Institute of Plant Breeding, Genetics and Genomics
College of Agricultural &Environmental Sciences
UNIVERSITY OF GEORGIA

POSTER ABSTRACTS

Organized by Competition and Author Last Name

Wednesday, May 17, 2023 – Friday, May 19, 2023

The Columbus Georgia Convention & Trade Center
in the historic Iron Works Building
801 Front Avenue | Columbus, GA 31901

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Characterization of *Arachis cardenasii* Introgression in Cultivated Peanut, *Arachis hypogaea*

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***MS**

Peanut root knot nematodes (PRKN), *Meloidogyne arenaria,* are microscopic roundworms that infect peanut, *Arachis hypogaea.* To combat this pest, peanut growers can use both nematicides and crop rotation. A more desirable method would be to grow peanut cultivars that have resistance to PRKN. No strong resistance was discovered in cultivated peanut; however, strong resistance does exist in a wild relative, *Arachis cardenasii.* An interspecific cross made using this wild source as a donor for PRKN resistance led to the development of 'COAN' which has strong PRKN resistance but lacks tomato spotted wilt virus (TSWV) resistance, which is another significant problem in peanut. Cultivar improvement for the southeastern US resulted in the development of 'Tifguard' which is resistant to both PRKN and TSWV. 92% of chromosome A09 in Tifguard is derived from *A. cardenasii.* Prior studies have indicated that strong resistance is conferred by the top portion of this introgression and moderate resistance by the lower portion. The combination of introgressed regions potentially could impact durability of resistance. Further characterization of these introgressed regions has resulted in identification of additional recombinants that are being tested for PRKN resistance. The combination of genotypic and phenotypic data will guide the selection of markers for breeding to incorporate only essential portions of wild segments. Furthermore, the resistance genes for PRKN will potentially be discovered.

In vitro Inoculation and Field Evaluation of Peanut Lines in Search of Resistance to Aflatoxin Contamination

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***MS**

Aflatoxins are a toxic, carcinogenic secondary metabolite that is produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin resistance in peanut has been sought after for many years but has remained elusive in cultivars because of large amounts of variance due to genotype-by-environment interactions, making it difficult to capture. Previously, four Quantitative Trait Loci (QTLs) have been identified on chromosomes A01, A02, B03, and B10 of the peanut genome. We evaluated several lines from a population (ICB population) derived from a cross between the resistant genotype ICG1471 and the resistant line Carolina Black, and one line from a population made from a cross between ICG1471 and the susceptible Florida-07. We also evaluated the parents of our 18-parent and 8-parent MAGIC (Multi-parent Advanced Generation Intercross) populations, since it would be beneficial for us to see the resistance levels of the parents as we test the progeny for aflatoxin resistance. Seeds from these lines were inoculated with *Aspergillus flavus*. The aflatoxin was then extracted using methanol and quantified using Aflatest affinity columns and a fluorometer. We compared the Fisher's Least Significant Difference groupings to the genotypes of each line to see how they differed with the log-transformed aflatoxin production levels. Through this *in vitro* inoculation of lines, we were able to see how the groupings corresponded with the QTLs for resistance in the ICB population, but more testing needs to be done to confirm the QTLs. There was also good differentiation between many of the MAGIC parents. From the ICG1471 x Carolina Black population, line ICB_11_02 shows promise as a germplasm with resistance to aflatoxin production.

Mapping of candidate QTLs for Ramularia Leaf Spot resistance in cotton in two resistance sources: BRS 372 and 7-7-1020CT

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***MS**

Ramularia Leaf Spot of cotton (RLS) is one of the most economical important cotton diseases in South America, especially in Brazil where severe incidence of the disease causes early defoliation and reduced yields between 20% and 60% have been reported. RLS is of increasing concern for the U.S. cotton growing regions because new disease incidence has recently been reported a number of states. RLS has two causal agents: *Ramulariopsis gossypii* and *Ramulariopsis psuedoglycines*. Chemical fungicides are required in Brazil to control for RLS, which is the main means of control of RLS. Because of the heavy reliance on fungicides there are fears that the two causal species of RLS will develop resistance. Consequently, there is great interest in developing varieties with genetic resistance to RLS. In Brazil Dr. Nelson Suassuna has developed two resistant lines; BRS 372 and BRS 416. We have created six biparental mapping populations with BRS 372 and another resistant line; 7-7-1020CT crossed to three susceptible cotton lines. The first year of phenotypic data seems to show that the causal genes have a different mode of inheritance. Mapping of the resistance gene(s) can assist in marker assisted selection of new varieties of cotton adapted to cotton growing regions in the U.S. where RLS is present.

Obtaining Dinitroaniline Resistance in *Schedonorus arundinaceus* (Tall Fescue)

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***MS**

Dinitroanilines have been used globally for decades as pre-emergence weed control. Currently there are twelve different weed species that have dinitroaniline resistance. The reported cases of resistance credit mutations within the alpha-tubulin protein that confer resistance to dinitroaniline herbicides. The reported mutations are Leu-125-Met, Leu-136-Phe, Val-202-Phe, Thr-239-Ile, Met-268-Thr, Arg-243-Met/Lys. There are no reported cases of dinitroaniline resistance within turfgrass species. A dinitroaniline resistance turfgrass would help with weed control during establishment on both the homeowner and industrial level. The goal of this research is to find and identify a mutation within the alpha-tubulin protein of tall fescue (*Schedonorus arundinaceus)* that confers resistance to pendimethalin and other dinitroaniline herbicides. Seed from tall fescue was screened using callus from tissue culture at 3 µmoles pendimethalin growth medium. Each screen occurred at either 2 or 3 cycles and each cycle was approximately one month. The callus that demonstrated growth were selected to regenerate new plants. The regenerated plants were then screened for pendimethalin resistance using hydroponics. Possible resistant genotypes selected from the tissue culture screen were separated into tillers and placed in Hoagland solution containing 0 or 2 µmoles of pendimethalin. After a month the tillers were harvested and root weight, root length, and visual ratings were recorded. After several screenings the most promising genotypes that exhibited significantly different root length, root weight, and visual ratings were recorded. The next step in this research is to extract DNA from these genotypes to perform PCR and gel electrophoresis. The intended goal is to separate the 700bp-800bp DNA bands to send off for sequencing to determine the presence of a mutation and mechanism of resistance.

Efficient transformation and regeneration of morning glory

Chad Hawkins*

***MS**

Rationale:

The genus *Ipomoea* has great potential as a tool to understand the genetic causes of phenotypic diversity. Members of *Ipomoea* genus span from cultivated species such as sweet potato and water spinach, to weeds such as the various morning glories. Within this genus there is great diversity in floral color, growth form, mating system and adaptations to environment. To discover the genetic underpinnings of this diversity, genetic resources must be developed to further our ability to study their genomes. Morning glory is native to Mexico and remains a recalcitrant species in tissue culture. Few genetic resources have been developed to study morning glory. Reports of embryogenesis from immature embryos have been made in some genotypes. It remains to be seen if *Agrobacterium*- mediated transformation is a potential tool we can use to introduce novel genes. Beyond this, regeneration protocols for transformed tissue must be developed in order to make use of transformations. Both will likely be possible if embryogenic calli can be maintained.

Objectives:

To develop efficient transformation and regeneration protocols for morning glory.

Methods:

Six genotypes are being tested on MS media supplemented with 4-Fluorophenoxyacetic acid (4FA) to determine if they will produce embryogenic calli. In order to determine if each of the genotypes is compatible with several *Agrobacterium* strains, an initial agroinfiltration test will be conducted. Once compatibility is established an attempt to transform calli will be made. In addition, a kill curve will be established using kanamycin and spectinomycin for plant selection. Post transformation, calli will be moved to half-strength MS media for regeneration.

Conclusions:

So far one of the six genotypes have produced yellow and friable calli. Once a sufficient calli population has been established, transformation work can begin.

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

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***MS**

The cowpea curculio, *Chalcodermus aeneus* Boheman, has been one of the most destructive insect pests of black-eyed peas or cowpea, *Vigna unguiculata* L., in the southeastern United States. Cowpea production in these areas has decreased significantly, and even with moderate levels of infestation, over 50% of losses have occurred due to insect damage. The cowpea curculio causes damage to both the pods and the peas inside, through feeding and ovipositioning. Control of the cowpea curculio is difficult, the adults mainly feed upon the pods and hide within the foliage, and the eggs and larvae are protected within the pod where the larvae cause the most damage. Currently, the best pest management practices involve insecticide application or avoidance of planting in areas where this insect is most prevalent. The objective of this research is to evaluate cowpea germplasm for resistance to the cowpea curculio and to identify resistant genotypes. Fourteen cowpea genotypes were selected and evaluated in three replicated field-choice experiments, one in 2021 and two in 2022. Insect damage was recorded on samples of 100 mature fresh green pods, measuring the feeding and oviposition marks on pods and peas and the number of larvae present. Preliminary results from the data analysis confirmed the potential presence of resistance reactions, tolerance, antibiosis, and antixenosis. Based on these results, five genotypes were selected for a no-choice greenhouse experiment in 2023 to investigate which mechanism or combination of mechanisms are present. An additional replicated field-choice experiment of the 14 genotypes will be conducted to verify results. Research results will assist in future development of varieties resistant to the cowpea curculio.

Capturing diversity from an uncharacterized wild peanut collection

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*** MS**

Genetic diversity of the allotetraploid cultivated peanut (*Arachis hypogaea*) is limited due to a domestication bottleneck, which hinders the improvement of peanut cultivars. To overcome this limitation, wild diploid relatives can be used to bring in useful traits, such as disease resistance. The objectives of this research are to generate wild-derived allotetraploids that are compatible for crossing with cultivated peanut so that disease resistance traits can be introduced to the cultivated peanut germplasm and to identify QTLs conferring resistance to tomato spotted wild virus (TSWV) in wild peanut. In the 1940s, James Louis "Cowboy" Stephens collected wild peanuts and other plant germplasms during his exploration in South America. Some of these peanuts have been growing on the Tifton Campus since that time, but no research has been done on them and their passport information was not preserved. Out of eight morphologically distinct accessions, six were genotyped using the Axiom_Arachis2 SNP array and identified as A-genome species. A pilot study indicated that four of these accessions exhibited strong field resistance to both TSWV and leaf spot diseases. All accessions were crossed with B-genome species, and the hybrids will be used to generate novel allotetraploids. TSWV-resistance QTLs were previously identified in progeny of *A. hypogaea* x (*A. ipaensis* x *A. correntina*). To investigate the potential source of resistance, two IpaCor4x hybrids were sequenced, then the assembled genome sequences were aligned to *A. ipaensis* to subtract out the B-genome sequence. The remaining sequences were assembled to construct putative *A. correntina* genomes. The assembly will be used to develop SNP markers across the QTL for QTL validation. This study provides valuable resources for peanut breeding programs by generating novel allotetraploids and identifying disease-resistance QTLs in wild peanuts. Utilizing these genetic resources can potentially lead to the development of more resilient peanut cultivars.

QTL validation and identification of candidate genes in *qFL-Chr.25,* a *G. barbadense*-sourced QTL conditioning for increased fiber lengths in four diverse *G. hirsutum* backgrounds

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*** MS**

Stagnation in fiber quality improvement created by the narrow genetic base of elite cotton germplasm has hindered long term improvement of Upland cotton (*Gossypium hirsutum* L.). A closely related species, *G. barbadense*, has been used with some success to transfer favorable alleles for fiber quality into Upland cotton. An obsolete Upland line with introgressions from *G. barbadense*, Sealand 883, was shown to carry a quantitative trait locus (QTL) for fiber length (*qFL- Chr.25*). This QTL was later transferred into four diverse genetic backgrounds (Acala SJ-4, Deltapine 50, GA 2004089, and Paymaster HS-26) that represented four major cotton-growing regions of the United States Cotton Belt. To more precisely determine the effect of the QTL, it necessitated the development of near-isogenic lines (NILs). A three year, multilocational study was conducted to test the deployment of *qFL-Chr.25* into the four different backgrounds. The fiber analysis results showed a significant positive effect with the introgression of the *qFL-Chr.25* locus on the length of fibers in all four backgrounds. In tandem with the field evaluation study, a transcriptome profiling study via RNA sequencing (RNASeq analysis) was conducted to identify putative candidate genes for the causal fiber length gene. The RNASeq analysis revealed three potential candidate genes (Ghir_D06G000180, Ghir_D06G000680 and Ghir_D06G000880) that showed significant down-regulation during early fiber elongation stages in lines carrying the *G. barbadense* alleles. The three candidate genes in the *qFL-Chr.25* region provide targets for functional validation using reverse genetics approaches.

Improving *Arachis hypogaea* for Tomato Spotted Wilt Virus resistance through QTL based selection and field evaluation

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***MS**

Tomato spotted wilt virus (TSWV) can be highly detrimental to susceptible *Arachis hypogaea* (cultivated peanut) cultivars in the field. Breeding for resistant varieties is a favorable way to reduce yield loss from this virus. PI203396 has been a source of resistance for many years. More recently, PI576638 (SSD6) was identified as another source of TSWV resistance. NC94022, a highly resistant offspring of SSD6, was used to produce a mapping population for identifying a quantitative trait locus (QTL) for TSWV resistance on chromosome A01. Two regions of interest within this QTL have been identified for further analysis. This study aimed to evaluate the influence of the A01 QTL regions on field resistance of eight F_2 populations derived from a recombinant inbred line containing the QTL as the male and eight unique female parents. F_2 seed was genotyped for presence of the QTL regions and selected individuals were observed for TSWV incidence in 2022. Individuals showing TSWV resistance and desirable agronomic traits were harvested as F_3 seed to be either advanced to the F_4 generation in the winter nursery or maintained as F_3 for further evaluation in 2023. A subset of advanced lines was further genotyped for other traits of interest potentially obtained from the maternal genotypes, including leaf spot and nematode resistances and high oleic acid. Current results show that individuals containing both regions of the QTL present significantly lower TSWV scores than those with only one. And individuals in these populations are likely to contain at least one other trait of interest for breeding. Ongoing evaluations will further confirm the QTL's influence on TSWV resistance and select germplasm useful for producing improved peanut cultivars.

Linkage Drag Associated with Introgression of *Meloidogyne incognita* Resistant Genes from Wild Relatives into Upland Cotton

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***MS**

Meloidogyne incognita (southern root-knot nematode - RKN), is the most economically detrimental endoparasitic pathogen to cotton. Cotton germplasm resistant to RKN has been available since the 1970s and genetic markers linked to major resistant QTLs have been developed, yet a majority of the modern cotton cultivars are susceptible to RKN. The objective of the present study was to determine the potential yield and fiber quality linkage drag associated with the incorporation of the resistance QTLs. We developed eight resistant experimental lines possessing either both resistance QTLs *qMi-C11* and *qMi-C14* or only a single QTL. Field evaluation conducted in multiple Georgia environments showed that a number of the resistance lines had higher lint yield than both the donor parent and commercial resistant check cultivars. In addition, the experimental lines performed exceptionally well for fiber quality traits such as length and strength. Hence, the data suggests that while linkage drag due to incorporation of the resistance QTLs may affect yield and quality components, such negative associations could be overcome by breeding with elite germplasm and rigorous selection to produce high yielding lines with excellent fiber quality.

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Analysis of a Hessian fly resistance gene in soft red winter wheat in Georgia

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*** PhD**

Wheat is among the largest global commodity crops concerning production. 211.43 million metric tons (MT) are expected to be exported worldwide during the 2022-2023 growing season. Soft red winter wheat, used for sponge cakes and other confectionary foods, is the wheat class with the highest production in the southeastern region of the United States. However, pathogens and insect pests like Hessian fly (HF) can severely lower wheat yields. Over the years, HF has cost southeastern wheat growers millions of dollars in lost yield. Compared to controls such as insecticides, genetic resistance is the most ecofriendly and economical solution. A population of 225 F6 recombinant inbred lines was developed from crossbreeding resistant UGA 111729 and susceptible AGS 2038. From the 2019- 2022 growing seasons, this population was tested for adult resistance in the field (F) at the Southwestern Research and Education Center in Plains, GA and Bledsoe Research Farms in Williamson, GA. Seedling studies were performed in growth chambers (GC). To find quantitative trait loci (QTL), the following traits were analyzed with inclusive composite interval mapping: percent resistant plants, percent infested tillers, and numbers of pupae or larvae per tiller and infested tiller for each entry. Averaging across all traits, a major QTL on chromosome 3D explained 10.43% (F) and 42.27% (GC) phenotypic variance (PV) within a 9.86 cM region. Marker IWB65911 was associated with the QTL peak that explained up to 67.45% PV. HF resistance gene *H32* co-localized with SNP IWB65911 and it was confirmed to express HF resistance in UGA 111729. KASP marker validation demonstrated that UGA 111729 and KS89WGRC06, the ancestor of UGA 111729, express *H32*. KS89WGRC06 has been reported to carry *H24*, which is approximately 20 centimorgans away from *H32*. Future projects should include the introgression of *H32* into new varieties.

Pyramiding of resistance loci to root-knot nematode and foliar diseases from wild relatives in cultivated peanut

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***PhD**

Peanut is a major crop in Georgia, with 680,000 acres planted and a production value of more than $710 million in the state. Globally, peanut production generated an estimated $38 billion dollars in 2021; moreover, peanut is a critical source of calories and protein in many regions of the world. However, peanut yield is threatened by several pests and pathogens to which no sources of strong genetic resistance exist in the primary gene pool of cultivated peanut. The objective of this project is to combine sources of genetic resistance previously identified in wild peanut relatives to two major pests/pathogens, southern root-knot nematode (*Meloidogyne incognita*, RKN) and the early/late leaf spot (ELS/LLS) disease complex (caused by *Passalora arachidicola* and *Nothopassalora personata*, respectively), into a common elite genetic background. Parental lines were chosen from two previously developed populations. Homozygous BC_3 lines from an [*Arachis batizocoi* x *Arachis stenosperma*]^{4x} x *Arachis hypogaea* backcross population were chosen based on the presence of the RKN resistance locus as determined by KASP genotyping. Homozygous BC_1 lines from a TifNV-High O/L x [Bailey x IAC321] population were similarly chosen based on the presence of three leaf spot resistance loci derived from *Arachis cardenasii* as determined by KASP genotyping. From April to June 2022, RKN-resistant lines were manually emasculated and pollinated with pollen from leaf spot-resistant lines. From 329 total crosses performed, 19 F_1 progeny were confirmed by KASP genotyping to have inherited the RKN resistance locus at least one leaf spot resistance locus, with 15 progeny having inherited all three. Currently, these F_1 progeny are being genotyped by Thermo Fisher 'Axiom_Arachis' 58K SNP array to confirm the presence and size of the desired introgressions, are being grown in greenhouse conditions to self-pollinate. F_2 progeny homozygous for target introgressions will be evaluated nematode/disease resistance and field performance.

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

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***PhD**

Soybean varieties are categorized into relative maturity groups (MGs), which are estimates of photoperiod sensitivity and time to maturity. A variety's MG also equates to the approximate geographic zone that it is best adapted. Maturity is the most important trait that growers consider when deciding which cultivars to plant and is used in breeding programs as a covariate to draw meaningful comparisons among genotypes for traits of interest. Accurate phenotyping of maturity is an important task during line development but is a labor-intensive process. High-throughput phenotyping (HTP) of soybean maturity using unmanned aerial systems (UASs) provides an opportunity to reduce the human resources, time commitment, and error associated with manual maturity notes to support breeding efforts. An HTP pipeline for maturity will provide higher quality maturity data that will greatly improve breeders' ability to evaluate the performance of breeding lines and more efficiently develop elite cultivars for growers. Several studies have utilized spectral measurements, mainly with UASs, to estimate soybean maturity of lines from Midwestern MGs (0–IV). However, these methodologies suffer from lack of public availability, scalability, and/or adoptability. The objective of this study is to develop an intuitive, accessible, and accurate HTP pipeline for determining the maturity of soybeans in later MGs VI– VIII, predominantly grown in the southeast region of US. A residual network (ResNet) is a type of deep learning neural network that has become very popular for image classification and regression tasks. In this study, a pretrained ResNet50 model was trained on plot imagery of two breeding stages across two years to estimate the time difference between the image date and plot maturity. Various train-test partitioning strategies indicated that ResNets exhibit promising and robust validation accuracies (83-90%) for estimating the correct plot maturity date and that this algorithm can be further developed for breeding applications.

Keywords: High-throughput phenotyping (HTP), maturity group (MG), unmanned aerial systems (UASs), soybean

Breeding Better Zoysiagrass: Harnessing Somaclonal Variation to Enhance Seed and Pollen Sterility through Tissue Culture

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*** PhD**

Zoysiagrasses (*Zoysia* spp. Willd.) are allotetraploid (2*n*=4*x*=40) perennial warm season grasses that require little maintenance and tolerate drought, low soil fertility, and saline environments. However, the presence of fertile inflorescence in zoysiagrass can lead to unwanted seedling establishment and cross pollination with other grasses. To address this issue, we explored the use of tissue culture to induce somaclonal variation in an effort to induce sterility in zoysiagrass. We tested different explants, including seeds, nodes and immature inflorescence to establish callus cultures. For surface sterilization all the explant materials were treated with 70% ethanol for 2 min followed by treatment with varying concentration of sodium hypochlorite (2-4%) and 0.1% Tween 20 solution and rinsed 5 times with distilled water. Different explants were cultured on basic Murashige and Skoog medium, 3% sucrose, 0.5% gelrite supplemented with different concentrations of growth hormones- for seeds and immature inflorescence (1 mg/L to 5 mg/L 2,4-D, 0.01 mg/L to 0.1 mg/L 6-BA, 4 mg/L Thiamine-HCl, 100 mg/L α-Ketoglutaric acid), for nodes (1 mg/L to 2 mg/L 2,4-D, 0.01 mg/L to 0.1 mg/L 6-BA, with or without 0.0082 mg/L ABA, 1-2 mL/L Plant Preservative Mixture), pH-5.8. All the explants were cultured in the dark at 26±°C for 1 month (nodes), 2 months (immature inflorescence), 3 months (seeds) for callus induction. The frequency of callus formation varied among different explants with highest in case of seeds (44%) followed by nodes (7%) and then by immature inflorescence (2%). The calli will further be classified into three types: type I as embryogenic with bright yellow color and firm texture; type II callus as friable without secretion; type III callus as white, highly friable, and non-embryogenic. Type I and II embryogenic calluses will be transferred to different regeneration media for shoot and root induction after which their phenotype will be observed for pollen and seed sterility in multiple environments over time.

Characterization of A Novel Fruit Flavor Aroma Locus in Tomato

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***PhD**

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. In the future, we aim to perform gene validation by gene editing and incorporate the beneficial allele into modern tomato varieties. 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.

Loss of function of *GmSNAP02* on chromosome 2 confers resistance to soybean cyst nematode

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***PhD**

Fine mapping of a major novel quantitative trait locus on chromosome 2 in PI 90763 identified *GmSNAP02*, an α soluble NSF attachment protein gene paralogous with *GmSNAP18* (*rhg1*) and *GmSNAP11* (*rhg2*), as the best candidate for resistance to soybean cyst nematode (SCN) HG type 1.2.5.7 (Race 2). An insertion in *GmSNAP02* in PI 90763 was confirmed, but how the function of *GmSNAP02* contributes to resistance is unclear. Here we investigated the functional consequence of this insertion on the transcription of *GmSNAP02*. For this, PI 90763 (resistant) and PI 548402 (Peking, susceptible) differing for the insertion at *GmSNAP02* were inoculated with SCN HG type 1.2.5.7. Root tissues of mock-inoculated and infected roots were harvested at three days post-inoculation for RNA isolation followed by qRT-PCR analysis using *GmSNAP02*-specific primers. *GmSNAP02* expression was significantly upregulated in Peking, but not PI 90763, in response to SCN infection. In addition, we were unable to amplify *GmSNAP02* full-length transcripts from PI 90763. CRISPR-Cas9 was used to delete portions of *GmSNAP02* in Peking and PI 90763. Peking-edited composite plants exhibited reduced susceptibility to SCN HG type 1.2.5.7 whereas resistance in PI 90763-edited composite plants remained unchanged. Our results demonstrate that the insertion in *GmSNAP02* in PI 90763 leads to a loss of function of *GmSNAP02* conferring resistance to SCN HG 1.2.5.7.

Resistance to Meloidogyne incognita-1 from cultivar Forrest is located on *Glycine max* Chromosome 10

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***PhD**

The southern root-knot nematode (SRKN), *Meloidogyne incognita*, poses a major threat to soybean production in the Southern United States, causing an estimated $227 million yield loss in 2022. The first soybean resistance gene identified, *Resistance to M. incognita-1* (*Rmi1*) was previously identified as a single additive gene for SRKN resistance using the resistant cultivar Forrest and the susceptible cultivar Bossier, but never mapped to a specific genomic region. Four hundred seventy-four F_3 progeny derived from a cross between Bossier and Forrest were evaluated for SRKN resistance using gall scoring and genotyped using KASP markers previously identified for SRKN resistance. SRKN resistance is significantly associated with the marker on chromosome 10 ($P<0.001$;$R2 = 0.5$). Eighteen $F_{2:3}$ families segregating for this marker were phenotyped for resistance and genotyped, confirming the association with the marker on chromosome 10 ($P<0.001$; $R^2 = 0.6$). These results indicate that *Rmi1* is located on chromosome 10, in the region most frequently identified as a major QTL for SRKN resistance. Despite the prevalence of the QTL, the causal gene(s) for SRKN resistance are yet to be identified. To finer map the causal gene(s) in *Rmi-1*, 883 Bossier x Forrest F_5 lines were genotyped to identify possible recombination events between five candidate genes. Identified lines will be evaluated for SRKN resistance. These findings, coupled with CRISPR gene editing, will be used to identify the causal gene(s) for SRKN resistance.

Identification of branched-chain amino acid derived volatile loci in tomato (*Solanum lycopersicum*)

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***PhD**

Volatiles are a class of chemical compounds that readily diffuse in air at near ambient temperatures. Along with sugars and acids, a variety of these compounds affect the way humans perceive flavor through their olfactory receptors. The focus of this study was to investigate the genetic aspect of the branched-chain amino acid (BCAA) volatiles, derived from L-valine, L-isoleucine and L-leucine. It is generally considered that these BCAA-derived volatiles contribute positively to overall liking, because these are essential amino acids required by the human diet. We investigated four F2 bi-parental populations generated in the lab from a unique and diverse collection of ancestral tomato accessions. 11 volatiles from the BCAA pathway were analyzed in red-ripe fruits using Gas-Chromatography Mass- Spectrometry (GC-MS). The four F2 populations were genotyped by sequencing (GBS). The raw reads obtained were aligned to SL4.0 reference genome and variants (SNPs and INDELs) were called. Genetic maps were constructed with roughly 130 markers for each population. Composite interval mapping identified 39 quantitative trait loci (QTLs) associated with BCAA volatiles among four populations.

Several of the QTLs overlapped with known genes like FLORAL4 and various branched-chain aminotransferases (SlBCAT-3, -1, -7, -2). This was compared to the 113 previously identified BCAA QTLs from GWAS results in the lab. Seven loci in the F2 population overlapped with the GWAS study. We identified candidate genes underlying the loci, based on annotated gene function. We aim to validate and fine map these QTLs, and meanwhile, knock-out the candidate genes using CRISPR-cas9 to confirm their function in the biosynthesis of BCAA-derived volatiles in tomato fruits.

Genomic Selection in Switchgrass - Multi-location Phenotypic Trait Analyses

Jazib Ali Irfan*, Shiva Makaju and Ali Missaoui

***PhD**

Switchgrass is a warm-season perennial grass that has potential of providing feedstock for biofuel production. It has been a matter of great concern that with the increasing world population and the excessive use of fossil fuels, fuel reserves will be exhausted in near future and there is a dire need of replacing fossil fuels with renewable sources of energy like edible and forage crop biomass. The hurdles that bioenergy production from switchgrass faces is yet to achieve a greater yield of plant biomass, increasing fertilizer use efficiency, improvement of harvesting and transportation along with efficient conversion technologies. Forage Breeding lab at UGA has been working in collaboration with the Centre for Bioenergy Innovation (CBI) that is one of four Bioenergy Research Centers (BRCs) within the Department of Energy's (DOE's) Office of Science Biological and Environmental Research (BER) program created to lay the scientific groundwork for a new robust, biobased economy. Main goal of forage breeding lab is to improve the biomass yield and related agronomic traits in half-sib families of switchgrass produced from high yielding parental population with two distinct features of early and late flowering. Progenies of late and early flowering parents will be analysed and subjected to genomic prediction to identify high yielding cultivars. The project is focused on improving biomass yield to produce sustainable aviation fuels, more environment friendly that will replace fossil fuels with the renewable biofuels. Present study shows the results from the half-sib family performance grown at two different locations of Georgia (Athens and Tifton). The phenotypic traits under study (tiller height, tiller diameter, biomass yield, crown perimeter, spring emergence and flowering time) have been analysed using statistical tools and the best performing half-sib families/progenies have been identified based on phenotypic data compiled from the year 2022 harvest.

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

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***PhD**

Solanum microdontum is a wild Andean relative of potato that has shaped the domestication and adaptation of modern cultivated potato to diverse environments. *S. microdontum* is a diploid species that crosses readily with cultivated potato and has the potential to provide a wealth of untapped genetic material for use in modern potato breeding. Advances in potato breeding are becoming especially critical as climate change brings about new challenges including elevated temperature, increased frequency of extreme weather events, and evolving disease pressure. This project addresses these needs by providing breeders with genetic, molecular, and germplasm resources to be used within the context of newly developed diploid potato breeding programs. This project has two objectives, the first of which is to identify accessions of wild *S. microdontum* with characteristics that make them favorable for crossing with cultivated potato (*S. tuberosum*). Traits of interest include resistance to the late blight pathogen (*Phytophthora infestans*) and tolerance to heat stress. 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 of *S. microdontum*. This project will contribute to much-needed publicly available potato genome resources and permit robust data mining of *S. microdontum* trait loci. Access to these phenotyped and genotyped accessions will allow breeders to cross key *S. microdontum* lines with cultivated breeding lines to create more resilient varieties. These efforts are underway and will ultimately become a rich resource that can be used to help address major challenges in potato production.

Genetic Engineering of Antioxidant Genes for Aflatoxin Resistance

Prasanna Kharel*, Baozhu Guo, Jake Fountain & Peggy Ozias-Akins

***PhD**

It has been shown that reactive oxygen species (ROS) produced due to drought stress are linked with aflatoxin production in peanuts. Considering this, developing plants with high antioxidant enzymes may reduce aflatoxin contamination. As genetic gain through traditional breeding methods remains slow and partial due to high G*E interactions, genetic engineering may provide this avenue.

The objective of this research was to develop genetically engineered plants with different antioxidant capacities and link the antioxidant capacity of the host plant to its resistance against aflatoxin contamination. Gene encoding antioxidant enzyme catalase (CAT), ascorbate peroxidase (APX), or superoxide dismutase (SOD) were overexpressed using overexpression cassette containing kanamycin and hygromycin selection markers, eGFP, actin2 promoter, complete gene encoding enzyme, and transcription terminator. In addition, the gene encoding the catalase enzyme was mutated using CRISPR/Cas9 to disrupt its function. The constructs were delivered using biolistic method. Successful transformants (T0) were identified on a Hygromycin-selective medium and planted in the greenhouse to produce T1 seeds. GFP-positive T1 seeds were selfed to produce T2 seeds. A high level of antioxidant enzyme activity and reduced aflatoxin contamination is expected for overexpression lines while the reverse is expected for CRISPR/Cas9 edited lines. So far, only catalase concentration has been measured in the T2 seeds of the catalase overexpression lines. The results showed that the catalase content in the overexpression lines was not greater than the untransformed line, most likely due to the dryness of the seeds. So, T2 seeds will be planted, and leaf tissues will be used for the enzyme measurements. T3 seeds from homozygous T2 lines will further be used to characterize their response to *Aspergillus flavus* infection. This experiment will provide evidence that enhancing antioxidant capacity is a valid approach to improving resistance.

Three-dimensional, high-throughput phenotyping of peach tree architecture utilizing terrestrial laser scanning.

Jordan Knapp-Wilson*, Alexander Bucksch, Dario Chavez

***PhD**

Temperate fruit trees, such as peaches [Prunus persica (L.) Batsch], have been developed throughout history to improve fruit quality, yield, and resistances to disease and frost. The area of tree growth and morphology however, also known as tree architecture, has long relied on training systems to control the innate vigor and branching patterns of fruit trees. Training systems direct crown and branch growth to specific configurations, resulting in better light penetration and higher yield. Characterization and quantification of tree architecture requires significant amounts of branching data, which is difficult to collect. Traditionally, branching data have been collected manually, but this process is tedious, timeconsuming, and prone to human error. These barriers can be circumnavigated by utilizing terrestrial laser scanning (TLS) and three-dimensional modeling to obtain a digital replicate of the real tree. To facilitate the use of these tools for peach, we selected sixteen young peach trees scanned in 2021 and 2022, which are all of the same variety (‘Julyprince’) but differing rootstocks (‘Guardian’ and ‘MP29’).
These sixteen trees were then modeled and quantified using the open-source software TreeQSM. As a result, the computational (‘in silico’) branching and biometric data for the young peach trees were calculated and compared to field (‘in situ’) measurements. Mean average deviation when comparing all young tree height was approx. = 4.74%, with crown volume was approx. = 8.31% across both 2021 and 2022. Mean average deviation was also calculated for branching data collected in 2021, with the average across the available trees = 21.67%. This scoring was found to be smaller at lower branching orders, being 15.08% and 18.22% at the primary and secondary branching orders, respectively. The phenotyping and characterization of tree architecture could aid the selection of trees that require less pruning or that naturally excel in specific growing/training system conditions.

Reinventing Peanut: Origin, Evolution, and Domestication of [*Arachis ipaënsis* x *Arachis duranensis*]^{4x} neopolyploids

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***PhD**

Allotetraploid peanut (*Arachis hypogaea* L.; 2n = 4x = 40; AABB), originated less than 10,000 years ago from the spontaneous hybridization of two diploid wild *Arachis* species, *A. duranensis* (2n = 2x = 20, AA) and *A. ipaënsis* (2n = 2x = 20, BB), and a consequent whole genome duplication event. Given the narrow origin base, the polyploidization process generated reduced genetic diversity in peanut, which also found itself isolated from the other diploid *Arachis* species due to ploidy inequalities. Conversely, the merging of different genomes during peanut origin caused a genetic shock, which produced different types of genetic modifications and rearrangements in the peanut genome and gene expression alterations. These genetic instability phenomena increased the overall peanut phenotypic variability and conferred to peanut the phenotypic plasticity and adaptability characteristics typical of polyploid plants. Therefore, regardless of the adverse initial conditions, *A. hypogaea* ended up being domesticated by humans and is currently the only *Arachis* species cultivated worldwide. In this study, we are recreating peanut's evolution and domestication processes in controlled greenhouse conditions using colchicine induced [*Arachis ipaënsis* x *Arachis duranensis*]^{4x} (IpaDur) neopolyploids. IpaDurs have been advanced for about ten generations and showed increased variability for several agronomic traits, such as stem length, flower color, flowering time, seed weight, seed number, growth habit, leaf shape, and leaf color. Selection for contrasting seed weight phenotypes has been carried out for four generations with IpaDur plants and wild diploid ancestors to investigate early peanut genetic and phenotypic plasticity and recreating the human domestication process. As hypothesized, we identified increased allelic variability in IpaDur plants. Additionally, IpaDurs'overall response to selection for contrasting phenotypes through generations was greater than the diploid progenitors confirming the presence of an evolutionary advantage caused by polyploidization in peanut crop.

Characterization and study of different isolates of tomato spotted wilt virus (TSWV) of peanut found in Georgia, USA

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*** PhD**

Tomato spotted wilt virus (TSWV; Family *Bunyaviridae*; Genus *Tospovirus*) is a disease agent that significantly reduces peanut yield. It is single-stranded RNA virus with tripartite genome (L, M and S segment). It is primarily transmitted by viruliferous thrips with a wide-host range (>900 plant species). This virus quickly develops new strains due to its rapid adaptability and high degree of genetic heterogeneity resulting from genetic reassortment and replication within the vector, thus leading to resistance breakdown in resistant cultivars. However wild species of peanut have been reported to confer varying degree of resistance against TSWV. Understanding the constantly evolving genome structure of this virus in cultivated and wild-derived induced allotetraploids peanut is important for developing breeding strategies against this disease. This experiment aimed to assess the genetic diversity of TSWV by characterizing isolates from different induced allotetraploids and cultivated peanut. In 2022, TSWV infected leaves were collected from eleven cultivated and eight induced allotetraploids peanuts from Midville, GA. An initial assessment was performed in four samples: two cultivars (GA-06G and Bailey II), and two wild-derived induced allotetraploids (GregSten1 and ValsiWilliamsii). Total RNA was extracted, rRNA depleted, and sequenced (Illumina NovoSeq4). Multiple alignment and phylogenetic analysis showed variation among the isolates from the four samples. Furthermore, phylogenetic analysis was performed with complete genome segment of TSWV isolates available in NCBI, which showed clustering of four isolates from our study with other isolates collected from the USA. In addition, peanut mottle virus (PMV) was also detected in all four samples. Future plans include characterizing TSWV isolates collected (from cultivated and wild-derived lines) from different locations in GA with the aim to perform comprehensive assessment of the genetic diversity and population structure of TSWV in Southeastern GA. This finding will be valuable for the development of effective breeding strategies against the disease.

Screening of a Zoysiagrass mapping population (*Z. japonica* × *Z. matrella*) for physiological parameters related to salt tolerance

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***PhD**

Crop cultivation and turfgrass management are presently facing many challenges due to soil salinization. The major contributor to soil salinization is sodium chloride (NaCl). Plants encounter various physiological and biochemical alterations under salinity stress, which greatly reduce yield and productivity. Zoysiagrass is an important turfgrass species that is used widely in the US. 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 tolerant *Z. matrella* acc. PI231146 (narrow leaf) and susceptible *Zoysia japonica* var. Meyer (broad leaf) has been developed. Around 200 F_2 plants were screened under control (no NaCl) and 30 dS/m levels of salinity (added NaCl). The mapping progeny along with their parents have been phenotyped for morpho-physiological parameters such as maximum quantum yield of photosystem II (F_V/F_M), cell membrane stability via electrolyte leakage, and visual rating (0-9 scale). Plants showed a wide range of phenotypic responses from extreme susceptibility to high tolerance. A strong negative correlation (r^2 = -0.65) was observed between cell membrane stability and visual rating. It was interesting to identify progeny with broad leaves along with higher salt tolerance from the mapping population. The association of phenotypic data with genotypic data will identify key Quantitative Trat Loci responsible for salt tolerance. A wide phenotypic separation and consensus among parameters allowed us to identify accessions at the extremes of the distribution for salt tolerance and susceptibility. This will allow us to identify differentially expressed genes and metabolomic patterns between tolerant and susceptible genotypes through Bulk Segregant RNA sequencing and Metabolomics approaches, respectively.

Sources of whitefly-transmitted virus resistance identified in *Citrullus* crop wild relatives

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***PhD**

Watermelon (*Citrullus lanatus*) production in the Southern United States has been vulnerable to whitefly (*Bemisia tabaci* Gennadius) transmitted viruses such as Cucurbit Leaf Crumple Virus (CuLCrV), Cucurbit Yellow Stunting Disorder Virus (CYSDV) and Cucurbit Chlorotic Yellows Virus (CCYV). Although the management of whiteflies is highly insecticide dependent in the field, the management tactic is becoming ineffective due to their ability to evolve quickly. An effective management strategy could be the development of varieties resistant to whitefly-transmitted viruses. To evaluate whitefly-transmitted virus infections we conducted a field trial with 21 *Citrullus* genotypes in the field. Our trial followed a randomized complete block design with three blocks and three replications. We scored virus disease severity in the field and collected tissue samples from each plant for quantification of CuLCrV, CYSDV and CCYV through quantitative Polymerase Chain Reaction (qPCR). We also scored each plant for downy mildew symptoms in the field. For virus disease severity and downy mildew scores, Area Under Disease Pressure Curve(AUDPC) was calculated. Our results show low virus disease severity, CuLCrV concentrations and downy mildew severity for the genotypes PI 494528 and Grif 16444 We also observed low CYSDV and CCYV virus titers in genotypes PI 494528 and PI 595203. These accessions identified in our current study can be potential sources of resistance to whitefly and whitefly-transmitted viruses.

Generation of Auxotrophic *Agrobacterium* Strains using a CRISPR-mediated Base-editor

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***PhD**

Agrobacterium-mediated transformation is an essential tool for plant genetic engineering. Most optimizations surrounding *Agrobacterium*-mediated transformation have traditionally focused on tissue culture and infection parameter modifications. There has been an increased adoption of auxotrophic *Agrobacterium* strains in both the public and private sectors as a means of controlling bacterial growth post-infection. Auxotrophic strains help mitigate the use of antibiotics which can have undesirable effects on plant tissue. Historically, auxotrophs have been produced through random mutagenesis, often with unintended consequences, or through homology directed repair, which can be time consuming. Recent developments in *Agrobacterium* engineering have demonstrated the utility of CRISPR-mediated base- editors for targeted gene knockouts in chromosomes and the Ti(Ri) plasmid. Here, we used the CRISPR base-editing architecture, Target-AID, to induce nonsense mutations in the coding regions of genes relevant for metabolite biosynthesis in laboratory *Agrobacterium* strains. The Target-AID editor was introduced to *Agrobacterium* through a binary plasmid via electroporation. Prospective gRNAs were selected either manually or via an in-house developed Geneious Prime wrapper plugin to target codons amenable to nonsensical mutation with minimal predicted off-target binding. In one example, we targeted the thymidylate synthase, thyA/Atu2047, gene in each *A. rhizogenes* K599-, *A. tumefaciens* C58-, and transconjugant R1000-derived strains for induced thymidine auxotrophy. Incorporation of a chromoprotein reporter in tandem with sucrose counter-selection enabled rapid identification of putative *Agrobacterium* mutants that had evicted the editor. Given the shared chromosomal backgrounds of many laboratory *Agrobacterium* strains, the same gRNA could be reused to produce auxotrophic derivatives in both a time and cost-efficient manner. Further, this strategy could be used to rapidly stack multiple auxotrophies in one strain spanning a variety of metabolites.

An efficient approach for tetraploid peanut genome assembly

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***PhD**

The cultivated peanut (*Arachis hypogea)* is an allotetraploid derived from two ancestral species, *Arachis duranensis* and *Arachis ipaensis*. Genome assembly of tetraploid plant genomes can often be difficult. Chimeras and sub-genomic collapse at positions where homoeologous chromosomes are similar has been observed, particularly with short read sequencing technology. Likewise, older long-read technology can produce more contiguous genome assemblies but the higher error rate also disrupts proper repeat assembly. Recently developed PacBio HiFi reads are long (~15kb) as well as accurate. We assembled and scaffolded peanut HiFi reads to the reference 'Tifrunner' genome as well as a synthetic progenitor genome. Our pipeline involves adapter filtration, genome assembly, scaffolding and dot plots for visual curation. Resulting scaffolds do not show any sub-genomic collapse. The pipeline has been tested with the genomes of three different peanut varieties and shows consistent results. We also report on comparisons across these three genomes as well as ancestral species and discuss structural implications for genetics and breeding.

Identification and validation of novel QTLs for rice blast (*Magnaporthe oryzae*) resistance using genome- wide association study approach

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***PhD**

The rice blast pathogen, *Magnaporthe oryzae*, is a major biotic constraint to the global production of rice, a staple food and economically important crop worldwide. It is known to affect more than 50 grass species, and among other agriculturally important crop species, it infects wheat, ryegrass, barley, pearl millet, and finger millet. Many quantitative trait loci (QTLs) have been identified in rice for blast resistance. However, the molecular interplay between the host and pathogen and the genetic architecture of the blast-resistant genes needs more attention. This study conducted a genome-wide association study (GWAS) using 134 parental inbred lines developed by the International Rice Research Institute (IRRI), Philippines. These lines were genotyped with a 7K Infinium SNP Panel and were characterized phenotypically with a rice blast isolate, M64-1-3-9-1, which can overcome the *Pia, Pik-s, Pit, Pi3, Pii, Pik, Pik-h, Pik-m, Pik-s,* and *Pi20(t)* resistance genes in rice. Two susceptible checks, LTH and CO-39, and 30 IRRI-bred blast resistance monogenic lines (IRBL) with identified target genes for rice blast disease were also screened for comparative analysis. The blast symptoms appeared as spindle-shaped lesions with a grey/ tan- colored center and dark brown margins and were evaluated six days post-inoculation under controlled conditions. The disease was examined and rated on a scale of 0 (resistant) to 5 (susceptible), and quantitative traits, like severity (percentage of infected leaf area) and number, length, and width of the lesions, were also measured. A GWAS was initiated using general and mixed linear models, and twelve potential candidate SNPs ($p < 0.01$) associated with rice blast resistance were identified on chromosomes 3, 7, 8, and 9. With the novel QTLs identified, we aim to augment the existing information on rice blast resistance genes and contribute further to developing blast-resistant rice varieties.

Validation of introgressed QTLs alleles effects in fiber fineness in Upland Cotton (*G. hirsutum*)

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*** PhD**

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 germplasm, resulting in a high degree of relatedness among cultivated germplasms. Other *Gossypium* species are known to have longer, stronger, and finer fiber than elite Upland. 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. As a foundation for this study, SL883 has been crossed with four important cultivars: Acala-SJ4, Paymaster HS26, Deltapine 50 and GA2004089, transferring the QTL regions to the progeny. In this project, the objectives are to validate and develop new germplasm with improved fineness to enhance the diversity of Upland Cotton gene pool. The genetic populations were planted in 2020, 2021 and 2022 at the Gibbs Farm, Tifton, Georgia. The results from 2020 show that the fiber collected is not as fine as the SL883 parent, but there is improvement of the progeny lines when comparing fineness to the four elite backgrounds. Our current effort is to characterize regions from *G. barbadense* that confer the finer fiber trait using SNP markers to, ultimately, map and identify the genes that are located in those regions.

Variation in Ethylene Insensitive 4 (EIN4) orthologs at the Rpp6 locus is associated with resistance to soybean rust

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***PhD**

Soybean rust, caused by the fungus *Phakopsora pachyrhizi*, is one of the pathogens that can significantly decrease productivity. Development of soybean cultivars using disease resistance genes is the most effective way to control this disease. The *Rpp6* locus was initially discovered on chromosome 18 in PI 567102B that provides superior resistance to soybean rust in the southern region of USA and a resistance allele at this locus was also identified in PI 567068A. However, it remains unknown whether the alleles from PI 567102B and PI 567068A are the same. Functional markers are needed to perform effective marker-assisted selection for rust resistance in breeding programs. This research aimed to fine map the *Rpp6* locus, identify the gene controlling the resistance and develop robust SNP marker assays for marker-assisted selection. Two populations were developed using PI 567102B and PI 567068A, respectively. The populations were phenotyped for rust resistance in the greenhouse using the GA-12 isolate. After the genome resequencing of two PIs and two susceptible parents G00-3213 and Prichard, SNPs were identified from the target region and SNP marker assays were developed to genotype the populations to identify recombinants. The recombination analysis narrowed down the Rpp6 locus to an interval of 0.43 Mb on chromosome 18. In this interval, four *Ethylene Insensitive 4* (EIN4) genes were identified, two of which were polymorphic between resistant and susceptible parents. An RNAseq and RT-qPCR validation experiments were conducted in order to identify genes with differential expressions. RT-qPCR analysis indicated that the two genes, *Glyma.18g069300* and *Glyma.18g069802*, expressed in different times in susceptible and resistant genotypes after the inoculation and are the best candidates for the *Rpp6*. The candidate genes identified will allow researchers to understand resistance mechanism and the KASP markers developed will become important tools for introgressing the resistance into elite soybean lines.

Keywords: *Phakopsora pachyrhizi, RNAseq, RT-qPCR, fine-mapping, KASP markers.*

Genetic Analysis of Major Agronomic Traits in Soft Red Winter Wheat using Genome-Wide Association Study

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***PhD**

Wheat is a crucial crop for global food security, and its production must increase by at least 50% by 2050 to meet the growing demand. Grain yield is a complex quantitative trait, heavily influenced by environmental factors, making it challenging to improve through conventional breeding. However, breeding for correlated secondary traits, such as plant height and kernel characters, has shown success in indirect selection for improved yield. The objective of this research is to identify novel genes that govern agronomic traits in SRWW and to develop markers for marker-assisted breeding. We evaluated 236 genotypes of SRWW for eight major agronomic traits across two locations over two years and genotyped them using GBS libraries, resulting in 27,466 SNPs after marker filtration. GWAS was performed using the FarmCPU model in R, reporting markers above -$\log_{10}$ p-values of 5.74 based on Bonferroni correction. Population structure and linkage disequilibrium were also estimated.

Seven MTAs were identified for yield and 29 more for rest of the seven agronomic traits. Eight QTLs showed major effects and explained > 10% PV for respective traits. A stable QTL for yield, *QYld-2A*, explained approximately 12-20% PV in two tested environments and is potentially associated with the gene producing disease resistance protein RGA3. Additionally, another major QTL for yield, *QYld-5A*, was also identified that overlapped the gene producing MYB transcription factor during heat and drought stress. Two major QTLs were identified for heading date, *QHd-5B* and *QHd-7D*, explaining 14 and 10% PV, respectively. Further research is needed to identify the novelty of these identified QTLs. The QTLs/genes identified in this study can be considered as targets for improving the SRWW cultivars for southeast.

Bulked Segregant Analysis of F3 segregating populations to identify major genomic regions containing potential QTLs for resistance to Cotton Leafroll Dwarf Virus

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***PhD**

Cotton Leadroll Dwarf Virus, caused by a single-stranded non-enveloped RNA virus from the genus *Polerovirus* and family *Solemoviridae* is transmitted through aphids (*Aphis gossypii* Glover) and is highly prevalent worldwide, including the USA. Virus infection can be asymptomatic but susceptible genotypes can produce symptoms such as stunted growth, reddening of leaves, petioles and stems, v-shaped curling, drooping, and disappearing of wilting symptoms in the non-peak heating hours. The objectives of this research are to detect QTL and candidate gene for the resistant gene(s). A total of 60 F3 populations developed from different parental combinations were planted in 2022, of which 31 showed symptomatic plants. Based on the pedigrees of the segregating populations, the parental lines contributed towards the resistant or susceptible alleles were inferred by disease ratings. Based on the disease rating phenotypic data, 6 populations were selected for downstream QTL analysis. Our approach to map resistance alleles utilized a modified bulk segregant analysis (BSA) based on the allele frequency of sequencing data (Takagi, Abe et al. 2013). The F3s with diseased phenotypes were selected to form the Resistant (R) and Susceptible
(S) bulks and each bulk was genotyped using Illumina Short-Read Sequencing. By comparing the genotypes of the bulks to the parents, we are expecting the BSA to identify genomic regions containing potential QTLs. The expected outcomes of this study will complement other QTL mapping approaches to identify QTLs associated with resistance against CLRDV.

Takagi, H., et al. (2013). "QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations." The Plant Journal 74(1): 174-183.

Genome-Wide Association Study of Seed Oil Content and Weight in a Subset of USDA Cultivated Peanut Collection

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***PhD**

Demand for peanut oil is expected to increase in many countries in the future. In order to characterize USDA genetic resources for oil yield potential, individual inventories from the peanut collection housed at the Plant Genetic Resources Conservation Unit in Griffin, GA were phenotyped for oil content and seed size. A large amount of variation was observed in these traits. In addition to phenotypic data, genotypic data were also collected on ~2,400 accessions using the Axiom_Arachis2 SNP array. In these accessions, the mean seed weight was 47.08 g and ranged from 21.84 – 117.29 g, while the mean oil was 48.96% and ranged from 39.5 – 58.48%, both of which are similar in range to the entire collection. The observed variation and large size of the dataset suggested that meaningful information could be obtained on the genetic basis of the measured traits. We conducted a genome-wide association study (GWAS) to do a preliminary investigation into the genetics of seed oil content and weight. We found multiple markers that were significantly associated with each trait, and we show examples of differences in the distribution of the traits based on genotype at these loci. Using these results, markers can potentially be developed to assist in the selection of seed oil content and weight in breeding programs to improve peanut oil yield.

Targeted Mutagenesis to Develop Seedless Muscadine

Katie Toomey, Patrick Conner, & Wayne Parrott

***PhD**

Muscadines, *Vitis rotundifolia*, are native to the southeastern US and have been cultivated for hundreds of years. Georgia is one of the main producers of this uniquely flavored fruit, which has been commercialized for its jams, jellies, juice, and wine. With its various health benefits, there's room for market expansion as recent consumers' lifestyles change to reflect healthier lives. While breeders have created varieties with more desirable fleshs and skin textures, the large seeds in muscadine are considered undesirable. Stenospermocarpy, a type of seedlessness, has been well-characterized in grape and has been attributed to a single guanine-to-thymine point mutation in *VVag11*. To maintain all the high-quality traits of the muscadine, prime editing utilizing a modified CRISPR/Cas9 could be used to replicate the single point transversion mutation in muscadine. While the goal of the project is to use prime editing to create stenospermocarpy-derived seedless muscadines, the groundwork for tissue culture and editing must be established initially. Creating protocols for micropropagation, somatic embryogenesis, transformation and acclimatization must be in place before editing can be implemented. Our group has thus far developed protocols for quicker regeneration of tissue for eembryogensis. Further expansion of the propagation, embryogenesis, and transformation pipeline is underway. This research will be advantageous to farmers and will further advance CRISPR prime editing in plants, which is still incipient technology. With the growing utility and expansion of prime editing, the successful implementation of stenospermocarpy seedlessness in muscadines using prime editing could serve as a model for other plant species. Additionally, this research could lead to the development of new varieties of muscadines with improved consumer appeal and higher commercial value, thus contributing to the economic growth of the southeastern US region.

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

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***PhD**

Soybean southern stem canker (SSC) caused by a fungal pathogen, *Diaporthe aspalathi,* is an economically important disease in the southern region of the United States. Five loci conferring resistance to SSC, namely *Rdm1* through *5,* have been named based on segregation analysis and reactions with different isolates. The *Rdm3* locus carried by the SSC-resistant cultivar Crockett provides good comparable resistance to SSC when compared to the cultivars possessing multiple *Rdm* loci. However, the genomic location of this locus is unknown, and sources of resistance to SSC used in the breeding program are undetermined. This study aims to map the *Rdm3* locus from Crockett and determine the key sources of resistance to SSC in the Georgia Soybean Breeding Program. Using a recombinant inbred line (RIL) population derived from a cross of G81-2057 (susceptible) ◌ Crockett (resistant), genetic mapping identified the *Rdm3* locus on chromosome 14 that explained 55% of phenotypic variation. The genomic position of the *Rdm3* locus is overlapped with *qRdm14,* a QTL conferring SSC resistance from soybean line PI 398469, a plant introduction from South Korea. To determine the key sources of resistance to SSC in elite germplasm, a panel consisting of 485 experimental lines from the Georgia Soybean Breeding Program was selected to perform a genome-wide association analysis. The resistant allele at the *Rdm3* locus provides a major source of resistance to SSC in this elite germplasm pool. GSM_975, a marker tightly linked with the *Rdm3* locus, could accurately distinguish soybean lines based on their SSC resistance provided by the *Rdm3* locus. The results revealed the prevalence of the *Rdm3* locus resistance allele in the elite soybean germplasm. The QTL and flanking marker information will provide useful information and tools to assist breeders in developing SSC-resistant cultivars.

Keywords: Southern stem canker, *Rdm3* locus, Genetic mapping, Genome-wide association analysis, Marker-assisted selection.

Variation in gene expression associated with peanut reproductive phenology

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*** Staff/Postdoc**

The cultivated peanut (*Arachis hypogaea* L.) ssp. *hypogaea* never produces any flowers on the main stem whereas ssp. *fastigiata* produces flowers on the main stem. Despite the economic importance of peanuts, there are few studies directed toward investigating the molecular regulation of flowering in peanuts. The present study aims to find key genes to control peanut flowering regulation and how they coordinate the flowering pattern between ssp. *hypogaea* and ssp. *fastidiata*. We used a transcriptional approach from 132 RNAseq libraries to compare samples of Tifrunner (ssp. *hypogaea*) and GT-C20 (ssp. *fastigiata*) at six time points and four tissue type. Our results showed the gibberellin (GA) pathway genes *GA20ox1*, *GID1* and *DELLA-GAI1* were differently expressed between Tifrunner and GT-C20. *EIN3* an ethylene (ET) response showed low expression at just preceding the flowering (T2), and increase during flowering (T3 and T4) for GT-C20. *FT*, *TFL1* and *AGL42* showed difference of expression when compared main stem and lateral stem samples between Tifrunner and GT-C20. DEGs between the beginning of vegetative fazes (T1), precede of flowering (T2), and initiation of flowering (T3) showed more up regulated genes (*JAZ*, *MYC4*, *AOS3*, *AOC4*) associated to the jasmonate (JA) for Tifrunner. Associated to ABA, *PYR1* was down-regulated in Tifrunner and *NCED5* up-regulated in GT-C20. *SOC1, AGL24, GIGANTEA*, and, *DELLA* were DE between Tifrunner and GT-C20 at the vegetative stage (T1 and T2), and beginning of flowering (T3). Gene co-expression showed as hub genes, *FRIGIDA* and two *JAZ*s. The tan module of leaf samples showed high number of genes associated to JA pathway, which were more expressed in Tifrunner compared to GT-C20. Our finds show that JA, ET and ABA could be important to peanut flower regulation, and, together with *TFL1* and *AGL42* can be responsible for the absence of flower in the ssp. *hypogaea* main stem.

Engineering a terpene-depleted chassis in tomato fruit for production of high value terpenes

Natalie C. Deans* and C. Robin Buell

*** Staff/Postdoc**

Plants produce numerous specialized metabolites that function in plant defense, as attractants to pollinators and symbionts, and in cell wall strengthening. Over 50,000 plant terpenoids are known which are synthesized from universal C_5 building blocks via the activity of terpene synthases, cytochrome P450s, and glycosyl transferases. A subset of these specialized metabolites are highly valued for a range of properties including insect repellants, fragrances, antimicrobial compounds, low calorie sweeteners, and medicinal properties. It is challenging to economically or sustainably produce many of these compounds from their native species due to the low abundance of the compounds or the time and space required to grow sufficient plants for production of the natural product. In addition, heterologous expression of terpenoid biosynthetic genes in plants is problematic due to the tendency for conversion of the activated products into conjugates or other derivatives via endogenous cytochrome P450s and glycosyl transferases. In this project, we will use gene editing and transformation technologies to overcome these challenges by creating a novel tomato chassis with minimal terpenoid biosynthetic capacity in fruit through removal of native terpenoid biosynthetic pathways, that will enable engineering of high value terpenoid molecules in tomato fruit.

Bladderwort short sequences work as insulators in *Nicotiana* plants.

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*** Staff/Postdoc**

Future approaches in plant synthetic biology will rely on the ability to stack multiple genes of interest into compact multigene cassettes. However, enhancers in promoters alter the expression of other genes in the cassette, leading to unpredictable outcomes in gene expression. There needs to be a way to insulate the components of multi-gene cassettes and prevent their interference with each other. Hence a better knowledge of which *cis*-regulatory elements (CRE) that can be utilized to restrict expression of transgenes where it is not desired is needed. Bladderwort (*Utricularia gibba* L.), with one of the smallest known genomes among flowering plants (~82 Mb), is a potential source of compact CREs that could be used for assembling better transgene expression cassettes. Insulators are DNA sequences that could shield expression of genes of interest from spurious transcriptional signals coming from surrounding elements. In animals, many of such sequences have been identified, but only few have been found in plants, and these are too large for routine use in transformation cassettes. To test the potential of the putative bladderwort insulator sequences we used a series of plasmids where CaMV 35S promoter drives the expression of the mCherry fluorescent reporter protein on one direction and a root-specific gene promoter driving the expression of GFP. Bladderwort candidate sequences, as well as other previously described insulators, were placed between the two reporter genes. The constructs were tested in *Nicotiana benthamiana* leaves by agroinfitration and were evaluated based on their ability to mitigate the ectopic GFP expression caused by the CaMV 35S promoter. As controls, we tested random effects due to fragment size, sequence orientation, or any "*stochastic*" component. We identified 3 short (<1kb) bladderwort sequences that are the shortest and most effective insulators described to date, and that can be used as novel insulators in plant transformation.

Mining the *Utricularia gibba* Genome for Putative Insulator Elements.

Daniel Laspisa*, Sohyun Bang, Robert Schmitz, Wayne Parrott, Jason Wallace

*** Staff/Postdoc**

Gene expression is often controlled via *cis*-regulatory elements (CREs) that modulate the production of transcripts. For multi-gene genetic engineering and synthetic biology, precise control of transcription is crucial, both to insulate the transgenes from unwanted native regulation and to prevent bleedthrough or other incorrect transcription within a single cassette. To prevent this, chromatin insulators are inserted to separate the transgenes so that they are independently regulated. However, only a few validated insulators are available for plants, and they tend to be larger than ideal. To identify putative insulator sequences, we conducted a genome-wide analysis of *Utricularia gibba* (Humped Bladderwort), one of the smallest known plant genomes where its genes are naturally close together. We identified a total of 1,626 putative CREs, including 431 putative insulators. Comparisons of these CREs across 45 other plant species (representing Monocots, Asterids, and Rosids) suggest that insulators are the least conserved element when compared to other putative CREs (promoters and terminators). Genome wide analysis of unmethylated regions (UMRs) indicate 98% of the called putative CREs are unmethylated; however, interpretation of this is complicated because *U. gibba* has remarkably low levels of methylation across the genome, and large UMRs frequently extend over multiple putative CREs and genes. We could not identify any conserved motifs among the putative insulators, though short repeats are common. We also found no sequence conservation between these putative insulators and those reported in other studies. These putative insulators represent a set of new CREs for potential use in genetic engineering, and validating them will be in important next step to deployment.

Pre-breeding efforts in *Aronia* berry: Developing a New Native Crop for the Southeast US

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*** Staff/Postdoc**

Aronia (Rosaceae) is a genus of deciduous tree-like shrubs native to eastern North America. With an ORAC three times more than blueberries, and the increasing consumer interest in anthocyanin-rich foods, *Aronia* is an attractive new native fruit crop. The genus has two main species, *A*. *melanocarpa* (black chokeberry), and *A*. *arbutifolia* (red chokeberry). The cultivar 'Viking' is the primary cultivar grown for fruit production; 'Viking' is a tetraploid intergeneric hybrid of *A*. *melanocarpa*. *A*. *melanocarpa* grows natively in cold regions, and 'Viking' is commercially grown in the northern US. A notable characteristic of 'Viking', and both black and red chokeberry, is that tetraploids are obligate apomicts. *A*. *arbutifolia* has been collected through the eastern US and all plants have been tetraploid. Wild-collected *A*. *melanocarpa* have been diploid, triploid, or tetraploid. In Georgia, *A*. *arbutifolia* grows naturally throughout the State while *A*. *melanocarpa* grows only in the northern mountain region. *Aronia* plants have not been collected in the state of Georgia. Our goal is to develop a cultivar of *A*. *arbutifolia* that could be grow commercially throughout Georgia with high yields and high nutraceutical qualities. We evaluated the horticulture performance of *A*. *arbutifolia* and 'Viking' plants in Blairsville (Zone 7a, Blue Ridge region) and Griffin (Zone 8a, Piedmont region) during the 2022 season. Fruit yield of 'Viking' was higher in Blairsville (average 237 berries/plant) than in Griffin (119 berries/plant). *A*. *arbutifolia* also had a higher yield in Blairsville than in Griffin, with 1,086 berries versus 673 berries, respectively. Berry weight and diameter, as well as brix for both 'Viking' and *A. arbutifolia* were also greater in Blairsville. Better performance of *A*. *arbutifolia* in Blairsville could be attributed to germplasm adapted to colder climates, as seed material usually comes from northern locations. We also collected wild *A*. *arbutifolia* in the Piedmont and Coastal Plain regions. Although most genotypes were tetraploid, we identified two populations of triploid *A*. *arbutifolia* from two adjacent South Georgia counties. In one of those counties, we identified triploid and tetraploid genotypes, which could mean a diploid genotype is also present in the same area. *A*. *arbutifolia* plants collected in Georgia will enter the trial in 2023 to evaluate their horticulture performance. Our findings are the first report of a triploid *A. arbutifolia* population.

Identification of potential interactors from the *OFP*, *TRM* and *SUN* families to investigate the molecular mechanisms of organ morphogenesis in tomato

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Members of the *OFP*, *TRM* and *SUN* families are known to regulate plant organ morphogenesis. However, the interaction among members of these three families and their effect on shape is only partially understood. Moreover, the underlying molecular mechanisms of plant morphology that is controlled by these three families also remain unclear. For this, we analyzed a single-nuclei RNA-sequencing (snRNA-seq) dataset of shoot apical meristem (SAM) from 2-week-old tomato seedlings. We focused on genes from *OFP*, *TRM* and *SUN* families, and conducted hearmap clustering, correlation analysis, and Gene Ontology enrichment analysis for co-expressed genes and biological or molecular pathways the candidate genes could be involved. Importantly, we created nearly isogenic lines (NILs) carrying natural mutation alleles of the known tomato fruit shape genes in a common genetic background as well as knock-out mutations by CRISPR-Cas9 editing in other members of the family in the same background. Additional mutant combinations based on data analysis were also investigated in the leaf and fruit shape to validate the hypothesis that these coregulated genes interact to control organ morphology in tomato. Our data suggest that investigating coregulated genes from the vegetative SAM is a valid strategy in identifying genes functioning in leaf shape regulation. Given that organ shape regulation mechanism is conserved in diverse crops, furthering our understanding into the molecular mechanisms in tomato should provide insights into the plant organ shape regulatory. This work is funded by NSF IOS 2048425.