2025_IPBGG Retreat Poster Abstracts

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01 Jessie Adams

Evaluating applications in peanut breeding for NIR-based high throughput phenotyping of seed compositional traits

Brittany Davis, Jack Davis, Nino Brown

University of Georgia, Crop and Soil Science (Adams, Brown)
JLA International (B. Davis, J. Davis)

The peanut industry requires peanut cultivars with a range of seed compositional traits to satisfy specific product requirements. For instance, candy and snack manufacturers require high oleic acid concentration in the oil for improved shelf stability, whereas other parts of the industry prefer lower or normal oleic acid concentration. The use of non-destructive seed analysis is an advantage for peanut breeding programs. Near-infrared spectroscopy (NIR) is a non-destructive method that is currently used for indirect measurement of seed compositional traits such as oleic acid concentration, oil and protein percentage. However, the platforms that were available until recently were too slow for processing a large number of samples. The QSorter Explorer, recently developed by Ferrum, measures several traits including NIR-reflectance of individual kernels and allows sorting of seeds in real-time based on trait threshold values at speeds of 10-20 seeds per second. Calibrations for oleic acid concentration in peanut are well established for the device, however, sorting thresholds have not been optimized for the purpose of peanut breeding. Additionally, calibrations for oil and protein percentages have not been fully developed. Optimizing thresholds for oleic acid concentration will standardize the method used within our breeding program, and provide empirical data to inform other peanut breeding programs. Developing and validating calibrations for oil and protein percentage will help accelerate improvement for those traits. High throughput phenotyping of seed compositional traits will assist in the efficient development of improved peanut cultivars for Georgia and beyond.

02 Amelia Boettcher

Field Evaluation of Watermelon Backcross Lines for Resistance to Gummy Stem Blight

Samikshya Rijal, Winnie Gimode, Cecilia McGregor

IPBGG, CucCap

Watermelon is a key crop in Georgia, valued at $142 million in 2022. However, gummy stem blight (GSB), caused by Stagonosporopsis spp., threatens production, necessitating resistant cultivars. This study evaluated five BC_2F_3 and five BC_3F_3 lines with introgressed QTLs on chromosomes 5 (ClGSB5.1, PVE = 10.2%) and 7 (ClGSB7.1, PVE = 21.1%). The lines were derived from crosses between the susceptible 'Crimson Sweet' (Citrullus lanatus) and resistant UGA1081 (C. amarus). QTL introgression was carried out through marker-assisted backcrossing. Lines were evaluated in the field in Summer 2024 at the J. Phil Campbell Sr. Research and Education Center. Six seedlings were transplanted per plot in a randomized complete block design with three replicates per line. In addition to the backcross lines, resistant and susceptible controls and a resistant line from NCSU were also included. Plants were inoculated with S. citrulli isolate 12178A, and disease severity was recorded weekly for seven weeks. Yield and fruit quality were recorded at the end of the trial. Among BC_3F_3 lines, 62_BC3 showed significantly lower disease severity than 'Crimson Sweet'. All BC_3F_3 lines maintained yields similar to 'Crimson Sweet,' but quality traits like Brix remain lower, requiring further improvement. These results highlight BC_3F_3 lines as promising candidates for breeding efforts to enhance GSB resistance while maintaining yield and fruit quality.

03 Stephanie Botton

Unknotting a nematode: Exploring wild Arachis root knot nematode resistance in peanut

Stephanie Botton (1,2); Sameer Pokhrel (1); Lesley Schumacher (4); Walid Korani (3); Josh Clevenger (3); Patricia Timper (4); Ye Chu (1,2); C. Corley Holbrook (4); Peggy Ozias-Akins (1,2)

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Peanut root knot nematode (PRKN) (Meloidogyne arenaria) is a microscopic roundworm that primarily infects peanut. Peanut fields infected with PRKN demonstrate symptoms of malnourishment, reduced yields, and increased susceptibility to other opportunistic pathogens. In the state of Georgia, PRKN was responsible for $32.5 million in crop damage in 2022. For a state that is responsible for 55% of the peanut production in United States, this can be devastating. One of the most effective strategies to combat this pathogen is through growing cultivars with PRKN resistance. Currently, the only PRKN-resistant cultivars that are commercially available to growers have an introgressed region from Arachis cardenasii. However, PRKN resistance derived from A. stenosperma is also under investigation. Phenotypic experiments exploring both resistances have identified regions In the A. cardenasii introgression where the resistance gene(s) potentially resides. In addition, resistance derived from the recombination of both (A. cardenasii and A. stenosperma) introgressions have also been characterized. To validate these results, an additional assay and genotyping experiment will be performed.

04 Chloe DelaCerna

Identification of Novel Metabolite Associations with Insect Resistance in Tall Fescue-Epichloë Symbiosis

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Tall fescue (Lolium arundinaceum) forms a mutualistic symbiosis with fungal Epichloë endophytes, which enhance host resistance to herbivorous insects through the production of bioactive alkaloids. While previous studies have identified specific alkaloids such as lolines, peramine, and janthitrems as contributors to insect deterrence, the broader metabolomic basis of Epichloë-mediated insect resistance in tall fescue remains unexplored. This study integrates untargeted metabolomics with field observations and insect feeding assays to identify novel metabolites linked to fall armyworm (Spodoptera frugiperda) resistance. Defoliation ratings were recorded from Epichloë-infected tall fescue crossing blocks under high natural fall armyworm pressure to assess natural fall armyworm feeding preferences. The same endophyte/tall fescue combinations were utilized in a controlled insect feeding assay to assess effects on fall armyworm development and mortality. By comparing the metabolomic profiles of Epichloë-inoculated tall fescue strains with host plant defoliation and insect mortality, we confirmed the role of known alkaloids and identified additional bioactive metabolites strongly correlated with insect resistance. These findings suggest that Epichloë-mediated insect resistance is more biochemically complex than previously understood. Leveraging metabolomics for targeted selection of endophytes with optimal metabolite profiles empowers forage breeders to develop cultivars with enhanced insect resistance, reducing reliance on chemical insecticides and expanding the potential for Epichloë endophytes as an agent of biological control in forage production.

05 Anne Marie Gahagan

Identification of QTLs Associated with Nut Shape in Pecan (Carya illinoinensis)

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Pecan is a valuable North American nut crop, with most nuts mechanically shelled before reaching consumers. Shelling machinery requires uniform nut size and shape to minimize kernel shattering and maximize the production of complete kernel halves. Mixed-cultivar orchards, which could enhance pollination, mitigate pecan scab, and increase yields, are impractical due to cultivar variation in nut shape. This study aims to identify QTLs associated with nut length and width to support the development of cultivars with similar nut shape. Nuts from 119 individuals of a biparental (‘Pawnee’ × ‘Elliott’) mapping population were harvested over multiple years and analyzed using Tomato Analyzer software to assess maximum length, maximum width, and nut shape index (length-to-width ratio). QTL analysis was conducted using R/qtl with parental maps created via a pseudo-testcross mapping strategy. Phenotypic data were analyzed separately for each parental map across two near-complete yearly datasets, as well as a combined dataset spanning multiple years. Analysis revealed a QTL on ‘Pawnee’ chromosome 11 was consistently associated with length and nut shape index across all datasets. In the ‘Elliott’ map, analysis of the combined dataset as well as a single-year dataset revealed a QTL for nut shape index on chromosome 8 and a width-related QTL on chromosome 13. Additional potential QTLs were detected for each trait in single datasets. This analysis represents a first step towards the development of marker-assisted-selection of nut shape traits in pecan.

06 Jackline Litunya

Phenotypic Evaluation of Whitefly-Transmitted Virus Symptoms in Two Watermelon F2 Interspecific Mapping Populations

Alexander Luckew and Cecilia McGregor

IPBGG

Watermelon (Citrullus lanatus) faces significant challenges from whitefly-transmitted viruses, including Cucurbit leaf crumple virus (CuLCrV), Cucurbit yellow stunting disorder virus (CYSDV), and Cucurbit chlorotic yellows virus (CCYV). In Colquitt County, 81%, 79% and 2% of watermelon samples collected in 2019 were infected with CuLCrV, CCYV, and CYSDV, respectively. Managing whiteflies and the viruses they transmit requires multiple control methods, yet they do not eliminate the impacts since even a single whitefly could transmit these viruses. Developing resistant cultivars is the most effective management strategy. The aim of this study was to phenotype two F2 mapping populations for virus disease severity.

Field trials in Tifton, Georgia, and Live Oak, Florida, in 2024 evaluated two mapping populations developed by crossing susceptible cultivars Charleston Gray and Sugar Baby with resistant C. mucosospermus selection UGA172. Crumple-like and yellowing symptoms were evaluated for 5 weeks on a scale of 0 to 5, (0 = no symptoms, 5 = 100% symptoms), then used to calculate AUDPC.

F2 plants from both populations segregated for both traits. Susceptible parents showed higher symptoms than F1s and resistant parents, with Tifton showing more severe yellowing symptoms than Live Oak. Leaf samples were collected for virus quantification. Phenotypic and viral load data will be used to develop two bulks for QTL-seq to identify loci associated with observed traits, followed by KASP markers development for virus resistance selection in cultivated watermelon.

07 Caitlin McCann

High throughput genotyping and SNP discovery in peach using Capture Seq technology for genetic diversity studies

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Peach (Prunus persica) is one of the world's most widely produced temperate fruit tree crops. In addition, it is considered a model organism for fruit tree research by virtue of its small and relatively simple genome. The first goal of our project is to characterize the relatedness and diversity of worldwide peach populations, to assess their potential as sources of breeding material to improve American peach varieties. Currently, genetic studies of P. persica are facilitated by 9k and 16k SNP chip arrays, which can be expensive and inflexible. Another goal of this project is to develop a panel of 50k SNP markers for use in peach and related species. These can be used for genetic diversity analysis, GWAS, QTL analysis, and more. We acquired tissue samples from 250 unique peach genotypes from the University of Georgia's Dempsey Farm, USDA-GRIN, and collaborators in Australia. The samples represented 28 different countries and 17 Prunus species and interspecific hybrids. Tissue samples were sent to Rapid Genomics for DNA extraction and Capture-Seq analysis. Capture-Seq was carried out using 50k genetic probes, which were based on known SNPs from the 16k SNP array, as well as SSR markers and exonic regions. After processing, over 7 million variants were identified, 134,424 of which were biallelic SNPs with a read depth between 10 and 100, minor allele frequency of 10% or more, and linkage disequilibrium r2 less than 0.2. This panel of 134k SNP markers was used to run a principal component analysis, which revealed separation between North American and Australian populations, marking Australian peaches as a potential source of novel germplasm for American breeding programs. The most informative SNP markers have been identified for use in genotyping. These markers will be used to investigate the diversity of Australian peach populations.

08 Gema Nugraha

Exploring the Diversity of a Legacy Wild Peanut Collection to Enhance Cultivated Peanut

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Genetic diversity of the allotetraploid cultivated peanut (Arachis hypogaea) is limited due to a domestication bottleneck, which hinders the improvement of peanut cultivars. On the other hand, wild diploid relatives are highly diverse, and they can be used to introduce beneficial traits, such as disease resistance. In the 1940s, James Louis Cowboy Stephens collected wild peanuts and other plant germplasms in South America, some of which have been growing on the Tifton Campus without any research or preserved passport information. This study aims to characterize this peanut collection and generate wild-derived allotetraploids compatible with cultivated peanuts. Preliminary identification using the Axiom_Arachis2 48K SNP array identified six out of eight accessions as A-genome species, with partial morphometric analyses of leaves and flowers affirming these findings. Subsequent in vitro and field evaluations for early and late leaf spot, as well as tomato spotted wilt virus (TSWV), revealed strong resistance in these accessions. Crosses with B-genome species produced hybrids that were, then, treated with colchicine to create novel allotetraploids. This collection, along with 48 accessions representing 29 species in the section Arachis will undergo whole exome sequencing to obtain better genetic information. This study provides valuable resources for peanut breeding programs. Utilizing these genetic resources can potentially lead to the development of more resilient peanut cultivars.

09 Aasish Pokharel

Validation and Quantification of a Major Seed Size QTL in a Biparental Elite Peanut Population.

Aasish Pokharel1, Zack Myers2, Walid Korani2, Josh Clevenger2, Nino Brown1

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Seed size is a key trait in peanut production, influencing marketability, yield potential, seed processing, and breeding efficiency. In an F_2 mapping population, a large-effect QTL (qYC-C11) on chromosome 11 (B01) was identified for individual plant yield components, along with a major QTL for pod and seed size (qSZ-C16) on chromosome 16 (B06). Kompetitive allele-specific PCR (KASP) markers tightly linked to these QTLs were tested for validation in F2:3 segregating lines. Only one of the eight markers for yield components was polymorphic. Hence, additional polymorphic KASP markers for yield components need to be developed near the QTL region. However, all eight pod and seed size associated markers were validated.

In 2023, individual plants were selected from F2:3 segregating lines, and 12 exhibiting intermediate seed size phenotypes were advanced to the F_4 generation in 2024. KASP markers linked to qSZ-C16 were validated in these 12 intermediate segregating lines, with a few recombinants identified. Recombinants were harvested individually and will be included in a replicated trial in the next growing season to refine the QTL region further. Additionally, F3:4 lines were evaluated in a replicated trial, and shelling is underway to quantify the effect of qSZ-C16. Further quantification and fine-mapping of the qSZ-C16 will be conducted in replicated trials in the coming seasons to identify candidate genes controlling seed size in peanuts. Validated QTLs and their associated markers will help define the genetic basis of peanut seed size and enhance peanut breeding efforts through marker-assisted selection.

10 Skye Remko

Establishing Genotype-Flexible Agrobacterium-Mediated Leaf Transformation in Switchgrass

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The Sustainable Aviation Fuel Grand Challenge aims to make 3 billion gallons of cost-competitive sustainable aviation fuel available to U.S. aircraft operators by 2030. By 2050, the broader objective is to achieve a fully carbon-neutral aviation sector. In pursuit of these goals, switchgrass is under evaluation as a next-generation biofuel. To contribute to the vision of a carbon-neutral aviation sector, this project seeks to improve and optimize genetic transformation in switchgrass. The primary objective is to leverage recent advancements in plant transformation techniques, specifically focusing on expanding transformable genotypes through Agrobacteriummediated leaf transformation. The overarching goal of this project is to conduct a comparative analysis of four sets of morphogenic genes, aiming to establish a more genotype flexible switchgrass leaf transformation system. The first set involves the overexpression of maize-derived morphogenic regulators, Babyboom and Wuschel2. This approach enables the transformation process to operate on leaf tissue, departing from the conventional use of embryogenic callus or immature embryos. The second set 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. The subsequent two sets incorporate the switchgrass morphogenic regulator PvWox2a: Wuschel-like homeobox 2a. All constructs use the RUBY reporter. Early stage testing in cv. Performer, using Babyboom and Wuschel2 resulted in transformation efficiencies as high as 45% (RUBY calli produced/ plants used as explants). The calli still need to form plants to determine the final efficiency.

101 Emile Barnes

Construction of an Introgression Line Population for Cultivated Peanut (Arachis hypogea) to Facilitate Breeding With Wild Relatives Arachis batizocoi and Arachis stenosperma

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EB: PBGG; SLB: PBGG, Plant Pathology; DB: PBGG, Crop & Soil Sciences

Cultivated peanut is susceptible to several highly damaging pests, pathogens and abiotic stressors. Its unique genetic origin has resulted in an extraordinarily narrow genetic bottleneck within the primary gene pool, and consequently, wild relatives represent the most viable source of valuable alleles for many crop protection traits. However, a ploidy barrier exists between cultivated and wild peanuts, which makes the process of interspecific hybridization relatively laborious and beyond the scope of many peanut breeding programs. Thus, the peanut breeding community would greatly benefit from a germplasm collection that captures the diversity represented by wild peanut species in a format that is compatible with cultivated peanut and easily introduced into breeding programs. To this end, we report the construction of a structured introgression line (IL) population bearing introgressions from the wild species A. stenosperma and A. batizocoi. The IL population, which has 32 lines in total, has been constructed to maximize genome coverage across the cultivated peanut allotetraploid genome with complementary introgressed segments of a mean size of 34.7 Mbp. In this report, we detail the genetic and population structure as well as present phenotypic data that demonstrates the utility of the population for cursory detection of genetic loci for several traits of interest. This IL population will be released for public use to the broader peanut research and breeding community. The open-ended nature of this resource will allow researchers and breeders to exploit the genetic value of these two wild peanut relatives for a number of traits without themselves needing to take on the task of interspecific hybridization.

102 Nathaniel Burner Burner

Developing and Deploying a UAS-based Pipeline for Determining Maturity of Soybeans

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Soybean maturity is one of the most important traits that growers consider when deciding which varieties to plant and is used in breeding programs as a covariate to draw meaningful comparisons among genotypes. Accurate phenotyping of maturity is an important task during line development but is labor-intensive. High-throughput phenotyping (HTP) of soybean maturity using unmanned aerial systems (UASs) can reduce the human resources and error associated with manual maturity notes to support breeding efforts resulting in higher quality data that will improve breeders' ability to evaluate the performance of breeding lines. Several studies have utilized spectral measurements to estimate soybean maturity of lines from Midwestern MGs (0–IV). However, these methodologies lacked public availability, scalability, and/or adoptability and they also retroactively estimate relative maturity after the conclusion of the growing season instead of providing timely estimates as lines mature. The objective of this study is to develop an intuitive, accessible, and accurate HTP pipeline for detecting the maturity status of soybean plots in real time. Matti, a QGIS plugin, was developed to track the average green leaf index (GLI) of soybean plots during the senescence period. Segmented and local polynomial regression models monitor the senescence curve and provide maturity estimates when the GLI values are near a user-specified threshold. This algorithm resulted in moderate to high correlations (R = 0.52-0.96) between the ground truth and estimated maturity of soybean lines in both early and late maturity groups. Similar correlations (R = 0.43-0.72) were found for early generation materials that are rated on a different maturity schedule. Maturity estimates were generally timely and occurred close to the observed maturity dates. Results indicate that Matti can be easily implemented by soybean breeding programs to provide estimates of relative maturity in a timely manner.

103 Kamalpreet Kaur Dhillon1*

Inducing Male Sterility in Zoysiagrass via Gene Cloning and CRISPR-Cas9 Editing

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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. The presence of fertile inflorescences can lead to unwanted seedling establishment through self and cross pollination. We are exploring a reverse genetics approach to induce sterility in zoysiagrass to address this issue. Mutation of the Acyl-CoA Synthetase (Acos) gene confers male sterility in Arabidopsis, rice, corn, and wheat. Atacos5 and Osacos12 mutants produce no pollen in mature anthers, no seeds by self-fertilization, and are severely compromised in pollen wall formation. Phylogenetic analysis of different land-plant orthologs of Acos to Zoysiagrass identified ZjAcos24 as the potential ortholog based on high amino acid similarity and conservation of essential protein domains. The genomic sequence of ZjAcos24 was cloned and sequence verified by Sanger sequencing from 'Zenith' zoysiagrass. Two constructs have been developed one driven by 35S promoter and other by Arabidopsis Acos5 promoter in the background of pMDC32 binary vector to determine if ZjAcos24 can complement the Arabidopsis Atacos5 mutants. We have also developed a reproducible regeneration protocol for zoysiagrass from mature seeds and successfully regenerated plants using CRISPR-Cas9 mediated genome editing. These regenerated plants will be further evaluated for sterility in the greenhouse, including the assessment of the phenotype of Zoysia knockout plants and by sequencing to confirm that CRISPR-induced mutations lead to male sterility. These pioneering efforts hold significant promise for the zoysiagrass breeding community enabling controlled sterility to maintain genetic purity in commercial cultivars.

Key words: Arabidopsis thaliana (At), Oryza sativa (Os) Zoysia japonica (Zj)

104 Qian Feng

Characterization of A Novel Locus for Fruit Flavor Aroma in Tomato

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Tomato 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, leading to consumer dissatisfaction. While volatile compounds play an important role in defining the distinct tomato flavor, little is known about their biochemical pathways, making it difficult to build a desirable volatile profile. Identifying the genes involved in volatile production can help us better understand the biochemistry as well as accelerate the breeding process. This study focuses on two consumer-desired volatiles, 1-nitro-2-phenylethane and phenylacetaldehyde, and has mapped a novel QTL on chromosome 8 by combining results from linkage mapping and GWAS (genome-wide association study). A cluster of Amino Acid Decarboxylases (AADCs) were identified as the candidate genes underlying this QTL and a total of four SV haplotypes of the AADC cluster were found in the Varitome collection. Among these haplotypes, Type III was lost during domestication and is a likely beneficial allele to increase the concentrations of phenylacetaldehyde and 1-nitro-2-phenylethane in tomato fruits. Preliminary data of transgenic plants created by CRISPR/Cas9 suggested a positive involvement of this AADC locus in volatile production. Enzymatic analysis of the AADC proteins and incorporation of the beneficial allele into modern tomato varieties is in progress. The outcome of this study will provide breeders valuable tools to facilitate the selection process for better tomato flavor. Characterization of volatile pathways will also give us insights on plant secondary metabolite biosynthesis and the evolution history during adaption and domestication. This research is funded by NSF IOS 2151032.

105 Anita Giabardo

Another Brick in the Cell Wall: The High-Resolution Transcriptional Landscape of Xylan Biosynthesis

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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 ρ 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 in poplar 717, we generated two single-cell RNA-seq datasets encompassing two different growth stages, from primary growth to secondary growth. Known cell type markers from the literature were used to annotate the cell clusters detected in the analysis. We used a curated list of genes known to be involved in xylan biosynthesis to generate xylan-specific gene co-expression modules. We identified gene modules involved in the different steps of xylan biosynthesis, including reducing end sequence biosynthesis, backbone elongation and decoration. The gene expression profiles show clear cell cluster specificity, highlighting the quality of the data. This analysis will serve as foundation in the identification of targets for cell type-specific xylan engineering.

106 Austin Hart

Fine-Mapping of A Novel Locus Regulating BCAA-Derived Volatiles In Tomato

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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, contributed by our perception of aromatic volatiles present within the fruit. Even at nanomolar concentrations, aromatic volatiles can be perceived by the olfactory system and influence the liking. These aromatic volatiles can be derived from numerous metabolites, in particular the branched-chain amino acid (BCAA) volatiles are derived from L-valine, L-isoleucine and L-leucine. It is generally considered that these BCAA-derived volatiles contribute positively to overall liking in tomato fruits. The objective of this research is to identify QTLs affecting the biosynthetic pathway for the production of eleven BCAA-derived volatiles in tomato. An F2 population was derived from two semi-domesticated tomatoes, BGV008042 and PI487625. A genetic map was constructed from 327 markers across the genome using genotyping by sequencing. Phenotyping for the eleven BCAA-derived volatiles was analyzed in red-ripe fruit using GC-FID. Linkage analysis was conducted using the composite interval mapping method, under the r/qtl package. To validate the QTL, progeny testing was conducted with eight F3 families. A total of 12 QTLs were identified in the F2, in which five overlapped on chromosome 3. The progeny testing showed significance in three of the eight families, validating and further fine-mapping the QTL. The results of this research will aid in evaluating the effect of the QTL and identify genes responsible for the accumulation of these BCAA-derived volatiles.

107 Rachel Hill

Identification and Distribution of Ramulariosis Species in Georgia

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Areolate Mildew is a late-season disease affecting cotton foliage and is characterized by necrotic lesions, white mildew, and premature defoliation. This disease is caused by two pathogens, Ramulariopsis gossypii and 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% yield losses, revealed R. pseudoglycines as the causal agent for 94.4% of the naturally occurring infections. As the presence of Areolate Mildew in Georgia and surrounding southeastern states has increased, the identity and distribution of the causal species were brought into question. During the 2023 growing season, 165 infected leaf samples were collected from cotton fields across Georgia, and utilizing the tools and methodologies established by the Brazilian team, the causal species was preliminarily determined using PCR. In 2024, 36 of these locations were resampled to confirm the previous year's results. Species identification was confirmed via fungal culturing and Sanger sequencing. While PCR results indicated that both species were present, sequencing results revealed that only R. pseudoglycines was present on infected leaves from both years. Additionally, these fungal isolates are being screened for fungicide resistance mutation(s) and diversity within the R. pseudoglycines species. As Areolate Mildew continues to progress in incidence and severity, the results of this study represent an initial step towards a better understanding of the identity and distribution of the causal species of this disease, which will aid pathologists and breeders in developing resistant resources.

108 Raegan Holton Holton

Evaluating Diverse Peanut Genotypes for Feeding Preferences of Field Pests

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There are several economically important field pests of peanut. Of these, insect pests which compromise peanut hull and skin integrity pose the most serious threats to both growers and shellers. The result of extensive damage caused by pests such as peanut burrower bug (Pangaeus bilineatus) and rootworm (Diabrotica spp.) is a value reduction in the price peanut growers receive for their peanuts. Damaged hulls and kernels also pose a risk in warehouse storage as they are more susceptible to fungal colonization and aflatoxin proliferation prior to shelling. Research regarding these pests is becoming more critical as regulations on crop protectants such as chlorpyrifos become more stringent. Here we examine the feeding preferences of two rootworm species on various peanut genotypes in naturally infested fields and present a phenotyping method for assessing damage from burrower bug. Abundance data was collected on pests throughout the season as an indicator of pest pressure at multiple field locations. Our analyses identified some peanut genotypes which may be more susceptible to rootworm damage and demonstrated the efficacy of utilizing x-ray tomography for assessing burrower bug damage.

109 Anne Frances Jarrell

Mining the wild species Solanum microdontum for improvement of cultivated potato

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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, the first of which is to identify S. microdontum accessions with characteristics that make them favorable for crossing with cultivated potato (S. tuberosum). 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 with publicly available accessions. The resulting genome assembly has a BUSCO score of 99.0% and an N50 of over 57Mb, indicating a high level of completeness. The reference accession was found to have a relatively high level of heterozygosity. A k-mer based kinship matrix describing relatedness among accessions in the diversity panel was generated, which revealed an underlying population structure. Specimen collection data for the panel was mined and will be used explore the relationship between geographic proximity and population structure. The project will enhance publicly available potato genome resources and permit robust data mining of S. microdontum trait loci, providing breeders with genetic, molecular, and germplasm resources for newly developed diploid potato breeding programs.

110 Prasanna Kharel

Genetic Engineering of Antioxidant Gene for Aflatoxin Resistance in Peanut

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Aflatoxin production by Aspergillus sp. has been linked with the stimulating effect of reactive oxygen species (ROS) produced during heat and drought stress. Since ROS scavenging is associated with host antioxidant capacity, developing peanuts with high antioxidant enzymes may reduce aflatoxin contamination. Developing peanuts with enhanced antioxidant capacity through traditional breeding methods is slow and partial due to high G*E interactions. As such, genetic engineering could be a potential solution.

The research aimed to develop genetically engineered peanut lines with different antioxidant capacities and correlate their antioxidant capacity with their resistance against aflatoxin contamination. Gene encoding antioxidant enzyme catalase1 (CAT1) was overexpressed using overexpression cassette containing kanamycin and hygromycin selection markers, eGFP, actin2 promoter, coding sequence of the gene, and transcription terminator. In addition to the overexpression lines, knockout lines were developed using CRISPR/Cas9 technique that targeted the CAT1 gene with two guide RNAs. Both the constructs were delivered to the peanut embryogenic callus 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. Overexpression lines and CRISPR/Cas9 edited T2 lines have been identified. These lines will be used to measure the catalase content and to 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.

111 Jordan Knapp-Wilson

Analysis of Five Year Time-Series Phenotyping of Peach Tree Architecture Utilizing TLS technology and GAMMS

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Tree architecture is a complex trait of growing importance for horticultural production of fruit trees and for future automation. Several architectural traits were collected and analyzed from 3D quantitative structural models (QSMs) of 36 peach trees over 5 years spanning Winter 2019 to Winter 2023 (labelled here as 2020-2024). Traits that were evaluated included average number of branches, tree height, branch length, and crown volume. Each tree across 5 years was modelled 50 times utilizing the TreeQSM modelling pipeline method modified to suit peach trees grown in an open vase training system. Data collected from these 50 in-silico replicates were averaged and then analyzed to observe the potential environmental and genetic impacts on the overall tree architectural phenotypes in a time-series study. Spearman's rank correlation was performed for each trait for every year vs. year combination. For number of branches, the greatest Spearman rank-order correlation coefficient (ρ) among trees across all year comparisons was $\rho = 0.64$ and p-value ≤ 0.001 (year 2020 vs. 2021). For branch length, the greatest correlation coefficient across all years was $\rho = 0.60$ and p-value = 0.0001 (2021 vs. 2022). Tree height across all years, the greatest correlation coefficient was $\rho = 0.620$ and p-value ≤ 0.001 (2020 vs. 2021). Canopy volume across all years, the greatest correlation coefficient was $\rho = 0.836$ and p-value ≤ 0.001 (2022 vs. 2023). Across all 5 years, tree 17b, 'Durbin', was the most heavily branched tree with an average of 1109 branches. Tree 4a, 'Reliance' had the greatest average total branch length at 460.17 m. Tree 6a, 'Chui Lum Tao' had the greatest average tree height at 4.17 m across the 5 years. Finally, the tree with the greatest average canopy volume was tree 8a,'Redhaven', at 60.81 m^3.

112 Dallas Kreisa

Characterizing Soybean RILs for Drought Tolerance Through Canopy Wilting, Imagery, Root Traits, and Metabolomics

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Drought stress is the leading cause of soybean yield reduction, particularly in the U.S. where only 10% of soybean acreage is irrigated. Drought-tolerant plants can maintain health under reduced water conditions, making them crucial for sustainable agriculture. Evaluating the soybean germplasm and characteristics related to drought tolerance can help identify genes associated with drought tolerance to support breeding efforts. A recombinant inbred line (RIL) population derived from cultivar Benning × G18-DT100, possessing a slow canopy wilting trait, was developed. After evaluation for two seasons, four RILs with contrasting canopy wilting phenotypes were selected along with their parents for further evaluation under drought and non-drought stress environments. Canopy wilting and root traits including fibrous rooting scores were assessed in the field to understand the relationship between above- and below-grounds characteristics of these RILs and parents. Additionally, the RILs and parents were evaluated using imagery and metabolomics, where 26 target compounds potentially involved in soybean drought tolerance were selected. The results indicated that under drought stress conditions, RILs exhibited expected contrasting canopy wilting scores. Slow canopy wilting RILs produced more fibrous roots than fast canopy wilting RILs, however, no difference was observed under non-drought stress conditions. Metabolomic analysis revealed 10 target metabolites were well separated in a PCA between the drought-tolerant and susceptible groups under drought-stress conditions, but not in the non-drought stress environment. These results have provided robust phenotyping tools for understanding drought tolerance mechanisms, gene discovery and improving breeding selection strategies for drought tolerance.

113 Khyathi Madduru

Exploring the Relationship Between Zinc Concentration, Deficiency Traits, and Spectral Indicators in Pecan

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Pecan (Carya illinoinensis), a nutritionally rich, long-lived deciduous tree native to North America, is widely cultivated for its high-value nuts, which provide essential minerals such as magnesium and zinc. Zinc deficiency, a persistent issue in pecan cultivation since the early 1900s, causes pecan rosette, leading to stunted growth and deformed leaves. A genetic mapping population from a 'Lakota' × 'MX3-2' cross was used to explore associations between zinc concentration and deficiency-associated traits, biometric traits and spectral reflectance indices to determine their ability to detect zinc deficiency. Field data included severity and incidence of visual zinc deficiency symptoms and SPAD readings, while biometric traits (tree height, width, diameter, and canopy cover) and multispectral light reflectance indices were obtained via drone-based RGB and multispectral imaging. Biometric characteristics were extracted from drone-based (UAV) RGB imagery. Spectral indices derived from RGB based (visible light spectrum) imagery included the Visible Atmospherically Resistant Index (VARI), Green Leaf Index (GLI) and Triangular Greenness Index (TGI). The Green-Red Vegetation Index (GRVI), Normalized Difference Red Edge (NDRE), Normalized Difference Vegetation Index (NDVI), Soil Adjusted Vegetation Index (SAVI) and Optimized Soil Adjusted Vegetation Index (OSAVI) were derived from multispectral data. Leaf samples from each tree were analyzed for foliar zinc content. Pearson's correlation analysis revealed significant positive correlations between leaf zinc content and SPAD, biometric traits, and spectral indices (GRVI, TGI, NDVI, SAVI, OSAVI). Conversely, visual severity, incidence, and the red band showed significant negative correlations with leaf zinc content. These findings highlight the complex interactions between zinc concentration and spectral traits, emphasizing the potential of remote sensing for developing predictive models in pecan cultivation and breeding.

114 Namrata Maharjan

Field evaluation of peanut breeding lines from diverse genetic sources for resistance to tomato spotted wilt (TSW) and late leaf spot (LLS) diseases

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Tomato spotted wilt (TSW) and late leaf spot (LLS) are major peanut diseases that significantly reduce yield. Current cultivars rely on a narrow genetic base for resistance, limiting long-term disease management. Developing resistant cultivars using diverse genetic sources is crucial for sustainable peanut production. This study evaluated advanced breeding lines from diverse genetic sources to identify resistance to TSW and LLS. Genotypes from four populations were tested: two advanced backcrossed populations: BatSten and ValSten, derived from diploid wild species [Arachis batizocoi K9484 x A. stenosperma V10309](2n=4x=40)) and [A. valida GK30011 x A. stenosperma V10309](2n=4x=40)) respectively via a tetraploid route and two three-way cross populations: TBI and BBI, that incorporate A. cardenasii segments through cultivars Bailey, IAC322, and TifNV-HighO/L. Thirty genotypes, including controls, were evaluated across three field trials, with each trial separately assessing TSW severity, TSW incidence and LLS severity under natural disease pressure. Several advanced lines from all populations demonstrated significant resistance to both diseases compared to susceptible checks. Among these, some lines from BBI and TBI exhibited robust resistance against both diseases. BatSten-derived lines generally showed strong resistance to TSW, although their resistance to LLS varied, a subset of these lines showed moderate resistance to both diseases. Similarly, some ValSten-derived lines showing resistance against TSW, displayed moderate resistance to LLS. This study highlights the potential of diverse wild-derived genetic resources to enhance peanut disease resistance. The most promising lines will be advanced to yield trials, with the goal for future cultivar release.

115 Dionne Martin

Understanding Tuber Development through Single Nuclei Multiomics

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Department of Genetics

Solanum tuberosum L. (potato) is the world's third most consumed food crop with its tubers serving as an important source of calories for millions of people worldwide. Tuber development is a tightly regulated process involving the transition of a hooked stolon (a modified stem) to a tuber. A limited number of genes have been identified in tuber development including the flowering time homolog StSP6A, the transcription factors (TF) StPOTH1, StBRC1b, and StBEL5. Surprisingly, nothing is known about their role in tuber initiation and development at the cell-type-specific level. To further understand tuber initiation and development in potato, we generated a single nuclei multi-ome data (gene expression and chromatin accessibility) from hooked stolons of the tetraploid S. tuberosum cv. Atlantic. Nuclei (20,079) were assigned to 27 clusters based on gene expression representing nine annotated cell types. We also show that many previously characterized tuber development genes show larger expression differences across tissues rather than cell types. We attribute this to the lack of single-cell data and phased genome assemblies available prior to this publication. We show that our robust dataset could detect cell-type specific expression differences for various gene families, including the more well-studied BEL1-like TF family. Through motif enrichment, differential chromatin analysis, and gene co-expression, we identified novel cell type-specific genes with putative roles in tuber development. Overall, this novel dataset is a powerful resource for the discovery of novel developmental genes as it is the first of its kind to characterize the cell-type specific gene expression and chromatin landscape of early tuber development.

116 Daniel Matusinec

New Potential for Stem Rot Resistance in Wild Peanut Hybrids

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Peanut (Arachis hypogaea L.) is an important agricultural commodity in Georgia, which produces most of the peanuts in the United States. But, every year, farmers in Georgia lose millions of dollars due to a peanut disease: stem rot, caused by the fungus Agroathelia rolfsii. Peanut cultivars grown in Georgia are highly susceptible to stem rot, and because peanut has little genetic diversity, little resistance to this disease has been found within the cultivated germplasm. The objective of this research is to screen related wild peanut species to identify new sources of stem rot resistance that could be introgressed into cultivated peanut. 8 novel wild peanut hybrids created in the UGA Wild Peanut Lab were phenotyped for resistance to stem rot in a greenhouse evaluation, consisting of two bioassays in 2024 and 2025. Five of the hybrids were significantly more resistant than a commonly grown susceptible peanut cultivar in at least one of the bioassays, and one of these was even significantly more resistant than the most resistant cultivar currently grown. These results indicate that there is untapped resistance to stem rot in the wild peanut germplasm, and will direct future efforts to further investigate this resistance and produce stem rot resistant cultivars.

117 Saptarshi Mondal

Identification of QTL Associated with Salt Tolerance in Zoysiagrass Mapping Population (Zoysia matrella × Z. japonica).

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Turfgrass management is presently facing many challenges due to soil salinization. One of the major contributors to soil salinization is sodium chloride (NaCl). Salinity imposes ionic, osmotic, and oxidative stresses on the plants, resulting in several physiological and biochemical alterations. Zoysiagrass is an important turfgrass species that is used widely in the US. Despite a relatively higher salt tolerance than other turfgrass species, leaf firing and reduced growth are some of the most conspicuous symptoms in zoysiagrass under salinity stress, which leads to a drastic economic loss. An interspecific mapping population between susceptible Z. japonica var. Meyer (broad leaf) and tolerant Zoysia matrella acc. PI231146 (narrow leaf) has been developed. Around 200 F2 plants were screened under control (no NaCl) and 30 dS/m levels of salinity (added NaCl) in two consecutive years (2023 and 2024). The mapping progeny along with their parents have been phenotyped for morpho-physiological parameters such as maximum quantum yield of photosystem II (FV/FM), visual rating (VR), green cover percentage (GC%), dry biomass (DB), and Na+/ K+ content. While plants showed a wide phenotypic separation from susceptibility to tolerance in both years, plants screened in 2024 recorded significantly higher mean values for most of the phenotypic parameters than 2023, except for FV/FM. In 2023 a total of four QTL and in 2024 a total of nine QTL were identified, out of which colocalization of QTL for GC (chr 11) and FV/FM (chr19) across the years was observed. We also identified a gene coding for cytochrome P450 superfamily protein within the QTL region on the chr 19, that may have a potential role in chlorophyll biosynthesis and photosynthesis. Further validation of QTL and fine mapping of GC and FV/FM will aid Zoysiagrass improvement by promoting marker-assisted selection.

118 Rose Muruo

Developing a High-Efficiency Transformation Protocol for Genome Editing in Arachis hypogaea (Peanut)

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Peanut (Arachis hypogaea L.) is a globally important oil crop whose production is hampered by various biotic and abiotic constraints. Genome editing plays an important role in unravelling the mechanism of resistance against these stressors and to develop improved varieties. Though our lab has already established a peanut genome editing protocol, many cultivated peanut varieties are recalcitrant to in vitro regeneration and the transformation efficiency is dependent on choice of explants and mode-of-gene delivery. Among the popular methods for transformation, Agrobacterium-mediated transgene delivery is preferred due to higher T-DNA integrity, low copy number and simplicity of the protocol. Therefore, we aim to develop a robust and genotype-independent Agrobacterium-mediated peanut transformation protocol in this study. Expression of Growth Regulating Factor 4 (GRF4) and GRF-Interacting Factor 1 (GIF1) has been shown to improve plant transformation through regulation of cell proliferation and organ development. We are in the process of cloning and expressing multiple combinations of peanut GRF4 and GIF1 orthologs in the binary vector to test their effectiveness in improving regeneration and ultimately transformation efficiency. Additionally, we are also testing multiple explants and media components to complement the effect of GRF4-GIF1 chimera.

Key words: Genome editing, GRF4-GIF1, regeneration efficiency, Agrobacterium-mediated transformation

119 Sindoora Nalajala

Evaluating Phytophthora Root Rot Resistance in Blueberry and Optimizing Inoculation Protocols for Reliable Screening

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Phytophthora cinnamomi, a silent destroyer of blueberry that causes root rot is posing a severe threat to its industry and growers. Southern highbush cultivars are commonly grown in Southern U.S due to their early fruit production and low chilling requirements. However, these cultivars are vulnerable to root rot. No single management practice can completely abolish the disease or cause of the disease from the field rather having genetic resistance is the most effective means to control the disease. Two separate experiments were carried with 18-month-old and 5-month-old seedlings of cultivars ‘Emerald’ and ‘Rebel’ to develop a reliable screening protocol for Phytophthora root rot resistance. Blueberry plants and seedlings inoculated with P. cinnamomi displayed a range of symptoms over time with two inoculation methods using ground mycelia or pathogen-colonized millet grain as inoculum. Plants were observed weekly for disease progression and rated using a scale of 0-10, 0 being the healthy vigorous growing plant and 10 severe wilting or dead plant to estimate AUDPC. Relative difference in fresh and dry shoot and root weights between the inoculated treatments and control of both cultivars were determined to estimate disease severity. Negative correlation was observed between AUDPC values and dry root weight indicating reduction in root weight with disease progression. Similar results were obtained with old plants and seedlings. Moreover, using young seedlings with the optimized protocol is space-efficient, shortens the screening period and improves the throughput of disease screening.

120 Swikriti Pandey

Developing capture panel for targeted enrichment in mutant peanut lines

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Targeted enrichment through capture panel is an efficient method of investigating genomic variations in mutant population. Capture panels selectively hybridize and capture specific genomic regions, increases the coverage depth and enriching the targeted region. This is an efficient way of enhancing sequence depth of low pass sequencing strategies, which, although cost-efficient, often relies heavily on imputation in parallel to reference data to fill in gaps of missing information. However, imputation is counterproductive in detecting unique nucleotide variants which are often the result of induced variations and a major objective of this project. To investigate induced variations in 768 Ethly methane sulfonate (EMS) induced mutant lines, we developed a capture panel based on the reference genome of the Tifrunner cultivar, targeting exonic regions. Due to the allotetraploid nature of cultivated peanut, pre-filtering process was employed to increase specificity and eliminate repetitive sequences. Only exonic regions exceeding 500 base pairs (bp) were retained, resulting in 65,469 candidate sequences. To further refine the panel, BLAST was employed to identify and filter out highly repetitive sequences. Sequences with up to two BLAST hits and 99% similarity were retained to ensure specificity and reduce redundancy. The final capture panel encompasses a target size of 2,452,560 bp, comprising 20,438 distinct targets. The development of capture panel will facilitate high-throughput sequencing enabling detection of genetic variations in mutant population.

121 Anne Pavia

Biosynthetic pathway gene discovery in medicinal plants: a comparative single-cell transcriptomic approach for Mitragyna speciosa

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Many members of the Order Gentianales produce a structurally diverse class of compounds, monoterpene indole alkaloids (MIAs), many of which are clinically relevant for human health. Mitragyna speciosa (kratom) produces MIAs that have potential to treat pain and opiate withdrawals. Since the chemical synthesis of these compounds poses a challenge, increasing production through bioengineering strategies can improve their affordability and accessibility thereby benefiting human health. Since the biosynthetic pathways of the kratom specific compounds are yet to be fully elucidated, the goal of this research is to generate multi-omics data that will aid in gene discovery of genes, transporters, and transcription factors involved in their biosynthesis and transport. With the help of a high-quality genome assembly and single cell transcriptomic data from a range of tissues, single-cell and co-expression analyses will identify potential candidates for missing genes in these pathways. The PIP-seq platform and scRNA-seq was used to obtain transcriptomes of ~ 24,000 leaf and ~ 20,000 stem single cells derived from nuclei or protoplasts, respectively. These cells were clustered with PCA in Seurat, identifying 23 leaf cell cluster composed of 7 major cell types. Similarly, stem single-cells separated into 24 clusters, primarily vasculature cell types. Gene co-expression analysis was performed at the single-cell resolution to find genes that are co-expressed and physically clustered with known pathway steps in order to identify candidates for late-stage MIA biosynthesis, specifically those encoding enzymes with oxidase and methyltransferase activity. The single cell transcriptomes of these tissues reveal that the biosynthetic pathway is partitioned to specific cell types, starting out in IPAP cells for MEP and early iridoid pathways and diverging to different cell types for early and late-stage MIA synthesis.

122 Sameer Pokhrel

Testing the Pillars of Agile Genetics in Peanut PanMAGIC Platform

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Agile genetics utilizes a large, structured population, single-plant phenotyping, and comprehensive haplotype knowledge for trait discovery. We have developed an 8-way Multi-Parent Advance Generation Inter-Cross (MAGIC) population in peanut, using 18 divergent genotypes as the founder lines. Those lines exhibited disease resistance, drought tolerance, and other agronomically important traits. The pangenome encompasses both the conserved and unique genes within a population, offering a more comprehensive genetic diversity compared to a single reference genome. No pangenome has been published for peanut (Arachis hypogaea) yet. In this research, the MAGIC founder lines were sequenced, assembled, scaffolded to the progenitor peanut reference genomes, and aligned to develop a pangenome graph. The assemblies were highly contiguous, complete, and had reduced sub-genomic collapse.

In 2023 and 2024, F2 and F4 progenies of the MAGIC population were evaluated for drought tolerance and white mold disease resistance. The drought study included 900 and the white mold disease study involved 600 individual progenies each year. Progenies were selected based on the parental contribution to their pedigree. Leaf tissue samples were collected from all the progenies for low-coverage whole genome sequencing. Genotyping was conducted using the Khufu pipeline, developed by Hudson Alpha Institute for Biotechnology. This platform will also be employed to map the genetic loci associated with drought tolerance and white mold disease resistance. The genotyping accuracy was assessed using the founder lines on the progeny genotyping platform. We report on the genotyping accuracy, phenotyping, and genetic dissection of those traits.

123 Emily Powell

Effects of Nodulation on Soybean Yield, Agronomic Traits, and Seed Composition

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Biological Nitrogen Fixation (BNF) is a process for soybean nitrogen (N) acquisition, requiring nodule formation to host Bradyrhizobium japonicum, a symbiotic bacterium that fixes atmospheric nitrogen into ammonia. Eight QTLs, rj1 through Rj8 linked to rhizobium response have been reported. A recessive allele, rj1, is reported to cause ineffective nodulation. This study evaluated the impact of the rj1 allele on yield and agronomic traits using two backcross populations: AGS738RR(4) × Lee-NN and AGS828RR(4) × Lee-NN, advanced to the BC3F4 generation. Lee-NN is a near-isogenic line (NIL) of 'Lee' carrying the rj1 allele, while 'AGS738RR' and 'AGS828RR' are elite southern cultivars. Eight RILs per pedigree (four nodulating, four non-nodulating) and the recurrent parents were yield tested under two N treatments: 0 or 168 kg/ha applied at V6-V8 and R3-R4 growth stages totaling 336 kg/ha. Yield differences were observed between nodulation types and nitrogen treatment. Across both populations, non-nodulating NILs without nitrogen yielded lower than nodulating NILs and non-nodulating NILs receiving nitrogen. The non-nodulating NILs receiving nitrogen had yields comparable to nodulating NILs and recurrent parent. The magnitude of these differences varied between pedigrees, indicating that genotypic background plays a role in the effect of the rj1 allele on yield, however, the overall trend remained consistent. Non-nodulation decreases protein content, this reduction can be reversed with nitrogen supplementation. Non-nodulation increases oil content; this increase is reduced with the addition of nitrogen, suggesting that these effects result from nitrogen deficiency. These non-nodulating-NILs represent an important resource in quantifying BNF in modern cultivars.

124 Pallavi Rathore

Optimizing in-vitro cotyledon regeneration and determining antibiotics sensitivity level in watermelon.

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Watermelon (Citrullus lanatus) is an important crop because of its economic worth and appeal as a fresh fruit, which contributes to both domestic and foreign markets. Continuous cultivar development with improved traits like fruit quality, resistance to biotic and abiotic stresses, and yield is essential to ensure grower profitability and consumer satisfaction. CRISPR/Cas9 is a useful plant breeding tool that can accelerate breeding and be a source of novel variation. An important requirement for gene editing is an efficient plant regeneration protocol. This study focuses on developing and optimizing a watermelon in-vitro cotyledon regeneration protocol and evaluating the efficiency of different antibiotics for selection in future gene editing experiments. Different explants for watermelon regeneration were tested which included both in-vitro and in-vivo first true leaves and cotyledons. Among all these explants, in-vitro cotyledons demonstrated successful regeneration, from which micro-cuttings were cultured to generate full plants. The sensitivity of explants to Hygromycin and Spectinomycin were tested for concentration between 5mg/L and 40mg/L; 50mg/L and 200mg/L respectively. Weekly observations of yellowing in explants were made for 5 weeks. It was determined that 20mg/L and 100 mg/L of Hygromycin and Spectinomycin respectively, is optimal for explant selection. The results from this research lay the groundwork for future CRISPR/Cas9 gene-editing in watermelon.

125 Samikshya Rijal

Deciphering the Fungal Complex Behind Panicle Diseases: Ergot and False Smut in Georgia Switchgrass

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Smut and ergot diseases cause significant seed and grain losses in grass species, including switchgrass (Panicum virgatum L.), a key biofuel crop. Head smut (Tilletia spp.) has been reported in New York and Texas, while false smut symptoms have been observed since 2019 in a genome-wide association study (GWAS) panel of 285 switchgrass accessions grown at the University of Georgia's Iron Horse Farm (Watkinsville) and Gibbs Farm (Tifton, GA), as well as the University of Tennessee Knoxville (UTK). This study aims to identify fungal species associated with ergot and false smut in Georgia. Fungal isolates were characterized morphologically and phylogenetically, followed by pathogenicity tests on infected panicles and seedlings. A maximum likelihood tree, based on concatenated Internal Transcribed Spacer (ITS) and RNA Polymerase II (RPB2) sequences, confirmed Claviceps clavispora as the causal agent of ergot. Similarly, ITS, β-Tubulin, and Elongation Factor-1α sequences identified four Epicoccum species colonizing ergot-affected panicles. Artificial inoculation studies demonstrated that C. clavispora infects switchgrass panicles, whereas Epicoccum spp. did not induce panicle symptoms. However, E. spegazzinii caused necrotic lesions on seedlings, while E. sorghinum, E. andropogonis, and E. nigrum remained asymptomatic. Ongoing research aims to identify marker-trait associations for ergot and false smut resistance, supporting breeding efforts for disease-resistant switchgrass.

126 Iago Schardong

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

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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.

127 Gurjot Singh Sidhu

Evolution and diversification of the momilactone biosynthetic gene cluster in the Chloridoideae clade of Poaceae family

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Plants possess a remarkable ability to synthesize a wide variety of organic compounds. The genes responsible for the production of many of these specialized metabolites are often arranged together in groups within plant genomes, known as biosynthetic gene clusters (BGCs). Momilactones, first identified in rice, are diterpenoids that function as phytoalexins against pathogens and as allelochemicals against neighboring plants. In our previous studies on finger millet, we found that, on blast (Magnaporthe oryzae) infection, the genes involved in momilactone synthesis are highly expressed in a resistant genotype as compared to a susceptible one. Finger millet (Eleusine coracana), a subsistence crop grown in eastern Africa and southern Asia, belongs to the Chloridoideae clade of Poaceae (grass) family, alongside other important crops like teff (Eragrostis tef). Interestingly, in finger millet, the genes involved in momilactone synthesis do not follow the typical cluster-like organization and are located in non-syntenic positions compared to other Poaceae species, such as rice and wheat. It remains unclear whether the momilactone BGC evolved in Poaceae through vertical inheritance or horizontal gene transfer. Here, we propose to do comparative genomics across various species within the Chloridoideae clade to investigate the evolution of the momilactone BGC. Alongside genomics and transcriptomics analyses, we plan to examine the post-stress activation of momilactones in these species through mass spectrometry. These insights will shed light on momilactone synthesis in finger millet, ultimately aiding in the development of cultivars with enhanced resistance to blast disease.

128 Jake Spittle Spittle

Mining Novel QTLs for Insect Resistance from Wild Soybean

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In 2022, soybean [Glycine max] yield losses and management costs caused by insects in the U.S. totaled over $1.4 billion. Control can be achieved by using genes for increased plant resistance to leaf-chewing insects, which can minimize the need for insecticide applications and protect yield.Wild soybean [G. soja] has untapped genetic diversity that can serve as a source of insect resistance for breeders to complement the resistance found in G. max. Thus, 112 accessions comprising the soja core collection were screened for resistance to leaf-chewing insects. Lack of preference was evaluated in a greenhouse using soybean loopers [Chrysodeixis includens], and through an open infestation of Mexican bean beetles [Epilachna varivestis] in the field. Two years of field data were gathered, and the second year of greenhouse data is currently being generated. G24-W751 was the most resistant accession in the field studies, averaging 5.7% defoliation. This accession is over twice as resistant as the most resistant Glycine max check, Benning EMGH, which contains four stacked QTLs for defoliator resistance and averaged 12.2% defoliation. Additionally, G24-W751 was also the most resistant accession in greenhouse testing performed thus far. Haplotypes deduced from SoySNP50k chip data suggest that G24-W751 may have the two largest insect resistance QTL, M and E, but no other known QTLs for defoliator resistance that would explain the enhanced resistance. A biparental mapping population is being developed to identify novel QTLs from this wild accession.

129 Bukhtaawer Talat

Integrating High-throughput Phenotyping and Genetic analysis on resistance to CLRDV-Induced bronze wilting disease in Cotton

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Cotton leafroll dwarf virus (CLRDV) is a non-enveloped, single-stranded RNA virus from the genus Polerovirus (family Solemoviridae) that is transmitted by the cotton aphid (Aphis gossypii Glover), affecting various plant genotypes. While infections may be asymptomatic, susceptible genotypes often exhibit symptoms such as stunted growth, leaf reddening, V-shaped curling, reddening of petioles and stems, and wilting, with some symptoms disappearing during non-peak heating hours. The first objective of this study is to diagnose these symptoms using a combination of field observations and high-throughput phenotyping and correlate these symptoms with virus detection assays. To achieve this, we planted genotypes historically determined to be susceptible to bronze wilt and monitor plants which led to collecting our high throughput phenotypic data on a small subset of diseased and resistant lines. The second objective is to conduct marker-trait association using three F2:3 populations segregating for the disease. The preliminary results suggest that drone-based multi-spectral and thermal imaging can be very helpful in disease diagnosis; however, the data collection must be conducted early in the growing season and is most effective during the hottest part of the day from 11 am to 4 pm. Preliminary quantitative trait locus (QTL) analysis suggests that a resistant locus may be located in the telomeric region of chromosome 10. Future work will involve validation of the identified QTL using different genetic populations and additional mapping approaches, such as QTLSeq, Fine mapping. This research has the potential to better understand the genetics of virus resistance contributing to the development of resistant cotton cultivars.

130 Brandon Tonnis

Expanding peanut genetic diversity through the characterization of important seed traits in induced allotetraploids

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Identifying and expanding genetic diversity is necessary for improving crop traits through plant breeding. Peanut, Arachis hypogaea, is a major crop grown in the Southeast and is an important source of seed oil and protein. However, it has low genetic diversity due to its relatively recent origin and ploidy barrier with most of its wild relatives. Wild peanut relatives are known to harbor alleles for improving disease resistance in cultivated peanut, and they may be new genetic resources for improving the nutritional components of peanuts as well. To that end, six new allotetraploids were produced by crossing closely related diploid species and chemically doubling the ploidy of the hybrids to generate fertile tetraploids compatible with cultivated peanuts. All combinations included at least one of the peanut progenitor species, A. ipaënsis and A. duranensis. Our objective was to measure the newly produced allotetraploids for oil content, protein content, and fatty acid composition to characterize these new sources of genetic variation. WPL-BatDur1, WPL-BatDur2, WPL-MagDur1, WPL-IpaCor1, WPL-IpaDur1, and WPL-IpaVillo1 were grown in 2023 in a screenhouse in Griffin, Ga along with five common peanut cultivars. Harvested seeds were measured for oil content using NMR, protein content by combustion analysis, and fatty acid composition using gas chromatography. Protein content was higher in the allotetraploid lines compared to the peanut cultivars, while oil content was similar. The nutritionally important fatty acid, oleic acid, was lower on average. However, individual seeds of IpaDur1 were much larger and had higher oil and oleic acid compared to other allotetraploids. The results presented here suggest these new allotetraploids may be an important resource for improving seed traits in peanut cultivars.

131 Katie Toomey

Targetable Landing Pad in Poplar

Wayne Parrott, Pete LaFayette

BioPoplar
Department of Energy

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 would be facilitated by a genomic landing pad that facilitates targeted and repetitive insertion of specific elements into a into a previously selected locus.

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 multiple gene insertion into a single locus, and retain the ability to add additional genes in the future.

132 Samantha Wan

Phenotypic Validation and Candidate Gene Identification in qFL-Chr.25, a Gossypium barbadense-sourced Fiber Length QTL in Four Diverse Upland cotton (G. hirsutum) Backgrounds

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Low genetic diversity within the cultivated genepool of upland cotton (Gossypium hirsutum L.) has hindered significant improvement in fiber quality. To address this limitation, we have utilized the secondary genepool to enrich genetic diversity. Gossypium barbadense, known for its superior fiber quality, has been used to transfer favorable fiber quality alleles into upland cotton. Genetic evaluation of an obsolete upland line Sealand 883 revealed stable G. barbadense introgressions. Through QTL analysis, several fiber quality quantitative trait loci (QTLs) were identified, with one QTL in particular, qFL-Chr.25, demonstrating a significant positive effect on fiber length when introgressed into four diverse upland backgrounds. To validate the phenotypic effects of qFL-Chr. 25, eight pairs of nearly-isogenic introgression lines (NIILs) were developed, each carrying a QTL positive and a QTL negative allele for qFL-Chr.25. This study validated the phenotypic gain on fiber length from deploying qFL-Chr.25 using various fiber length measurements, including UHML, L(n), L(w), UQL(w), and L5%. Across all genetic backgrounds, fiber length increased by 1.1 mm, 0.6 mm, 0.1 mm, 1.1 mm, and 1.4 mm, respectively. Additionally, transcriptome profiling using RNA sequencing identified three putative candidate genes (Ghir_D06G000180, Ghir_D06G000680 and Ghir_D06G000880) as causal fiber length genes. Lines carrying the G. barbadense alleles showed significant down-regulation during early fiber elongation stages in all three genes. A functional validation study is in progress using reverse genetic approaches to determine whether silencing the G. hirsutum allele will result in increased fiber length.

133 Habib Widyawan

Two Novel QTLs Conferring Resistance to Southern Stem Canker

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Southern stem canker (SSC) is an economically significant soybean disease caused by the soil-borne fungal pathogen Diaporthe aspalathi. Five resistant loci, Rdm1 to Rdm5, confer resistance to SSC. Among these, Rdm3 is the only locus that has been mapped on the soybean genome and used as the primary source of resistance. However, using a single resistance source, such as the Rdm3, may lead to resistance breakdown and subsequent disease outbreaks. D85-10412 is a resistant line assigned with the Rdm2 locus and possesses a unique resistance distinct from Rdm3. In this study, a recombinant inbred line (RIL) population derived from G81-2057 ρ D85-10412 was used to map the resistant gene(s) from D85-10412. QTL sequencing (QTL-seq) analysis identified three putative genomic regions containing the resistance genes on chromosomes 2, 9, and 17. KASP SNP genotyping and linkage analysis confirmed two loci with major effects on chromosomes 2 and 17, designated as qSSC_2 and qSSC_17, respectively. qSSC_2 and qSSC_17 account for 71.0 and 26.5% of phenotypic variation in the RIL population, respectively. A combination of qSSC_2 and qSSC_17 increased phenotypic variation by only 0.3% compared to the qSSC_2 locus alone, suggesting that qSSC_17 contributes minimally to overall SSC resistance. Glyma.02G191950, a gibberellin-regulated family protein, is a strong candidate gene for qSSC_2 due to its proximity and role in regulating plant defense mechanisms. SNP assay, GSM1274, was developed for the qSSC_2 QTL to facilitate the selection of soybean lines with resistance provided by the qSSC_2 to develop resistant soybean cultivars and improve the durability of SSC resistance in soybean production.

201 Mounirou H. Alyr

Genetic analysis of Arachis magna reveals a new source of rust resistance for the peanut crop

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Leaf rust is one of the most important foliar diseases in peanut. Previous studies have shown that the wild diploid species Arachis magna is an important source of rust-resistant alleles. In this study, a population derived from the third backcross of cultivated peanut (Arachis hypogaea var. IAC OL4) and an induced allotetraploid (Arachis magna K 30097/PI 468340 x A. stenosperma V 7382/PI 497580)4x was evaluated in order to identify genomic regions controlling resistance to rust. This population was genotyped with the improved 48k SNPs array and phenotyped using the detached leaf technique. QTL analysis revealed a major QTL for rust resistance at the end of chromosome B08 in an interval of about 4.4 Mb. The QTL interval contained respectively 383, 265 and 229 predicted genes in A. magna, A. ipaënsis and in A. hypogaea cv. Tifrunner. Several disease resistance genes were found in the QTL region, including genes coding for terpene synthases, pathogenesis-related proteins, glucan endo-1,3-beta-glucosidase 5-like and transcription factors. KASP markers associated with the QTL were developed and validated using 66 progenies from 33 selected BC3 lines. This study confirms previously reported QTL for rust resistance derived from A. magna in diploid and tetraploid populations, and lays a solid basis for QTL fine-mapping and discovery of rust resistance genes.

202 Roth Conrad

A Reference Pangenome for the Pecan Provenance Collection Captures Common Pecan Diversity

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UGA, USDA-ARS

One of the most diverse collections of pecan germplasm (known as the Provenance Collection) is housed at the USDA-ARS Southeastern Fruit and Tree Nut Research Station in Byron, GA. This Carya illinoinensis collection consists of seed germplasm collected from nineteen distinct source locations (provenances) ranging from Northern Missouri to Oaxaca, Mexico. As such, the genetic diversity represented in the Provenance Collection also underlies numerous horticultural traits, including days to bud break, tree growth, resistance to key pests and disease, fruit development period, and yield, as well as, fruit quality traits such as nut shape and size, shell thickness, and percent kernel. The genetic basis of these traits in pecan is largely unknown due to limited genetic studies. Genome-wide variant identification will be a valuable genomic resource for carrying out genome-wide association studies and identifying allelic variants/candidate genes associated with the trait of interest. The associated variants can be used as DNA markers and implemented as valuable molecular tools in the marker-assisted breeding of pecans. Presently, we have sequenced and assembled haplotype-resolved genomes for 20 individuals (40 haploid genomes) using PacBio HiFi reads. Assemblies were aligned at the genome level, producing a pangenomic graph for variant characterization and future genotyping of the entire population. Rarefaction analysis indicates that sampling of core genomes was uniformly distributed across the phylogeny and that, though there is additional common variation unaccounted for, our sampling depth has entered a linear phase where additional sampling is adding mostly rare variants.

203 Shizhe Liang

An artificial intelligence (AI) based predictive breeding platform to improve plant breeding efficiency

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Improving yield is a primary goal of row-crop breeding, as yield is the main determinant of crops' profitability. Genomic selection (GS) has emerged as a powerful computational tool for improving breeding efficiency. It leverages past genotype-phenotype associations to predict phenotypic performance, enabling early-stage selection without extensive field testing. However, the integration of AI-driven methods in breeding remains underexplored. To address this gap, we are developing a two-stage machine learning framework for GS and a web-based interface to build a breeder friendly platform. This framework consists of a multi-task Lasso-based feature selection step, followed by applications of machine learning algorithms for direct prediction. It first identifies key molecular markers relevant to yield prediction, reducing dimensionality while preserving essential genetic signals. Then it applies various machine learning models to achieve maximal prediction accuracy. This study utilized nine years of multi-environment soybean breeding yield data as an example to perform robust validation across diverse environments. To facilitate adoption, we are developing an interactive, web-based software platform with a user-friendly graphical interface. It allows breeders to upload genomic and phenotypic data sets, perform GS, compare different prediction models, and visualize results intuitively. Designed for flexibility and scalability, the platform bridges the gap between AI advancements and practical breeding applications, making genomic selection more accessible in crops. Our research not only enhances genomic prediction accuracy through an AI-driven approach but also provides an intuitive software solution to support data-driven breeding decisions, adaptable across various crops. This will empower plant breeders to leverage cutting-edge methodologies to accelerate genetic gain.

204 Raissa Martins da Silva

Genetics of reproductive traits in Peanut: QTL Mapping Reveals Promising Targets for Breeding

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Peanut (Arachis hypogaea L.) is an essential legume crop, providing oil, protein, and economic value globally. Understanding the genetic basis of reproductive traits and plant architecture is crucial for improving yield and adaptability. Reproductive traits, which directly influence yield, are shaped by plant architecture through flowering patterns and resource allocation. Identifying genomic regions that control these traits can support high-yield, early maturing peanut breeding. In this study, SNP-based QTL mapping was performed using a recombinant inbred line population derived from a Tifrunner × GT-C20 (ssp. hypogaea × fastigiata) cross to identify key genomic regions regulating reproductive traits and plant architecture. A population of 123 RIL was phenotyped for reproductive and vegetative traits over two years. Genotyping was conducted using the Axiom_Arachis2 48K SNP array, and a genetic map with 3,969 loci was constructed using JoinMap v4.1. QTL mapping was performed with R/qtl using multiple QTL mapping (MQM) model. A promising, major, and stable QTL on chromosome B06 harbored genes potentially involved in flowering time regulation, including those related to signal transduction, light perception, hormone signaling, and transcriptional control. Additionally, major and stable QTL on A02 and B02 were linked to mainstem flower, with an additive interaction enhancing MSF- expression. A major and stable QTL on B05 controlled growth habit, primary lateral length, and main stem prominence, suggesting pleiotropic effects. The results of this research will aid in mapping QTLs controlling reproductive traits and plant architecture, which could be utilized to develop peanut cultivars with improved maturity, yield, and adaptability.

205 Sudeep Pandey

Unveiling Grafting-Induced Cold Tolerance Mechanisms in Cucumber via Transcriptomics

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Cucumber (Cucumis sativus) is a vital vegetable crop in the United States, with increasing production in controlled environment agriculture (CEA). Maintaining optimal temperatures (26–30°C) during colder seasons raises energy costs, making cold-tolerant grafting a promising alternative. However, the molecular mechanisms underlying graft-induced cold resistance remain unclear. This study utilized a transcriptomics approach to identify differentially expressed genes (DEGs) and pathways associated with cold tolerance in parthenocarpic cucumber (C. sativus cv. Diva) grafted onto fig-leaf gourd (Cucurbita ficifolia), Tetsukabuto squash (C. maxima × C. moschata), and self-grafted plants. Plants were exposed to three temperature treatments (D/N: G1 = 12/6°C, G2 = 18/12°C, G3 = 24/18°C) for 21 days, with leaf samples collected at Days 0 and 21 for RNA sequencing. High mortality in G1 led to its exclusion from analysis. DEG analysis revealed minimal overlap among all three graft types, with hetero-grafts sharing more DEGs than self-grafts. More underexpressed DEGs were identified in G2, while overexpressed DEGs increased at Day 21 in C. ficifolia and Tetsukabuto grafts but not in self-grafted plants. Gene Ontology (GO) and KEGG analyses identified key cold-responsive transcription factors (WRKY, MYB, NAC, AP2/ERF) and stress-related proteins (POD, prolines), varying across rootstocks. These findings enhance understanding of the molecular basis of cold resistance in grafted cucumbers, offering genetic targets for improving CEA cucumber production.

206 Yunus Sahin

How Magnaporthe oryzae loses its DNA

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The fungus Pyricularia oryzae, which causes blast disease, is a significant pathogen that affects cereal crops like rice and finger millet, leading to annual yield losses of 50%. Host resistance is typically driven by fungal Avirulence (Avr) – host resistance (R) gene interactions. Pathogens can evade detection by the host by losing Avr genes, thereby compromising the plant's innate immunity and facilitating infection. Sequencing of 226 blast strains revealed the presence of 5,647 deletions, ranging from 5 bp to 251,694 bp. Manual analysis of the breakpoints of 14 deletions revealed frequent association with transposable elements (TEs). A genome-wide study was then conducted to examine the prevalence of TEs associated with deletions in the genomes of 33 Ethiopian blast isolates. TEs belonging to seven families were identified in raw Illumina reads using BLASTn, the positions of the reads in the reference genome (generated in strain E2) were determined using Bowtie, and deletion breakpoints associated with a TE were identified using in-house scripts. Strain E15 exhibited the lowest frequency of TE-associated deletions (15%), while E49 had the highest frequency (80%). The deletions, which likely occurred by homologous recombination between TEs from the same family, resulted in removal of 416 genes, 13 of which were predicted effector genes. These findings provide evidence that TE-driven genomic loss may be an important mechanism through which the blast fungus overcomes single-gene resistance. Understanding the role of TEs in shaping the genomic landscape of P. oryzae offers valuable insights into pathogen evolution and adaptability.

207 Fiorella Spies

Advancing genome editing in poplar: Identifying and targeting safe harbors

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Poplar spp are beginning to emerge as a pioneering concept in bioproduct and bioenergy production. Their small genome, extensive genomic resources, and susceptibility to Agrobacterium tumefaciens make them amenable to transgenic experiments.

Our primary goal is to identify unique genomic regions within the Populus tremula x Populus alba (poplar 717) genome where foreign sequences can be inserted without interfering with the normal function of the cells, known as Safe Harbors (SH), and to develop site-directed genome-editing tools that enable precise integration of DNA sequences into these SH sites.

To help identify SH regions, 11 events that were stably expressed for over 10 years, without affecting the phenotype, were sequenced to determine the T-DNA insertion sites. These sites were further analyzed to identify those located away from highly methylated regions, miRNA, or tRNA putative genes, and not disrupting any coding genes. This analysis revealed three candidate SH regions, ranging from 11.1 to 13.2 kb in length, which will be further studied to determine their ability to uptake foreign DNA without disrupting the normal function of the host cell, thereby confirming their suitability as SH.

Two alternative site-directed editing approaches are currently under evaluation to insert transgenes into a SH: Since these plasmids are 21 and 20 kb in size, they were tested for stability in various strains of Agrobacerium, and found to be stable in GV3101 and GV3101thy-.

208 Yi-Wen Wang

k-mer Genome-Wide Association Study for Common Bacterial Blight Resistance in a Tepary (Phaseolus acutifolius) Diversity Panel

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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. For example, resistance to common bacterial blight (CBB) exists in tepary bean. The objective of this research is to augment knowledge of loci encoding bacterial blight resistant traits in tepary bean via genome-wide association studies (GWAS). Whole genome sequencing was conducted on 290 tepary accessions using Illumina NovaSeq 6000. CBB phenotyping data from a previous study evaluated disease severity on a 1–9 scale. A k-mer-based GWAS on 146 accessions identified CBB-associated k-mers. These k-mers were mapped across tepary genomes and visualized using R and Python tools. 1.08 billion unique k-mers were identified, of which, 5,241 were significantly associated with CBB. These k-mers were further mapped across various tepary bean genomes with different levels of CBB resistant level. 88% of k-mers were mapped to the accession TDP-154, which has high CBB resistance. The Manhattan plots revealed a k-mer peak of the significant k-mers in contig 750. A riparian plot further illustrated a syntenic region in multiple tepary bean genomes in which k-mers associated with resistance to CBB aligned. In conclusion, the significant k-mers associated with CBB resistance were identified in tepary beans. Additionally, potential candidate genes and genomic regions were discovered that could enhance disease resistance in Phaseolus breeding programs.

209 Lei Zhang

A QTL for fine tuning methyl salicylate level in tomato fruits

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Center for Applied Genetic Technologies

Methyl salicylate (MeSA) is an important signaling molecule within and between plants against biotic and abiotic stress. MeSA in tomato fruit is associated with low liking by consumer taste panels. The low level of MeSA in many tomato accessions is likely due to selection for superior taste. However, exogenous application of MeSA to tomato fruits was shown to reduce disease occurrence and cold damage during storage.

In tomato fruit, 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. 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, but loss of function in either gene will drastically increase the MeSA level. We aim to identify additional MeSA regulators that could function in fine-tuning MeSA levels to achieve the proper balance between the fruit flavor and defense.

We mapped a single MeSA QTL on the bottom of chromosome 3 (MeSA3.1) in an F2 population fixed at functional allele of both MES and NSGT1. Further fine mapping narrowed the MeSA3.1 to a 190 kb region, including a cluster of 20 cytochrome P450 genes and 11 other genes. The CYP71 group P450 synthesizes mandelonitrile from phenylalanine in peach and apricot. If the P450 plays the same role in tomato, it may involve in the MeSA production upstream of salicylic acid. Extensive structural variations were observed in the P450 clusters between the parental accessions which may cause differential expression of the causal P450. CRISPR-Cas9 mediated knock-out of possible P450 and a few other enzymes in the interval are underway to identify the causal gene.

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