pipelines (TrimGalore/Salmon and Cutadapt/Qiime2/DADA2/SILVA, respectively); due to the low quality of reads in the metataxonomic data, downstream analyses and generation of amplicon sequence variants (ASVs) were carried out using the only the forward reads. Large-scale correlations were computed between gene expression and ASV or phylotype abundance with CorALS using a single matrix generated by pairing RNAseq and 16S sequencing runs. Filtering out Pearson correlation coefficient > -0.6 and < 0.6 resulted in three and six pairs of transcript-ASV (one transcript) and transcript-phylotype (two transcripts) associations, respectively; next steps will include permutation analysis to determine the significance of these results. Given the sparsity of matrices, we expect that alternative filtering and refinements on how ASV placement steps are carried out and incorporating functional information associated with host genes or predicted from 16S sequences will improve analyses. Our group has also been working on the development of CoNekT Grasses Microbiome, a web platform that will enable exploitation of results from analysis of metataxonomics, transcriptomics, or their integration.

48. Daniel Laspisa

Postdoc

Genetic Determinants of Aerial Root Formation in Maize: Prospects for Harnessing Biological Nitrogen Fixation

Co-Authors: Daniel Laspisa 1, Kimberly Gibson 2, Rafael Venado 2, Jennifer Wilker 2, Valentina Infante 2, Claudia I. Calderón 2, Jean-Michel Ané 2, Jason Wallace 1

1) University of Georgia 2) University of Wisconsin

Indigenous maize varieties from the Oaxaca region of Mexico have been shown to support nitrogen-fixing bacteria in mucilage produced by their aerial roots. Maize plants with this trait can obtain 29-82% of their nitrogen from this symbiosis in the right environmental conditions. A better understanding of the genetic factors that contribute to this trait will enable greater use of biological nitrogen fixation to support the global demand for cereals and reduce dependence on synthetic nitrogen fertilizers. Here we assess population structure among eight populations of doubled haploids generated from inbred PHZ51 and three landrace parents. A two-location field trial of two doubled haploid populations shows heritability is generally low for the number of nodes with aerial roots, the number of roots per node, and the root diameter. However, noticeable differences are observed between

populations that are consistent across locations. Furthermore, we identify QTL associated with aerial root traits unique to each population and shared between populations. While additional research is needed to confirm and expand these results, our findings support pursuing the development and adoption of maize varieties with this trait to improve global food insecurity and reduce environmental degradation attributed to synthetic fertilizer use.

49. Fiorella Spies

Postdoc

Navigating safe harbors: Genome editing of poplar trees for sustainable bioproducts.

Co-Authors: Peter Lafayette, Wayne Parrott

Center for Applied Genetic Technologies, University of Georgia

The transition from an oil-based economy to the bioeconomy is based on the importance of plant-derived products. In turn, the bioeconomy depends on modifying plants for novel uses and for the production of useful metabolites. Poplar (Populus spp. and their hybrids) stand out as promising candidates for modification. They have a relatively compact genome, coupled with rich genomic resources. Additionally, certain poplar species are highly susceptible to Agrobacterium tumefaciens infection, facilitating transgenic experiments. Our primary objective is to develop advanced genome-editing tools to strategically modify the poplar genome by directing genes of interest to Safe Harbors (SH), which are specific genomic loci where the insertion of foreign sequences can occur without compromising the normal functioning of the host. Genomic DNA of a wide set of well-established poplar transgenic events will be analyzed to look for genomic sequences corresponding to TDNA insertions. The following selection process will consider regions away from telomeres and highly methylated regions, prioritizing regions of open chromatin and an absence of microRNA genes. Two alternative site-directed editing techniques approaches, CAST/Tol2 and PrimeRoot editing, are currently under evaluation. The former leverages the complementary action of Cas9 system and TOL2 transposase, enabling the integration of the gene of interest at the targeted SH. The latter involves the concerted action of nCas9, MMLV reverse transcriptase, and CRE tyrosine recombinase to achieve prime editing and subsequent DNA sequence insertion. Plasmids required for implementing these techniques are in the final stages of assembly. The culmination of these site-directed methods, in conjunction with the careful selection of SH, holds promise for re-engineering poplar as a versatile crop. The genetic engineering tools developed from this research should be applicable to other crops and help further the bioeconomy.

50. Yi-Wen Wang

Postdoc

Genome-enabled breeding across *Phaseolus* species

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Phaseolus vulgaris, common or dry bean, is the most widely cultivated dry seed legume and an important source of plant protein for human consumption. Its sister species, tepary bean (*Phaseolus acutifolius*), which is native to the Sonoran Desert, is closely related to common bean and is also cultivated. Common bean is susceptible to several diseases and abiotic stresses for which resistance is present in tepary bean. The objective of this research is to transform common and tepary bean breeding by exploiting their close relationship through the development of a knowledgebase that permits rapid trait discovery and subsequent breeding between the two species. We constructed a pan-Phaseolus knowledgebase for rapid causal trait identification and seamless breeding between *Phaseolus* species. By using GENESPACE, orthologs and syntelogs across legume genome assemblies were identified. To augment the knowledge of loci encoding agronomic traits, such as disease resistance in tepary bean, we sequenced a 290 accession tepary diversity panel via whole genome shotgun sequencing. In addition, k-mer based GWAS analysis was conducted to identify trait-associated k-mers. Using a subset of 146 accessions with phenotype data for common bacterial blight resistance, 1.08 billion unique k-mers were identified, of which, 5,241 were significantly associated with bacterial blight resistance. Separately, we identified six tepary accessions with key traits of interest and sequenced their genomes using ONT genomic long read sequencing data. All genome assemblies have at least 98% complete BUSCOs, indicating a high level of completeness. Further, to facilitate genome-enabled breeding of tepary bean, a Practical Haplotype Graph (PHG) is being constructed to store and use with reduced representation genotyping data to impute haplotypes. The resources we generated in this study will be useful for identifying genes and quantitative trait loci for traits of interest in common and tepary bean.

51. Lei Zhang

Postdoc

A QTL for fine tuning methyl salicylate level in tomato fruits

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Methyl salicylate (MeSA) is an important signaling molecule within and between plants during pathogen and herbivore attack. in the color fruited tomato, the accumulation of MeSA is mainly regulated by a methyl esterase locus (MES) and Non-Smoky Glucosyl Transferase 1 (NSGT1) and its levels can vary up to 9000-fold among tomato accessions. MES removes the methyl group from MeSA to produce salicylic acid whereas NSGT1 catalyzes an irreversible glycosylation of MeSA. Tomato with functional alleles of both genes generally present low level of MeSA. MeSA in tomato is associated with low liking by consumer taste panels. The low level of MeSA in many tomato accessions, except some modern types, is likely due to selection for superior taste. However, the volatile may play an important role in defense responses during fruit growth and/or after harvest. We aim to identify

additional MeSA regulators that could function in fine-tuning its levels to achieve the proper balance between the fruit taste and defense. In an F2 population developed from two tomato accessions fixed at the functional allele of MES and NSGT1, individual plants carry low but variable level of MeSA in their fruits.We mapped a single MeSA QTL on the bottom of chromosome 3 (MeSA3.1) in this F2 population.Progeny testing in six F3 families narrowed down the QTL to a 700 kb interval comprising about 100 genes. Further fine mapping and CRISPR-Cas9 mediated knock-out of a few candidate genes are underway to identify the causal gene. Funded by NSF IOS 2151032

52. Jianxin Zhao

Postdoc

single amino acid variant in a knotted-1 transcription factor modulates S/G lignin ratios in switchgrass

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Switchgrass (*Panicum virgatum*) is being domesticated as a sustainable bioenergy crop due to its wide adaptability, high yield, and low agricultural inputs. The S/G ratio of lignin, as a primary component, affects both lignin monomer yields and ethanol production We determined lignin monomeric composition using both pyrolysis molecular-beam mass spectrometry (PyMBMS) and thioacidolysis in an F2 population derived from a cross between the lowland genotype AP13 and the upland genotype VS16. Quantitative trait locus (QTL) mapping for the S/G lignin ratio obtained using both methods identified colocalizing QTL on chromosome 9N. This QTL region harbors the genes PvKNAT1, a member of the Arabidopsis TALE homeodomain transcription factor family. In Arabidopsis, KNAT family affects secondary cell wall biosynthesis, including lignin content. We transformed the AP13 (PvKNAT1AP13) and VS16 (PvKNAT1VS16) alleles of PvKNAT1, which differed by several non-synonymous single nucleotide polymorphisms (SNPs), into an Arabidopsis knockout mutant knat1. PvKNAT1VS16 but not PvKNAT1AP13 rescued the phenotype of the knat1. Moreover, PvKNAT1VS16 overexpression lines have higher lignin S/G ratios but similar total lignin content than PvKNAT1AP13 overexpression lines. Gene expression analysis showed that PvKNAT1 affects the expression of genes responsible for converting G to S lignin. We further demonstrated that the S214N substitution was critical for activity of PvKNAT1. Introgression of PvKNAT1VS16 is expected to further increase the S/G ratio in AP13, leading to improved lignin valorization and increased ethanol production. Our study demonstrates how combining genetic mapping in switchgrass with transgenic analyses in Arabidopsis can help uncover critical variants in genes contributing to traits of importance to the bioeconomy.

53. Tatyana Nienow

Staff

SNP Assays for Characteristic Traits in Soybean

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Soybean is a self-pollinating plant, so when crosses are made, it is important to be able to identify true F1 crosses from selfs produced by the female parent. Selections can be made based on phenotypic traits, such as flower or pubescence color, but that requires using a male parent possessing the dominant phenotype and a female parent possessing the recessive phenotype. SNP markers can greatly aid in making selections, but it can be difficult to find suitable polymorphic SNPs. Here, we developed two SNP markers associated with flower and pubescence color in soybean that can be used in marker-assisted selection of F1 crosses. Candidate SNPs near the flavonoid 3'5'-hydroxylase encoded by the main flower color locus W1 on Chromosome 13 and the flavonoid 3'-hydroxylase encoded by the main pubescence color locus T on Chromosome 6 were selected and KASP marker assays designed. These markers were tested on 68 parents used to make crosses during the summer of 2023 and the results compared to the phenotypes of the parent lines. Out of 68 parents tested, the marker results matched the flower color for all but two. The pubescence of 47 of the parents was known; out of those the markers results matched the phenotype for all but three lines. After testing the markers on the parents, the marker assays were then used to genotype the progeny from the crosses made in 2023 in order to identify true F1s. The results here show that both these markers have good performance and can be used to assist in selecting F1 crosses in soybean breeding programs.