## National Association of Plant Breeders 2018 Annual Meeting - **Abstracts**

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NAB

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### **Speaker Abstracts**

Pollinators and pollination under pressure: problems and progress with this predicament

#### Nigel Raine

Roughly one third of the food we eat depends on pollinators. Economically sustainable yields for 75% of global crops are byproducts of pollinator foraging activity (most importantly bees), moving between flowers collecting food and also carrying pollen to facilitate seed, fruit and nut production. Beyond crops, almost 90% of flowering plant species worldwide rely on animal-vectored pollination, making pollinators an essential part of natural ecosystem function and wider cultural values. Reports of global pollinator declines raise concerns for agricultural productivity, food security and reduced natural biodiversity. Declines seem to be driven by multiple, potentially interacting environmental stress factors. These include the loss and fragmentation of habitat, increased agrochemical exposure resulting from agricultural intensification, impacts of parasites and pathogens, invasive species and climate change. In this presentation I will review the evidence for, and impacts of, pollinator declines and discuss potential strategies to enhance pollinator health and sustainable agricultural production. Pollinators are beautiful, fascinating, diverse and essential creatures that we simply cannot afford to lose.

Phenotyping Apple Fruit (Malus x. domestica Borkh.) Using Portable Spectrometers

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Apple fruit is the second most consumed fresh fruit by weight in the USA, and repeat sales depend on consistent fruit quality. Traditional methods used to measure fruit traits can be destructive, susceptible to bias, and require extended processes. **Near-infrared spectroscopy** (i.e. the measurement of the interaction of near-infrared irradiation) is becoming an important tool for material evaluation outside lab environments. Although the efficacy of spectroscopy to assess many apple fruit traits has been extensively researched, very few studies have assessed the use of **portable** spectrometers in the orchard.

A <u>Felix Instruments F-750</u> handheld spectrometer was employed in 2016 and 2017 to assess fruit from 15 cultivars at the University of Minnesota Horticultural Research Center. In the second year, a <u>Consumer Physics SCiO</u> spectrometer was also used. Multiple traits were measured using traditional phenotyping methods. Each fruit trait was individually modeled with the same spectra by partial least squares regression. The traits most accurately predicted were soluble solids content, starch pattern index (a measure of **fruit maturity**), and firmness, while dry matter and titratable acidity were not predicted well. Possible reasons for low prediction accuracy include imperfect manual measurement methods, or that fruit contents, such as acids, constitute a small proportion of the total fruit matter when extracted.

Handheld spectrometers have the potential to estimate important traits in real-time **nondestructively** in outdoor settings. In this study, the traits that were predicted well (soluble solids content, starch pattern index, and firmness) are strongly related to fruit quality and are used as maturity indices. Additional data from germplasm with more genetic variation will be useful to confirm whether portable spectrometers can be used in segregating families in a **breeding program**. The application of portable devices, which contain robust prediction models, could also aid in **orchard management**.

Moving toward marker-assisted selection for carrot shoot traits in diverse crop management systems

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Carrot (Daucus carota subsp. carota L.) shoot morphology affects crop weed competitiveness, foliar disease tolerance, harvestability, and fresh market consumer appeal. Despite this impact on crop growth and quality, the genetic influences underlying phenotypic variation of carrot shoots remain poorly understood. Non-genetic factors that influence carrot shoot morphology (e.g. planting density, nutrient availability, and intra- and inter-species competition) further complicate breeding efforts to improve this suite of traits with accuracy. In this study, we leverage the quantitative heritability and differential expression of carrot shoot traits under organic and conventional management established in our preliminary work to accomplish two goals: a) detect QTL associated with carrot shoot traits to enable marker-assisted selection efforts, and b) characterize QTL x environment (QEI) and QTL x management system interaction (QSI) to inform breeding efforts for organic agriculture. We grew four segregating carrot populations in paired organic and conventional trials for multiple generations while selecting for high and low shoot biomass under both crop management systems, constructed high-density linkage maps for these populations, compared these linkage maps to the carrot reference genome, and mapped QTL for carrot shoot morphology traits including midseason shoot height, shoot height at harvest, and shoot biomass at harvest. We then compared the QTL identified for different trial locations and management systems. Most of the carrot shoot morphology traits measured showed significant QEI and QSI, and evaluation of this QSI indicates greater efficiency of marker-assisted selection for organic agriculture when QTL are identified based on phenotypic data collected from organically managed breeding trials. Taken together, these findings contribute to our understanding of carrot shoot morphology traits under diverse crop management systems and guide us in conducting effective trialing and selection when breeding for diverse crop management systems.

Homeologous epistasis in allohexaploid wheat: The search for an immortal hybrid

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The subgenomes of an allopolyploid crop will each contain complete, yet evolutionarily divergent, sets of genes. Like a diploid hybrid, allopolyploids will have two versions, or homeoalleles, for every gene. Partial functional redundancy between homeologous genes should result in a deviation from additivity. These epistatic interactions between homeoalleles are analogous to dominance effects, but are fixed across subgenomes through self pollination. An allopolyploid can therefore be viewed as an immortalized hybrid, with the opportunity to select and fix favorable homeoallelic interactions within inbred varieties. With the availability of affordable genotyping and a reference genome to locate markers, breeders of allopolyploids now have the opportunity to manipulate subgenomes independently and fix beneficial interactions across subgenomes. We present a statistical framework for partitioning genetic variance to individual subgenomes of allopolyploids, predicting breeding values for each subgenome, and determining the importance of homeologous epistasis. We also present a subfunctionalization epistasis model to estimate the degree of functional redundancy between homeoallelic loci and to determine their importance within a population. We search for genome wide patterns indicative of homeoallelic subfunctionalization in a winter wheat breeding population by anchoring homeologous marker sets to the IWGSC RefSeq v1.0 sequence. Some traits displayed a pattern indicative of homeoallelic subfunctionalization, while other traits showed a less clear pattern. Using genomic prediction accuracy to evaluate importance of marker interactions, we show that homeologous interactions explain a significant portion of the non-additive genetic signal. Allopolyploids have traditionally been treated as diploids in breeding programs because they undergo disomic inheritance. With modern DNA marker technology and ever increasing computational power, we provide a new tool for breeders of allopolyploid crops to characterize the genetic architecture of existing populations, determine breeding goals, and develop new strategies for selection of additive effects and homeologous epistasis in these ancient immortal hybrids.

Selection Index based on hyperspectral image data to increase genetic gain

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Large-scale conventional phenotyping can be costly and logistically difficult to implement. This imposes limits on selection intensity and on genetic progress that can be achieved through direct phenotypic selection. High-throughput phenotyping (e.g., crop imaging) can be used to phenotype large-number of genotypes over multiple timepoints of the crop phenology, and can be used for indirect selection. Reflectance of electromagnetic power at different wavelengths has been shown to be associated with physiological and agronomic traits. This information has been used to derive vegetation indices (VI) that are predictive of agronomic traits (e.g., green- and red-normalized difference, rNDVI and gNDVI, respectively). However, previous studies suggest that the genetic correlation between some VIs varies substantially between trials. To confront this problem, we propose to use Image-derived Penalized Selection Indices (PSI) that couple the well-established theory of selection indices with modern ideas emerging in the high-dimensional regression field. We present the derivation of the PSI and its application to data from CIMMYT's wheat breeding program consisting of 3276 records (1092 lines) of grain yield collected under 6 different environmental conditions. Reflectance data at 250 wavelengths was collected during 9 timepoints. We estimated the relative efficiency (RE=accuracy of indirect selection/accuracy of direct selection) for PSI and VIs. The difference in RE was high under heat-stressed environment (0.59 vs 0.35, for PSI and gNDVI, respectively) and in later timepoints under well-watered conditions (0.7 vs 0.5). The RE difference was smaller, yet always in favor of the PSI, in water-stressed environments (0.74 vs 0.69) and in the non-heat-stressed environment (0.87 vs 0.84). Estimated RE shows that PSIs are more efficient for indirect selection than traditional VIs. Moreover, RE for PSI suggests that if crop imagining enabled increases in the number of tested genotypes by at least 50%, then its use could substantially increase annual genetic gains.

Plant Breeders' Rights: What you should know

#### **Anthony Parker**

Plant Breeders' Rights Office, CFIA, Ottawa, Canada

The government has set an ambitious target of growing Canada's agri-food exports from \$55 billion in 2015 to over \$75 billion by 2025. A key component of increasing domestic agriculture productivity is creating a positive business environment for investment in plant breeding. Plant breeding is a highly specialized, time consuming, and resource intensive activity, often taking 7-12 years from an initial cross to commercialization of a new variety. Plants by their very nature are easily reproduced and multiplied. If new plant varieties are left unprotected, they can be propagated and sold without authorization and fair compensation to the breeder. Plant Breeders' Rights (PBR) is a form of intellectual property (IP) protection, specifically designed to encourage investment and innovation in plant breeding for the benefit of society. In 2015 Canada amended its Plant Breeders' Rights Actto conform to the 1991 Act of the International Convention for the Protection of New Varieties of Plants(UPOV'91). By strengthening our intellectual property protection law for plant varieties, new opportunities are created to support domestic plant breeding entities (private, public, partnerships, etc.) and access to foreign genetics. The fundamental basis to obtain PBR protection requires the breeder to demonstrate that their variety is new, distinct, uniform, and stable. Once PBR is granted, the breeder has the exclusive right to; produce, reproduce, sell, condition, store/stock, and import/export propagating material of the variety for up to 20 years (25 years for trees and grapevines). The breeder also has the right to collect a royalty on any sale of their variety and seek recourse (i.e. compensation) through the civil court system if an infringement occurs. Since the Canadian Plant Breeders' Rights Office opened in 1992, approximately 5400 new varieties have been granted PBR protection spanning over 340 different crop kinds.

Bringing Genomics and Modern Breeding Tools to a Budding Industry

#### **Greg Baute**

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Cannabis is projected to become one of the largest crops in Canada, generating domestic sales of up to \$8.7 billion annually. Canada is well-positioned to lead globally in both cannabis production and creation of value-added products, including cannabinoid-based pharmaceuticals. To capitalize on this opportunity, growers need access to high-quality varieties that are optimized for large-scale production. There is considerable genetic diversity within the genus Cannabis, which includes both industrial hemp and drug type genotypes with various metabolite profiles and adaptation to different growing conditions. In order to leverage this diversity to create improved varieties Anandia has been characterizing a wide selection of cannabis genotypes using genomics and chemical profiling. We contrast these data with current genotypic groups, genetic families, and strains and in order to build an objective framework for genotypic classification. Having a map of the genotypic and chemical landscape of this crop will be key in the creation of improved genetics for this new industry.

Asparagus Breeding: All-Male Hybrids in a Dioecious, Perennial Crop

#### David Wolyn

University of Guelph, Guelph, Canada

Breeding strategies for asparagus are influenced by its dioecious, perennial nature. Gender is controlled by a single locus, *M*, where male and female genotypes are *Mm*and *mm*, respectively. Development of *MM*supermales facilitates the production of all-male hybrids (*mmx MM= Mm*) which have a yield advantage compared those that are dioecious, as male plants are generally more productive than females. Longevity is an important trait for a perennial crop and a good asparagus cultivar should maintain high yields and survive 15-20 years. This requirement presents challenges and lengthens the timeline from crossing to commercial release. Additional traits important for selection include not only yield and disease resistance, but also spear diameter and tip quality, and 'replant resistance.' This presentation will review the main components of an asparagus breeding program and highlight the important considerations that distinguish this vegetable from annual field crops.

Opportunities, Challenges, and Wrinkles in a Plant Breeding Career

#### John Clark

University of Arkansas, Fayetteville, USA

It was a fabulous honor to receive the National Association of Plant Breeders Impact Award in 2017, and to follow up with a presentation on thoughts on a career in plant breeding. Having grown up on a dairy/beef/row crop farm in Mississippi, it was quite a career stretch to ending up working in fruit breeding. As with all careers, there are usually several twists and turns, some anticipated and some not. My greatest opportunity came at the University of Arkansas working with my PhD advisor and subsequent colleague James N. Moore. Dr. Moore began the Arkansas fruit breeding program in 1964, I joined him in 1980, and assumed the program leadership up his retirement in 1996. The opportunity to carry out our program dreams has extended well beyond what I envisioned in my early career. One of the larger challenges was in the area of funding for the program. From the program's beginnings until the 1990s, state funding provided almost all the support. Arkansas has no organized fruit industry, and no industry support to parallel his substantial effort. This appeared to be an unsustainable for the program and my career, and I figured that program support must be attained in some way. This led to the embracing of intellectual property rights as a means to generate support. This has worked out more successfully than envisioned, and allowed me to grow professionally in an area that I had little to no interest. The IP expansion turned out to expand program income, academic scholarship, and the increased use of the program's genetic resources. There were also some unusual wrinkles along the way, and I will share further on those that I was able to smooth out, and those that continue to be worked out.

Building upon the success of Brassica breeding at the University of Manitoba

#### Robert Duncan

University of Manitoba, Winnipeg, Canada

Brassica breeding at the University of Manitoba started in the 1950s and led to the development of the first canola cultivar (Tower) in 1974, the first high erucic acid rapeseed (HEAR) cultivar (Reston) in 1982 and the first low linolenic cultivar (Stellar) in 1987. Today, the breeding program focuses on HEAR hybrid development for commercial production in western Canada as well as Brassica napus trait development. Improvement in seed quality, disease resistance and agronomic performance represent key areas of research. Recently, emphasis has been placed on developing canola with enhanced protein and nutritional qualities. Canola meal has historically been a by-product and utilized only for animal feed and this provides an immense opportunity to expand the utilization of canola in Canada. Brassica napus value could grow several fold if high-quality protein products were developed for use in human food products. This presentation will also discuss current research on the challenges associated with canola and HEAR hybrid development and related discovery research on important traits required for B. napus production.

#### My wheat breeding journey among good company

#### Robert Allan

USDA-ARS & Department of Crops and Soil Sciences, Washington State University, USA

I am honored to receive the 2017 Lifetime Achievement Award from the National Association of Plant Breeders. My involvement in wheat genetics and breeding dates from 1954 to present. I spent my entire career as a Research Plant Geneticist with the USDA-ARS wheat research program at Pullman, WA. I interacted with many outstanding people. They included my mentors Dr. Elmer G. Heyne and Dr. Orville A. Vogel. I also had several exceptional graduate students and a dedicated support staff. I was fortunate to work with a number of first-rate, co-scientists on joint research projects. Pullman is an ideal place to conduct wheat research. It has a perfect environment, exceptional co-workers, good facilities, and strong stakeholder support. These advantages had a positive effect on my research. My main research accomplishments did not come via a structured or orthodox approach and serendipity occurred on occasion. Identifying and naming the two semidwarf genes that fostered the Wheat Green Revolution came about from an approach that initially failed. My discovery that these two semidwarf genes are insensitive to gibberellic acid ranks as one of my most cited publications. An unwanted plant physiology course my graduate committee made me take, gave me the idea to conduct the study. A paper I was assigned as a graduate student motivated me to develop multilines that successfully controlled the devastating stripe rust epidemics that were occurring in club wheat. The germplasm I acquired to develop my most successful wheat variety came about from a chance conversation with a French wheat geneticist at a social event. My brief newsletter note on a gene for resistance to eyespot foot rot led to collaborative research with a wheat biochemist that identified a molecular marker tightly linked to this gene for eyespot resistance.

Breeding strategies in long-lived species

**Barb Thomas** 

University of Alberta, Edmonton, Canada

Trees pose challenges for breeders that can often take a life-time to see the results from. In the boreal forest regions of Alberta, the rotation for white spruce (*Picea glauca*) or lodgepole pine (*Pinus contorta*), is between 80-105 years under current growing regimes. A classic tree improvement cycle can take 30-years to move from one generation of breeding and seed orchard development to the next, including breeding, testing and selection.

Coupled with this, is the economic burden imposed by the early investments required, a shrinking landbase for forestry operations due primarily to energy sector development, and more recently, the challenges of climate change increasing abiotic and biotic stresses. This presentation will present a case study applying a change in approach by incorporation of genomic selection into traditional tree breeding. A Genome Canada funded project entitled '*Resilient Forests (RES-FOR): Climate, Pests and Policy, Genomic Applications'* was initiated in 2016 with a 4-year mandate to unravel the challenges of genomic applications in the large and challenging genomes of white spruce and lodgepole pine.

Through the integration of the social sciences, natural sciences, genomics, bioinformatics, ecophysiology, entomology, chemical ecology and metabolomics, a paradigm shift is occurring as breeding programs in forestry move away from tradition volume based selection to an integrated approach with the goal of achieving forest resilience.

Apples: Harnessing Diversity for Genetic Improvement

Susan Brown

Cornell University, Ithaca, USA

Apple have outstanding genetic diversity, from the 25 to 40 *Malus*species to the heterozygosity evident in every cross. The sequenced genome and many multi-disciplinary, cooperative research projects, such as RosBREED and FruitBreedomics, further our resources to effectively harness this diversity for genetic improvement. Research on transcription factors and promoters has furthered our knowledge of color in leaves, peel and flesh. Dihydrochalcones and their role in health has promoted detailed studies of germplasm and genetic pathways. Interspecific hybridization has targeted only a few of the many *Malus*species, most notably for disease resistance and for ornamental and rootstock breeding. Different*Malus* species crossed to a common columnar (or reduced branching) selection provides opportunities to study genetic background effects. These populations are excellent resources for summer intern research projects and they provide important insight into fruit set differences, leaf morphology, plant architecture and the occurrence of common off-types. Cultivar development has benefitted from enhanced knowledge of, and markers for, fruit quality traits. Future releases will offer consumers enhanced aroma, distinctive appearance and unique quality attributes. Maintaining consistency of quality across growing regions will remain a research challenge, since it requires efforts to minimize the variability inherent in apple. Opportunities abound!

Cryopreservation of plant tissues for long-term germplasm storage

#### Max Jones

University of Guelph, Guelph, Canada

Genebanks are important to preserve genetic diversity of both wild and cultivated plant species, and represent a valuable resource for crop improvement. Most genebanks store plant genetics in the form of seed, but this is not a viable approach for plants that are seedless or produce recalcitrant seeds, and is not suitable for preserving cultivars that do not grow true to type from seed. While such species can be maintained in field collections, this represents significant costs and leaves the collections vulnerable to biotic and abiotic threats. An alternative method is to preserve plant genetics through cryopreservation of vegetative tissues, which ensures germplasm is maintained in a safe environment and can be more cost effective than field collections in the long term. Cryopreservation can be used to preserve various tissues including embryos, dormant buds, actively growing meristems, and even roots. However, each species/tissue have their own unique requirements and protocols must be developed on a case by case basis. This talk will introduce the Gosling Research Institute for Plant Preservation and some of the work we have done on plant cryopreservation of endangered species and economically important plants.

Flax Breeding in Canada: Challenges and Opportunities.

#### Helen Booker

University of Saskatchewan, Saskatoon, Canada

Canada has produced and exported more flax than any other country since 1994. Europe was the major export market prior to 2009, but transgenic flax discovered in a shipment of Canadian flax halted such exports, initially resulting in a steep decline in flax planted across the prairies. The entire flax industry worked together to institute the farm stewardship program, and the Crop Development Centre (CDC) reconstituted its commercial flax cultivars to alleviate concerns about transgene contamination. As a result, huge changes in flax production and export dynamics occurred over the past 3-5 years; Canada now accounts for ~30% of world production and Canadian exports for ~50% of global flax trade. Currently registered cultivars have both brown or yellow seed coat and high levels of alpha-linolenic fatty acid.Canada is the first country to allow a health-related claim for flaxseed use on food labels, linking ground whole flaxseed to lower cholesterol (a major risk for heart disease). Addressing key production issues would see flax more widely grown by Canadian producers and help to sustain conservation farming on the prairies. Breeding targets include crop traits associated with climatic adaptation, ease of harvest, increased yield (yield stability), and seed quality traits of interest to the market. New cultivar releases exhibiting improved yield potential across the prairies compared to popularly grown CDC Bethune include CDC Glas (103%) registered in 2012; CDC Neela (105%) and CDC Plava (106%; targeting the shorter growing season zone) registered in 2015; CDC Buryu (106%; 108% in the brown soil zone) registered in 2017, and CDC Rowland (112%; 117% in the longer season black and grey soil zone) registered in 2018. Specialty yellow seed coat high ALA CDC Melyn and CDC Dorado, registered in 2016 and 2017, respectively, were developed for the emerging human health and animal nutrition market.

Taking lentils out of their comfort zone: application of genomics to lentil breeding

#### Kirsten Bett

University of Saskatchewan, Saskatoon, Canada

Canada is the largest producer and exporter of lentil in the world, but the crop did not originate in a temperate climate with a long photoperiod. Lentil is grown in many other parts of the world where the photoperiod and temperature prior to flowering differ dramatically. Cultivars from one region struggle to perform well in other regions due to problems related to phenology, making breeders reluctant to use un-adapted material in their crosses with a subsequent loss of genetic variability. Lentil also has several wild relatives that offer useful genetic variability but using wild germplasm comes with a whole other level of 'unadaptedness'. To better understand how lentil is adapted to different macro-environments, and to identify markers for genes that control adaptation responses, we grew a diversity panel of 324 accessions for two seasons in nine locations around the world. We collected data on phenology and related traits as well as environmental data at all locations. Several interspecific populations have also been phenotyped for traits related to domestication, agronomy and seed quality to assess the consequences of using them in a breeding program. All lines have been genotyped to better understand the underlying genetic variability and identify regions of the genome that breeders should be aware of when using exotic germplasm.

Breeding Winter Wheat for Western Canada: Opportunities and Challenges

#### Anita Brûlé-Babel

University of Manitoba, Winnipeg, Canada

Western Canada is known for production of hard red spring wheat and amber durum wheat. Only 5% of the wheat acreage in this region is winter wheat. Harsh winters, variable snow cover, unpredictable weather patterns, and specialized production practices have often resulted in inconsistent production of winter wheat in western Canada. Despite the challenges, winter wheat production has a number of advantages over spring wheat. Winter wheat produces 25-40% higher yield than hard red spring wheat, makes better use of spring moisture, competes better with annual weeds, provides habitat for migrating birds, reduces erosion, and reduces fuel costs. Cultivar development has been a key factor in improving the viability of winter wheat production in western Canada. Improvements in leaf and stem rust resistance, Fusarium head blight resistance, agronomic performance, and production practices have contributed to the increased potential for winter wheat production. This presentation will focus on breeding successes for winter wheat, and discuss the opportunities and challenges to increasing winter wheat production in western Canada.

### **Poster Abstracts**

1

Homeologous pairing promoter chromosome in wheat (*Triticum aestivum*) from diploid wheatgrass *Lophopyrum elongatum* 

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Wild wheatgrass species can serve as a valuable genepool for wheat breeding. In order to develop wheat-wheatgrass amphiploids, crosses were made between Chinese Spring disomic substitution lines (for chromosome 1E-7E) and diploid wheatgrass (Lophopyrum elongatum, 2n=14). Five out of seven amphiploids for chromosome 1E, 3E, 5E, 6E and 7E were successfully developed. Each of these five amphiploid are octaploid (2n = 56) with four copies of respective wheatgrass chromosome. Meiosis of pollen mother cells (PMC) was analyzed in the F2 generation. The results showed complete pairing (all bivalents) for chromosome 5E, occasional multivalents (mostly one quadrivalent) for chromosome 7E, no multivalent with some univalent for chromosome 1E and frequent multivalents (1-3 quadrivalents) for chromosome 3E. Thus, out of five amphiploids, only chromosome 3E amphiploid promotes homeologous pairing in wheat. This is in contrast to earlier studies which showed that chromosome 3E and chromosome 5E of L. elongatum can promote homeologous pairing in wheat. One way to achieve homeologous pairing in wheat is by using ph1 (-) lines. However, use of this line is time consuming as the action of locus is recessive. Another homeologous pairing promoter locus Su1-Ph1 is identified in Aegilops speltoides. However, this locus promotes homeologous pairing mainly in tetraploid (4x) durum wheat but not in hexaploid (6x) wheat. Thus, the chromosome 3E amphiploid can be used as a valuable breeding source to promote homeologous pairing in wheat. This might facilitate QTL mapping for valuable traits from wheatgrasses such as salinity tolerance.

Characterization of Cruciferin Content in a Brassica napus Nested Association Mapping Population

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Canola and rapeseed (*Brassica napus* L.) are grown primarily for oil production while the residual protein-rich meal is used as livestock feed. However, with up to 50 % protein on a dry basis, *B. napus* meal is also a promising source of protein for human consumption. Of the seed storage proteins within *B. napus* meal, cruciferin makes up the largest proportion, accounting for 60-65 % of the total protein content of mature seeds. In addition, its distinct functional properties render it a valuable ingredient for food processors. To date, however, *B. napus* research has focused largely on oil content and quality and reducing undesirable compounds. As such, this research aims to evaluate the phenotypic variation and effect of genotype and environmental interactions on cruciferin content in the founder lines of a Nested Association Mapping (NAM) population. To this end, an enzyme-linked immunosorbent assay (ELISA)-based approach was developed to allow for the efficient screening of total cruciferin content. NAM founder lines were grown in field trials in Winnipeg, MB (2016, 2017) and Saskatoon, SK (2014, 2015). Phenotypic screening revealed significant variation in total cruciferin content amongst NAM founder lines. By improving the knowledge of existing phenotypic variation in cruciferin content, this research will facilitate breeding efforts for *B. napus* cultivars with unique seed storage protein profiles.

Analysis of the genetic diversity of NBS-LRR resistance genes in three maize lines

Amaja Andrews, Lorna Nissen, Shavannor Smith

University of Georgia, Athens, USA

The U.S. is ranked first in the world in corn (*Zea mays*) production, with 96 million acres of land reserved for corn production. One vital trait that affects maize and all plants grown in agricultural or natural environments is their ability to withstand disease. *Puccinia sorghi* is the causal agent of common rust of maize and is considered to be one of the most important production problems affecting maize. Plants use several different types of disease resistance genes to detect the presence of pathogens and induce defense responses. The largest class of resistance genes (R-genes) codes for proteins with nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains. For this project, a PCR-based approach was used to characterize the genetic diversity of NBS-LRR resistance gene homologues (RGHs) in three maize lines (*RP1-M*, H95 and B73) with different resistant phenotypes to *Puccinia sorghi*. RGHs were further analyzed through sequence alignment and phylogenetic comparisons. Phylogenetic analysis of the RGHs in the three maize lines indicated that the NBS-LRR resistance genes are evolving together (*RP1-M*, H95 and B73) and the nucleotide polymorphisms observed in the NBS-LRR resistant genes in the resistant line (*RP1-M*) may contribute to the resistance phenotype. Analysis of R-genes in previously uncharacterized *Rp1*maize genotypes will help identify resistance genes that will be potentially important for the production of disease resistant maize lines.

Genomic estimated breeding values in wheat breeding

M. Nelly Arguello-Blanco, Mao Huang, Daniel Borrenpohl, Clay Sneller

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The selection of parents to make a cross is the most important decision a breeder must make. The chosen parents are considered ideal if these generate progeny with enough genetic variance, as well as high mean performance, leading to genetic gain and attaining the breeder's goals. Currently, combining genotyping technologies and statistical modeling such as genomic selection (GS), provide the opportunity to simulate the progeny of a bi-parental cross and predict its performance. This can be a valuable cross-planning tool for breeders. This study utilized a training population (TP) of 470 individuals genotyped with genotyping-by-sequencing (GBS) markers and phenotyped for grain yield, softness equivalence, and fusarium head blight resistance. With this TP, a GS model was built using ridge regression and we obtained suitable prediction accuracies as revealed by 10-fold cross validation. Parents from the TP were selected based on individual's genomic estimated breeding value (GEBV) to create cycle 1 of selection (GC1). F1-plants from GC1 self-pollinated to the F2-generation. This F2generation of plants were genotyped with GBS and their GEBV were predicted using the GS model constructed with the TP. The same process was conducted for four subsequent cycles for a total of five cycles (GC1-GC5) aiming at assessing the genetic gain over the cycles of selection and to determine the best parental combination within the breeding program. Preliminary analysis show that GEBVs have increased for yield and decreased for Fusarium Head Blight, relative to the TP between and within environments. Population structure by principal component analysis performed with 3,239 SNPs distributed across the genome showed that individuals from the TP, GC2 and GC3 occupy the same genetic space, indicating that the selection process with GEBVs is maintaining the program's genetic diversity. Prediction of progeny variance will also be discussed in the poster presentation.

Rapid resistance gene discovery and cloning from a wild wheat

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Crop wild relatives represents a rich reservoir of genetic diversity for disease resistance. But introducing this variation into domesticated cultivars is hampered by sexual incompatibility, ploidy differences, and linkage drag. Additionally, single gene-based resistance is often overcome by resistance breaking strains of the pathogen. Cloning multiple genes and combining them as a cassette into a susceptible cultivar may provide more durable resistance. However, resistance gene cloning from crop wild relatives is complicated by a lack of genomics resources and poor agronomy. Here we report a method that allows the rapid discovery and cloning of resistance genes. It couples Association genetics with Resistance gene ENrichment SEQuencing (AgRenSeq) to exploit the natural pan-genome variation in resistance gene analogues. Using this method, we have cloned four stem rust resistance genes from a panel of 151 accessions of *Aegilops tauschii*, the wild D genome progenitor of hexaploid bread wheat. This method constitutes a major advance in the cloning of resistance genes from crop wild relatives. Moreover, the cloned gene sequences provide perfect markers which can be used by breeders to speed up gene introgression by marker assisted selection.

Developing the Genomic Resources for a Tetraploid Blueberry cv. 'O'Neal'

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Blueberry (Vaccinium sp.) is one of the recently domesticated fruit crops. In the United States approximately 314 M lb of fresh and 244 M lb of processed blueberry a produced annually. North Carolina ranks 6<sup>th</sup> in the nation in production of blueberry in over 7,000 acres with the farmgate value of \$70 M. In addition to yield, the demand for high quality blueberry cultivars is on the rise. The application of molecular breeding and genomic tools can help expedite the blueberry breeding process, to meet the market demands for higher yield and quality. However, the molecular breeding approach in blueberry is still lagging behind due to the lack of adequate genomic resources. Here, we present a nearcomplete genome sequence and transcriptome assembly of a commercially valuable blueberry cultivar, 'O'Neal'. 'O'Neal' is a tetraploid southern high-bush blueberry cultivar with unique characteristics such as high yield, good flavor, dry picking scar, and early ripening. We used Single Molecule Real Time (SMRT) sequencing technology (a.k.a PacBio) to obtain a high quality genome for tetraploid blueberry. We developed a 1.07GB draft genome with contig N50 of 180KB from a total of 130GB PacBio reads (>200X coverage of the estimated 670Mb haploid genome). The transcriptome profiling was done in various tissues including leaves, roots, and different developmental stage of flower and fruits using PacBio-isoseq and Illumina short read sequencing technologies. We generated 141,399 high-quality fulllength transcript reads from 52 PacBio SMRT cells. After collapsing similar isoforms using Cupcake ToFU package, 22,913 unique isoforms were retained. Furthermore, the mRNAs from each tissue were also sequenced using the latest Illumina HiSeq platform. A total of 219,842 transcripts were assembled form the 1.8 billion Illumina short reads. We will discuss the status of these genomic resources and their applications in the blueberry breeding program at NC State.

Identification of Aroma Differences in Norton and Cabernet Sauvignon Grapes and Wines and Using those Key Volatiles to Phenotype F1 Mapping Population of Norton and Cabernet Sauvignon for QTL Analysis.

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Grape-derived volatiles play an important role in wine aroma and contribute to the overall wine quality. Free volatiles and non-volatile precursors, including glycosides, are present in grapes. The aroma precursors upon release due to acidic or enzymatic hydrolysis during winemaking, give varietal characteristics to a wine. We investigated the grape-derived volatiles in Cabernet Sauvignon, a popular Vitis vinifera grape, and an interspecific hybrid, Norton. Although Norton is cold hardy and disease tolerant that have made it economically important to Missouri, they are less popular than the vinifera wines globally. We identified the differences in Norton and Cabernet Sauvignon grapes and wines using the non-targeted metabolomics approach. Both free and bound volatiles were profiled in grapes and free volatiles in wines. Twenty-one samples of Norton and Cabernet Sauvignon grapes, from different vintages and sites, along with their ten different commercial wines were analyzed using HS-SPME-GCMS. Data was processed using XCMS to identify features different between the two cultivars. 825, 697 and 403 features were found to be different for free grape volatiles, bound volatiles and wine volatiles respectively, at least at 0.05 significance level and with a 1.5-fold change. Those features were used to identify and quantify odor active compounds that varied in concentration, including  $\beta$ -linalool,  $\beta$ damascenone,  $\beta$ -lonone, Eugenol and Methyl salicylate. We did not find any compounds present in one that was absent in the other cultivar, however, the concentrations of the compounds identified were different in two cultivars. The identified compounds were quantified in F1 mapping population of Norton and Cabernet Sauvignon using GC-MS/MS. Metabolic QTL analysis will be done using R/qtl to identify DNA markers associated to the traits of interest. Identification of these QTLs will be useful in varietal development where the end goal is disease tolerant fruit with a widely accepted aroma profile.

Breeding innovations in wheat for resilient cropping systems (BRIWECS)

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Phenotypic and genotypic data were collected for 220 wheat cultivars in 2015 and 2016 under different N treatments as in other location of project partners. The genotypes were SNP typed Using 15k illumina chip. 9248 polymorphic markers were identified after imputation. Based on the gathered information, genetic association analysis was performed using SAS 9.2 Inc. A number of marker-trait association are identified for several traits like Heading (23), yellow rust (9) and yield (6) for data collected in 2015 and 5,11,3 for the same traits respectively in 2016. Significant differences in total N contents in grains and yield were obtained when comparing vegetation years and N treatments. It is concluded that the simulation of certain combinations may enable better understanding of plant mechanism in response to genotype interaction with environment and management.

Genetics by Processing Time Interaction: Will Faster Cooking Bean Varieties Benefit Canners?

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Dry beans are a nutrient-rich dietary staple that require processing prior to consumption. The two most common preparation methods are industrial canning of whole beans or home cooking of dry beans in boiling water. Long cooking times required for dry beans make canned beans an appealing and convenient option for many consumers. Genetic variability for cooking time may be of interest to the canning industry as an aspect of canning quality.

In this study, we evaluated fast and slow cooking yellow bean germplasm for canning quality under five retort processing times between 10 and 45 minutes. We found that faster cooking beans were fully cooked after 10 minutes at 250 °F in the retort, while slower cooking beans required up to 20 minutes to cook fully. While canning protocols may vary across processors and market classes, this finding indicates faster cooking times may be beneficial to the dry bean canning industry by reducing the processing time and energy expenditure required to can beans.

# BACKGROUND GENOME SELECTION FOR RAPID INTROGRESSION AND EVALUATION OF QUANTITATIVE TRAIT LOCI (QTL) FOR RESISTANCE IN TOMATO TO MULTIPLE *XANTHOMONAS SPP*.

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Bacterial Spot of tomato is a foliar disease caused by four species of Xanthomonas. Identification of genetic resistance in wild tomatoes and breeding has been a focus of our control strategy. Three independent sources of resistance, Hawaii 7998, PI 114490 and LA2533 have been discovered, and genetic studies have identified a major QTL mapping to the same region on chromosome 11. Genome resequencing and analysis suggests that these loci are not identical, though current resolution does not allow us to distinguish alleles from linked genes. To assess whether one QTL provides better resistance to multiple species, we developed near isogenic lines (NILs) using marker-assisted selection and background genome selection. The resistant sources were independently introduced into a susceptible parent, OH88119. Insertion/Deletion markers were used to select for the QTL region and a panel of SNP markers assayed on the KASP platform were used for background genome selection. This approach allowed us to rapidly develop NILs that are 95%-99% genetically identical, except for the QTL on chromosome 11. In 2016 and 2017 we assessed multiple lines developed from each source in independent field trials inoculated with three species causing bacterial spot (X. perforans, X. euvesicatoria, X. gardneri). The NILs were evaluated using the Horsfall-Barrat Scale (1-12), which estimates the percentage of disease on the foliage. Linear Models were used to make comparisons between QTL sources and allelic effects within source. The results show that there are significant differences in both cases, with Hawaii 7998 providing the highest level of resistance to all three species. NILs resistant to multiple species will be released for use by private and public breeding research programs.

Loci and candidate genes controlling root traits in wheat seedlings - a wheat root GWAS

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Two hundred and one hexaploid wheat accessions, representing 200 years of selection and breeding history, were sampled from the National Small Grains Collection in Aberdeen, ID and evaluated for five root traits at the seedling stage. A paper-roll supported hydroponic system was used for seedling growth. Replicated roots samples were analyzed by WinRHIZO. We observed accessions with nearly no branching and accessions with up to 132 cm of branching. Total seminal root length ranged from 70 to 248 cm, a 3.5-fold difference. Next-generation sequencing was used to produce genomic libraries that were aligned to the wheat reference genome IWGSCv1 and call single nucleotide polymorphism (SNP) markers. After filtering and imputation, a total of 20,881 polymorphic sites were used to perform association mapping in TASSEL. Gene annotations were conducted for identified marker-trait associations (MTAs) with  $-\log_{10}P > 3.5$  (p-value < 0.003). In total, we identified 63 MTAs with seven for seminal axis root length (SAR), 24 for branching (BR), four for total seminal root length (TSR), eight for root dry matter (RDM), and 20 for root diameter (RD). Putative proteins of interest that we identified include chalcone synthase, aquaporin, and chymotrypsin inhibitor for SAR, MYB transcription factor and peroxidase for BR, zinc fingers and amino acid transporters for RDM, and cinnamoyl-CoA reductase for RD. We evaluated the effects of height-reducing Rht alleles and the 1B/1R translocation event on root traits and found presence of the *Rht-B1b* allele decreased RDM, while presence of the *Rht-D1b* allele increased TSR and decreased RD.

#### SoySNP Registry: a database of soybean variant data

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There are over 230,000 soybean accessions in germplasm repositories worldwide, making the identification of truly unique accessions difficult. High throughput genotyping costs have dropped sufficiently to enable dense genotyping of large germplasm collections. Nevertheless, large challenges remain due to the sheer volume of such genotype data. Comparisons between genotyping projects are additionally complicated by lack of common markers among data sets, differences in accession names, SNPs called from different reference genomes, and by inconsistent data formats. Here we describe a new database for soybean genotyping data. All SNPs are assigned a new SNP ID and SNPs common between multiple datasets will have the same identifier. Old names (ex. rs and ss IDs) are kept and will be searchable. This database will be the foundation for developing new interactive tools for soybean breeders at SoyBase.

#### Genomic and Transcriptomic Analysis of Salt and Heat Stress in Carrot Seed Germination

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Carrot is a widely grown and economically important vegetable that provides a rich dietary source of vitamin A to much of the world. Carrot has long been observed to be one of the most salt and heat sensitive vegetable crops, and production is restricted in many parts of the world due to these abiotic stresses. To date there have been few phenotypic and genetic evaluations for traits related to salt and heat tolerance in carrot. Screening for tolerance at the germination stage is the first step in the identification of tolerant genotypes as it is a critical stage for crop development. The development of molecular markers would be a useful tool to select genetically tolerant lines for breeding improved carrot tolerance to abiotic stress at the germination stage. In order to identify regions of the genome associated with abiotic stress at the seed germination stage, we have utilized a combination of RNA sequencing and genome-wide association analysis on a subset of 294 previously phenotyped diverse germplasm accessions for both salt and heat stress. We have identified a wide range of phenotypic diversity for these traits and potential candidate genes that warrant further investigation.

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Trends in soybean phenotypes through decades of breeding at the University of Guelph

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The study of phenotypes in a breeding program is important for understanding the impact of breeders' selections and trait changes over time, while providing a retrospective look at the history and foundation of the program. The objective of this study was to establish a reference database of phenotypic data across the pedigree-related germplasm in the 296-genotype University of Guelph Germplasm Panel (UGGP). One hundred and eighty genotypes of Guelph Campus and historical germplasm were tested in the field at two locations, Woodstock, ON and St Pauls, ON in 2015 and 2016. The germplasm studied was split into three groups: historical, Guelph Experimental and Guelph Cultivars. Phenotypic traits studied included agronomic traits (yield, plant height and days to maturity (DTM)) and seed traits (oil, protein, fatty acid profile and sugars). Trends over 100 years of soybean breeding covered by the panel demonstrated increasing oil levels in modern soybeans (0.023% year<sup>-1</sup>) and yield (18.8 kg ha<sup>-1</sup> year<sup>-1</sup>), as linolenic acid decreased (0.01% year<sup>-1</sup>). Experimental cultivars had the widest range for all fatty acid components, while cultivars had the narrowest range; a similar trend was observed for the sugar components. Yield per DTM per year of release was 0.11 kg ha<sup>-1</sup> day<sup>-1</sup> indicating that yield increased without lengthening of the maturity. A negative correlation was observed between protein and oil (r=-0.68, p<0.0001), as previously reported in soybean. A high broad-sense heritability was found for 100 seed weight ( $H^2=0.93\pm0.008$ ), oil ( $H^2=0.88\pm0.013$ ) and protein concentration (H<sup>2</sup>=0.81±0.019), while lower estimates were found for other agronomic and seed traits. Overall a complete database for the breeding program's historical phenotypes has been established to guide breeding efforts for future cultivar development.

Spring Wheat Resistance to the Herbicide Clethodim

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One fifth of the calories which make up the human diet worldwide are derived from wheat. Adequate weed control in such an important crop is essential to meet the rising demands of a growing world. Weed control within the wheat cropping system is dependent on the use of new herbicides as a response to the high rate of herbicide resistant weeds which have developed within the wheat system. The release of completely novel herbicides is infrequent; thus it is beneficial to select for increased resistance to currently available herbicides which are not yet labeled for use in the wheat system. An understanding of herbicide resistance traits is vitally important to reach this goal. To this end, recently identified resistance to the herbicide clethodim has been investigated in spring wheat. Several resistant lines were discovered in a screen of the Washington State University Core Germplasm Collection. The resistant lines were further investigated through dose response evaluations in the greenhouse. These investigations were expanded to include dose response evaluations of biparental F<sub>2</sub> populations developed using these lines as the resistant parent. Concurrently, downy brome response to clethodim was evaluated, revealing sufficient control at levels below the threshold for the resistant wheat lines.

Phenotypic and Genotypic Profiling of Heat Stress Tolerance in Blueberry Species to Increase Survivability

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Abiotic stress tolerance increases survivability of plants in changing climates. Blueberries are a heat sensitive crop where a narrow temperature threshold can cause severe stress, yielding stunted plants. In extreme cases, plant death is directly connected to prolonged heat stress exposure. Identifying genotypic and phenotypic traits associated with heat stress tolerance may enable development of plants adapted to higher temperature environments. This project evaluates heat stress tolerance of clones of two divergent diploid Vaccinium species, V. darrowii a subtropical species and V. corymbosum a northern temperate species. Heat stress quantified over a two-week period using chlorophyll and catalase protein assays, and mRNA analysis to identify putative genes associated with heat stress tolerance. Next generation RNA sequencing analysis followed by gene expression profiling should identify the differentially regulated genes during heat stress in both temperate and sub-tropical species, and their corresponding phenotypes profiled. Partnering with Delaware State University Cooperative Extension, findings of this study will be provided to growers, submitted for publication and placed on our laboratory website. The intension is to reach growers who can use this information to improve their blueberry growing experience. Simultaneously adding scientific value for marker assisted selection breeding programs by identifying heat stress tolerant genes that can be incorporated in breeding efforts to improve survivability of blueberry populations globally.

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Quantitative phenotyping method for skin set and color in red potatoes using image analysis.

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Two of the primary desirable traits in the red potato market are having an attractive red skin color and good skin set; unfortunately, the genetics of these traits are not well understood. To facilitate QTL mapping and improve selection accuracy, our goal was to develop a quantitative phenotyping method for skin set and color using image analysis. To validate our method, we evaluated 14 red potato varieties and advanced breeding lines in 2015 and 2016. After mechanical harvest and grading, a set of 7-8 representative tubers per plot was photographed, and photos were analyzed using ImageJ software to measure the percent skinned area (0–100%) and skin color using the hue, chroma and lightness representation. The plot-based heritability was high for all traits (>0.82); the genetic correlation between years was also high, ranging from 0.84 to 0.90 across the traits. The color traits were highly correlated with each other (>0.92) but not with percent skinned area (<0.41). The effects of season duration and storage time on skin set and color were evaluated in 2016. The percent skinned area was reduced as we extended the growing season, reflecting the increase in tuber maturity. The skin color became lighter as the growing season was extended and was darker when images taken six weeks after harvest were compared to one day after harvest. The precision, ease of use, and close agreement with human perception for the image analysis lead us to conclude it is a valuable quantitative phenotyping method for potato breeding. Our hope is that the use of new phenotyping technologies will accelerate the breeding of new potato varieties to improve marketability and meet the needs of growers.
Characterization of the Genetic Basis of Winter-Survival of Canadian Winter Wheat

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The harsh and unpredictable winters in the high latitude regions of the northern hemisphere often leads to high risk of winterkill for winter wheat (Triticum aestivum L.). This emphasizes the need for more winter-hardy varieties that are adapted to specific winter-wheat growing regions to reduce winterkill incidents. The goal of this research is to investigate the genetic basis of winter-survival in Canadian winter wheat and to identify the combination of key candidate gene alleles that is optimal for Canadian winter wheat growing regions. A diversity panel of 450 winter wheat genotypes from Canada, with various levels of winter-hardiness, was planted in October 2016 and 2017. Normalized difference vegetation index (NDVI) of each individual plot was extracted from multi-spectral imagery captured by unmanned aerial vehicle (UAV) as a measurement for winter-survival. The diversity panel was genotyped for allele variation of candidate genes that have demonstrated to have significant effect on flowering time and frost tolerance. Our result has been able to identify the optimal allele combination for the genes Vernlaization-1 (VRN1), C-Repeat Binding Factor (CBF)-12 and -15 for winter survival in Canada. Furthermore, a genome-wide association study was conducted. Two major quantitative trait loci on chromosome 5A that correspond to Frost Resistance-A1 and Frost-Resistance-A2 were shown to be associated with winter survival of Canadian wheat. The influence of copy number variation of VRN-A1 and CBF-A14 on winter-survival will be further investigated in this study.

Effect of G×E interactions on  $\beta$ -Glucan Content in Barley in the Palouse Region of Eastern Washington

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Increases in the craft beer and baking industries have solidified a market for malt and human food grade barley which sell at a higher price than barley grown for animal feed. Spring barley is frequently grown in rotation with winter wheat in dryland farming systems in eastern Washington to break up disease, weed and pest cycles. However, 90% of Washington barley is grown for feed, the remaining 10% is split between malt and food.  $\beta$ -glucan is one of the deciding factors for the end-use of barley grain: low  $\beta$ glucan is recommended for malt (2-3%w/w), moderate to high  $\beta$ -glucan for heart-healthy food (4-10%w/w). Enzymatic assays are commonly used today to detect  $\beta$ -glucan levels, but the process is slow. Near infrared spectroscopy (NIR) has been studied previously for its accuracy predicting water-soluble fiber in cereal grain products, including those that contained barley  $\beta$ -glucan. The objectives of this study were to (1) determine the correlation between  $\beta$ -glucan measured using the Megazyme enzymatic assay kit in relation to  $\beta$ -glucan measured by the NIR; (2) understand the stability of  $\beta$ -glucan of food, malt and feed barleys across grain-producing dryland environments in eastern Washington. We tested 24 diverse spring barley lines and released varieties across eight locations in 2017. A calibrated NIR was used to measure  $\beta$ -glucan levels. Our preliminary results at one location showed a range of  $\beta$ -glucan from 2.28 to 6.43 (%w/w), with a mean of 4.05 (%w/w). The data generated through this research will provide a better understanding of which cultivars farmers can grow in their area that will meet industrytargets and standards for both food and malt barley. Additional results including the R<sup>2</sup> value for the wet chemistry and NIR correlation and; the effect of  $G \times E$  on  $\beta$ -glucan content; will be presented in poster.

Analysis of the *Or* Gene and its Role in Carotenoid Accumulation in Carrot <u>Kevin Coe<sup>1</sup></u>, Shelby Ellison<sup>1,2</sup>, Doug Senalik<sup>1,2</sup>, Julie Dawson<sup>1</sup>, Philipp Simon<sup>1,2</sup> <sup>1</sup>University of Wisconsin-Madison, Madison, USA. <sup>2</sup>Agricultural Research Service, USDA, Madison, USA

Carrot (*Daucus carota*) is one of the richest sources of the vitamin A precursor  $\beta$ -carotene in the human diet. Two genes, Y and  $Y_2$  have been previously identified to be responsible for the majority of carotenoid accumulation in carrot roots. Y conditions all carotenoid accumulation in carrot roots, and the allele present in orange and yellow carrots harbors a 212 bp insertion in the gene.  $Y_2$  is known to condition the accumulation of  $\beta$ - and  $\alpha$ -carotene in carrot roots. The identity of  $Y_2$  is unknown, but  $Y_2$ has been fine-mapped to a 650-kb region on Chromosome 7. Recently, the Or gene was identified by a genome-wide association study (GWAS) to also be significantly associated with carotenoid accumulation in carrots roots. During plant growth, Or stimulates chromoplast biogenesis, thereby creating a sink for carotenoids to accumulate. Additionally, OR has been shown to stabilize PHYTOENE SYNTHASE (PSY), the rate limited enzyme in the carotenoid biosynthetic pathway. A Serine to Leucine amino acid substitution within the OR protein has been shown to be associated with increased  $\beta$ - and  $\alpha$ -carotene in orange carrots when compared to wild carrots. It is our hypothesis that during carrot domestication, a mutated Or allele was selected, alongside Y and  $Y_2$ , for its unique ability to increase carotenoid accumulation in root tissue. A sequenced panel of wild and domesticated plant introductions (PIs) will reveal if there are other polymorphisms associated with  $\beta$ - and  $\alpha$ -carotene accumulation in carrots. Additionally, patterns of Or expression are being analyzed in a mapping population of carrots identified to be fixed for Y and  $Y_2$ but still segregating for orange and yellow root color. Functional studies using an Arabidopsis model system will support the hypothesis that Or is a major gene responsible for increased carotenoid accumulation in carrot roots.

High Oleic, Non-GMO Soybean Breeding

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High oleic soybean oil is a new food product which is projected to grow extremely rapidly over the next years. Unlike conventional soybean oil, high oleic soybean oil does not require partial hydrogenation to have a high oxidative stability and long shelf life, and is therefore free of trans fats. The projected growth of this new product is due to many factors, including an FDA ban on trans fats in effect beginning in June 2018. High oleic soybean has been developed using several different methods by several different groups. DuPont Pioneer and Monsanto have used transgenic RNAi approaches to develop GMO high oleic soybeans, Plenish<sup>®</sup> and Vistive<sup>®</sup> Gold, respectively. Calytx<sup>™</sup>, a publicly traded biotech startup, has released a gene-edited high oleic soybean as their first commercial product. Many public soybean breeders are breeding non-GMO high oleic soybean varieties using FAD2 mutants developed by Kristin Bliyeu at the USDA. Most public breeders are using a winter nursey, marker-assisted backcross procedure towards introgression of the high oleic trait from FAD2 mutants into elite varieties. The Soybean Breeding Program at Michigan State University has used a single-seed descent approach with early generation, phenotypic selection. Compared to marker-assisted backcrossing, this approach allows us to improve yield and oil quality simultaneously. It also allows us to select for low linolenic acid and low saturated fat using the same GC-MS phenotyping data. Essential to this strategy is a highthroughput oil extraction procedure used to screen over 10,000 breeding lines every year. Commercialization partnerships, as well as past, current, and future research will be discussed.

Increasing the Inclusion Rates of Soybean Meal in Shrimp Diets through Trait-Enhanced Soybeans

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The use of soybean as a protein source in aquaculture is confounded by the presence of anti-nutritional trypsin inhibitors. Using specialty trait soybeans low in trypsin inhibitors developed at Virginia Tech's soybean breeding program, we aim to compare the effects of low trypsin inhibitor soybean-based ration on the growth of pacific shrimp in indoor aquaculture tanks. The growth of the shrimp fed normal soybean, low-trypsin inhibitor soybean, and soybean protein concentrate based rations will be compared to determine the effect of this quality trait on shrimp aquaculture.

We will also investigate the effects of roasting the low soybean meal at different temperatures, lower than the standard 110 C used in the main experiment and industry. We will investigate different inclusion rates of low trypsin inhibitor soybean meal roasted at 80 C to determine if we can lower the heat treatment for the specialized soybean variety in feed preparation. The feeding trials are planned to begin in June at Virginia Tech's main campus in Blacksburg, VA.

Identification of a Unique Spectral Signature of Black Layer Formation in Maize (Zea mays L.)

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Physiological maturity in maize is reached at the developmental stage black layer, where photosynthates are no longer able to move into the developing kernels. Currently there is no high-throughput fieldbased phenotyping method available for detecting black layer, although remotely sensed spectral data may offer a solution to this problem. The aim of this project is to identify a unique reflectance signature associated with physiological maturity in maize. Being repeatable across genotypes, different environmental conditions, different senescence patterns, etc., is essential. Several types of remotely sensed data have been used including hyperspectral data generated with a dual-channel reflectance spectrometer and an unmanned aerial vehicle (UAV) mounted multispectral camera. Accompanying the remotely sensed data are ground-truthed data consisting of visual determination of black layer and chlorophyll readings using a SPAD meter.

Initial experiments consisted of two planting dates at one location and involved four short-season hybrids that exhibited two different senescence patterns at maturity, a rapid "die and dry" phenotype and an extended "stay green" phenotype. We have tentatively identified a region of the spectrum that exhibits a change as black layer approaches. This region is consistent across the four genotypes and the two planting dates. To confirm our initial findings, we are now examining additional genotypes and adding a second growing season to the data set.

Seed Composition and Amino Acid Profiles for 100 *Chenopodium quinoa* Accessions Grown in Washington State

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Quinoa (Chenopodium quinoa Willd.) is a pseudocereal originating from the Lake Titicaca region of Peru and Bolivia. Quinoa is celebrated for its excellent nutritional food quality and ability to improve food and nutritional security, especially in marginal environments. However, minimal information is available on how genotype influences seed composition, and thus, nutritional quality. This study aimed to characterize seed composition via proximate analysis (e.g. crude protein, moisture, fat, fiber, and ash), and profiles of 23 amino acids for 100 accessions. The accessions examined represent commercial varieties and advanced breeding lines of quinoa adapted to Washington State. The Agricultural Experiment Station Chemical Laboratories at the University of Missouri-Columbia conducted proximate analysis and determined amino acid profiles according to AOAC official methods. Uncooked, whole grain quinoa samples were ground into a flour before analysis. All results are reported as grams per 100 g sample. Preliminary results indicate that crude protein ranged from 10.04 to 13.68 (mean = 11.77), crude fat ranged from 4.56 to 7.19 (mean = 5.89), crude fiber ranged from 1.79 to 4.76 (mean = 2.40), and total amino acid content ranged from 7.96 to 11.94 (mean = 10.25). The most abundant amino acids were glutamic acid (mean = 1.56), aspartic acid (mean = 0.94), and arginine (mean = 0.90). Of the nine essential amino acids, leucine (mean = 0.74), lysine (mean = 0.67), and valine (mean = 0.56) were the most abundant. These results provide insight into nutritional differences among the selected genotypes, thus providing a fundamental baseline for any meaningful future studies on how genotype influences seed composition and nutrition. This study identifies germplasm with high nutritional value that can be successfully grown in the U.S., and supports international research efforts in which quinoa is a key component in increasing food and nutritional security.

## Characterization of Vernalization in Winter and Spring Camelina sativa Biotypes

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The vast majority of camelina varieties are spring biotypes. However, winter biotypes also exist that require vernalization to flower and consequently exhibit different patterns of growth. As a result, winter varieties are better adapted and higher yielding than spring varieties in some environments. Although phenotypic differences between spring and winter biotypes have been reported, genetic differences between the two biotypes have yet to be explored. A single gene, flowering locus C (FLC), is primarily responsible for regulating vernalization in *Arabidopsis thaliana*. Since camelina is genetically closely related to *Arabidopsis*, we hypothesized that the FLC gene also plays a primary role in regulating vernalization in camelina. The purpose of this experiment is to elucidate genetic control of the vernalization trait in camelina via phenotypic analysis of the vernalization requirement in a winterXspring bi-parental population.

Our bi-parental population was derived from an initial cross between Joelle, a winter variety, and WA-HT1, our recently released spring variety, and contains 185 F4-derived lines. Two replicates of the F4derived lines were evaluated in a climate-controlled greenhouse trial to ensure the plants were not exposed to cold temperatures that would *satisfy* the vernalization requirement and trigger flowering. The initiation of flowering in the non-vernalized plants was evaluated to characterize the vernalization requirement in each of the progeny. <u>Analysis of several F1 plants revealed that vernalization is a semidominant trait. Furthermore, chi-square analysis of flowering initiation in the bi-parental population indicated that vernalization is primarily controlled by a single-gene. This understanding of the genetic differences between winter and spring biotypes will greatly expand current camelina production through the creation of varieties better adapted to more diverse environments.</u>

Genetic Components of Unmanned Aerial Systems Phenotyping Variability for Maize Breeding <u>Nathalia Penna Cruzato<sup>1</sup></u>, Seth Murray<sup>1</sup>, Dale Cope<sup>1</sup>, Anjin Chang<sup>2</sup>, Jinha Jung<sup>2</sup> <sup>1</sup>Texas A&M University, College Station, USA. <sup>2</sup>Texas A&M University Corpus Christi, Corpus Christi, USA

Variability is a key factor in genetic improvement and valuable in studies of environmental effects in genotypic expression. However, taking phenotypic measurements manually in the field is a laborious and time-consuming task. Through the last decades, remote sensing techniques have been applied to crop sciences to decrease time, labor, and improve precision in crop monitoring. Lately, with the advent of high-resolution sensors and unmanned aerial systems (UAS, i.e. drones), remote sensing is proving to be an efficient tool for high-throughput field phenotyping (HTFP). In this context, the present research aims to explore the capability of six phenotypic metrics obtained temporally through HTFP (from multispectral imagery: NDVI; from RGB imagery: Excess Green and Excess Red difference; and from both multispectral and RGB: plant height and canopy cover) in expressing the genetic variability of hybrid maize (Zea mays L.) genotypes; all metrics have been shown to correlate with grain yield. 250 hybrid maize genotypes in three treatments (optimal dryland, optimal irrigated, late planted heat stress) each with two replications, belonging to the Genome to Fields (G2F) GxE project were imaged in College Station, TX, weekly through the growing season and twice a week during the flowering period. An Unmanned Aerial System (UAS) composed by a Tuffwing fixed-wing drone, a Sony high resolution RGB camera and a MicaSense RedEgde multispectral camera, were used in the acquisition of the aerial images. An analysis of the variance components showed that the phenotypic variance explained by the genotypes fluctuates throughout the growing season, reaching its maximum around 60 days after sowing (DAS) for vegetation indices and canopy cover, and around 114 DAS for plant height. The results indicated that the studied metrics have a significant potential in supporting breeding decisions.

Whole genome sequencing of putative somatic hybrids of Puccinia graminis

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In Australia, isolates of the cereal stem rust pathogen Puccinia graminis have been found with unusual pathogenicity profiles that cannot be categorized into any known forma specialis; these have been referred to as 'scabrum' rusts. Scabrum rusts are believed to have arisen via somatic hybridisation between the special forms of *Puccinia graminis* virulent on wheat (*Puccinia graminis* f. sp. tritici (Pgt)) and cereal rye (Puccinia graminis f. sp. secalis (Pgs)) as sexual recombination within this species in Australia is considered to be extremely rare or absent. Somatic hybdirisation between isolates of Puccinia graminis has been proposed to involve exchange of haploid nuclei and possibly parasexuality. Should this hypothesis hold true, it would have large implications for the selection and deployment of resistance genes introgressed from different species and in hybrid crops such as triticale. This study will for the first time examine putative hybrid isolates of *P. graminis* at the genome level to determine if they truly have originated from a somatic hybridisation event between Pgt and Pgs. To test this hypothesis, genome sequence information of selected isolates of Pgt, Pgs and the 'scabrum' rust will be compared. Reference isolates of both Pgs and the 'scabrum' rust have been sequenced using PacBio long-read sequencing and will be assembled de novo, and the degree of similarity of the scabrum rust genome to the Pgt and Pgs genomes will be determined. Understanding the mechanisms driving genetic variability of highly-mutable pathogens such as Puccinia graminis are vital to the success of cereal breeding programs worldwide and will influence the choice of new resistance genes to incorporate into new resistant cereal varieties. This project is currently underway, and preliminary results of these experiments will be presented in a poster.

Soybean Iron Deficiency Chlorosis High Throughput Phenotyping Using an Unmanned Aerial System

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Many field tasks in a soybean breeding program are labor intensive, time consuming, and subjective. These tasks include the phenotyping of thousands of plots for traits such as plant health, height, maturity, and lodging. In recent years, there has been a growing interest in the use of aerial high throughput phenotyping (HTP) platforms to assist in making field scoring faster, more accurate, and more objective. The goal of this project is to use an unmanned aerial system (UAS) to improve field screening for tolerance to soybean iron deficiency chlorosis (IDC). During the summer of 2017, 3,386 plots were visually scored for IDC stress. In addition, images were captured with a DJI Inspire 1 platform equipped with a modified dual camera system which allows image capture in the near infrared (NIR) and better detection of plant health. A pipeline was created for image capture, orthomosaic generation, processing, and analysis. A total of six predictors were extracted from the imagery of each plot including spectral and canopy properties. Random forest and neural network algorithms resulted in a misclassification rate of 0.31 for random forest and 0.29 for neural network. Overall, the results obtained show promise in utilizing a UAS for efficient IDC field screening in a high throughput system.

# An R2R3-MYB Protein Functions as a Positive Regulator of Proanthocyanidin Biosynthesis in Common Bean (*Phaseolus vulgaris*) Seeds Leading to Seed Coat Postharvest Darkening

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Seed coat postharvest darkening (PHD) of legumes, and in particular pinto beans and cranberry beans, two market classes of dry beans, is attributed to proanthocyanidin accumulation and its subsequent oxidation in the seed coat. The *J* gene is an uncharacterized classical genetic locus known to be responsible for PHD in common bean (*Phaseolus vulgaris*) and its recessive allele results in the non-darkening (ND) seed coat phenotype. The objectives of this study were to identify a gene associated with seed coat postharvest darkening in common bean and understand its function in promoting seed coat darkening, through *in silico* analysis. Amplicon sequencing of 21 candidate genes underlying the QTL associated with the ND trait revealed a single nucleotide deletion (c.703delG) in the candidate gene Phvul.010G130600 in nondarkening Witrood. *In silico* analysis indicated that it encodes a protein with a high amino acid sequence identity (70%) to a R2R3-MYB type transcription factor MtPAR, which has been shown to regulate proanthocyanidin biosynthesis in *Medicago truncatula* seed coat. It is likely that a transcription activation EDLL motif in the C-terminal region is disrupted in the nondarkening allele of the R2R3-MYB mutant, due to the frameshift mutation caused by the single nucleotide deletion.

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# Exploring the Diversity of Root System Architecture in Soybean using Plant Phenomics and Machine Learning

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Root system architecture (RSA) studies are tedious, susceptible to introduced variation and the extracted features may not translate to a meaningful outcome. With the advent of high-throughput phenotyping, computer vision and machine learning there is a renewed interest in uncovering "the hidden half". Our study included 300 diverse soybean accessions from a wide geographical distribution (19 countries) of which genotypic information is available. We deployed a 2-D (in controlled conditions) and stereo imaging platforms (field tests), image processing algorithms and data analytic tools to deep phenotype for RSA traits using in-house software. The 2-D platform developed is non-destructive, adding observations throughout seedling growth and development. The stereo imaging platform of multiple cameras at multiple angles allows creation of a 3-D point cloud of a mature root. Tens of thousands of images were collected from thousands of plants using the imaging platforms developed in this study. Moving forward, we are adapting machine learning techniques via convolutional neural networks will allow for the extraction and prediction of novel root architectural information. Utilizing phenotyping techniques has allowed us to capture tremendous RSA variability of these 300 diverse genotypes throughout various stages of development that will drive gene discovery and breeding methods forward.

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Screening grapevine accessions as a potential source of resistance to powdery mildew

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*Erysiphe necator*, the causal agent of grapevine Powdery Mildew (PM), co-evolves with its host, *Vitis vinifera* L. (2n = 38), and rapidly overcome grapevines' resistance mechanisms. Screening for grapevines with durable disease resistance, is one of the main concerns of grapevine breeders. In this study, we aim to explore PM disease response of 213 accessions, including species from wild *Vitis spp*. and wild and cultivated Muscadine (2n=40) to identify potential candidates of resistance to use in our PMD resistance breeding program. These accessions were collected from southwestern, southeastern and south-central United States and Asia and screened for PM resistance by *in-vitro* detached leaf assay in the laboratory. A five-category grading scale was utilized for the phenotypic screening and evaluation on four leaves replicates of each accession. Susceptible cultivar Carignan was used as a control to estimate relative infection. The virulent PM C-isolate, collected from California, elicited susceptible to moderate disease pressure on the wild *Vitis* spp, whereas the wild Muscadine has shown fair resistance to PM. Results showed programmed cell death mediated resistance in wild Muscadine. The screened resistant grapevine accessions would contribute to grapevine breeding for PM resistance. Moreover, these results may play an essential role in viticulture and PM disease management. Further studies will aim to identify and characterize the resistance as a race-specific or non-race specific disease resistance.

Genetic Analysis of Sucrose Concentration in Soybean Seeds using a Historical Soybean Genomic Panel

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Soybean (*Glycine max* (L.) Merr) is a crop of global importance for both human and animal consumption. Since its domestication more than 3000 years ago in China, soybean has lost some of its genetic variability as a result of decades of breeding. To improve and develop new cultivars, it is critical for breeders to know the genetic variability available in their breeding germplasm. This study focused on sucrose concentration, a trait that has been shown to play a role in the taste and overall quality of food grade soybean cultivars. The objective of this study was to do a genetic analysis of sucrose concentration using a historical panel of the University of Guelph's soybean breeding programs at the Ridgetown and Guelph Campuses. A genomic panel of 282 soybean genotypes was grown in four Southern Ontario field locations from 2015 to 2017. Sucrose concentration was determined using a Perten NIR analyzer, which has been calibrated for sucrose measurement. Haplotype groups were identified for the GAPC1 gene, which was found within a previously identified QTL for sucrose concentration. Allele fixation in the program was also used to determine the genotypes that possess variability. Based on the field studies, genotype, environment and genotype-by-environment interaction effects have been determined as affecting overall seed sucrose concentration in the seed. This information will facilitate the breeders' efforts to increase the overall genetic variability for sucrose concentration and develop new and improved high-sucrose soybean cultivars suitable for the soy food industry.

Assessment of Genetic and Environmental Variation for Flavonols in American Cranberry

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Flavonoid compounds have been attributed to a multitude of human health benefits, including urinary tract and cardiovascular health, as well as neuroprotective activity. Within the flavonoids, the flavonol class has been shown to have higher bioavailability than proanthocyanidins and anthocyanins, and is found in especially high levels in cranberry (Vaccinium macrocarpon) fruit. Eight principal flavonols are present in cranberry fruit: myricetin-3-galactoside, myricetin-3-arabinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-xyloside, quercetin-3-arabinopyranoside, quercetin-3arabinofuranoside, and quercetin-3-rhamnoside. To determine the potential for genetic enhancement of flavonol content in cranberry, cranberry germplasm and breeding material was screened to obtain estimates of genetic variation, narrow sense heritability, and genotype by environment interaction. A collection of about 300 germplasm accessions at the Marucci Blueberry and Cranberry Research Station in Chatsworth, NJ was phenotyped for fruit flavonols in 2016 and 2017. Germplasm with significantly higher flavonol profiles were identified in wild accessions. Berry weight was negatively correlated with total flavonols, as larger fruit had less epidermis to pericarp ratio. A separate analysis of pericarp and epidermis showed that most of the flavonols are found in the epidermis. To determine if flavonol content was heritable, 24 breeding populations were analyzed using a mid-parent offspring regression and gave a narrow-sense heritability  $(h^2)$  of 0.47. Further analysis of seven varieties in four growing regions (NJ, WI, WA, OR) indicated significant regional variation in the fruit size and flavonol content in the different regions and no genotype-by-environment effect. These findings suggest the potential for enhanced flavonol content in cranberry through genetic manipulation.

Genomic regions associated with milling and baking quality of elite soft red winter wheat (SRWW)

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Genome wide association studies was performed to identify the genomic regions associated with the milling and baking quality traits in the elite soft red winter wheat (SRWW). To generate phenotypic data for quality traits, two hundred and seventy elite lines of SRWW from seven different breeding programs were evaluated in two location for two years for three milling quality traits- flour yield, softness equivalent, and flour protein and for four solvent retention capacity tests (lactose, sodium carbonate, sucrose, and water retention capacities). High quality markers (27,449 SNPs) developed using genotyping-by-sequencing (GBS) and 90K SNP array technologies were used for association analysis by fitting a linear mixed model that accounts for population structure and kinship. We have identified highly significant ( $-\log(P) >= 4.0$ ) 18 genomic regions in 12 different chromosomes. Most significant associations were identified in chromosomes 1B, 2A, 4B, 7A, and 7D. We have identified potential candidate genes in the periphery of QTLs that are associated with cell wall formation in seed and auxin synthesis/response pathway.

Dissecting the Genetic Mechanism behind Glandless Cotton

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Cotton (Gossypium spp) is predominately used as a fiber crop, but the cottonseed byproduct is a potentially cost-effective rich source of vegetable oil and high-quality protein for feed. However, the presence of small darkly pigmented lysigenous glands containing deposits of gossypol limits the amount of cottonseed meal that can be consumed by monogastric animals. Similarly, the uses of cottonseed oil prior to substantial processing in the food industry are limited by the presence of gossypol due to its human toxicity. In nature, gossypol plays an important role in the plant's defense against pests and insect vectors. Therefore, reducing gossypol in seeds while maintaining its level in other tissues would allow wider use of cottonseed meal and oil. In order to achieve this goal through breeding of improved cotton varieties, understanding the genetic control of the glandless trait in cotton is critical. We will present our work on the genetic architecture of the glandless trait through genetic mapping of F2 populations. Populations are being analyzed to generate a high-density genetic linkage map of singlenucleotide polymorphisms (SNPs) using the CottonSNP63K array, which will be followed by a mixedmodel single-trait quantitative trait loci (QTL) mapping. We will use these results to enhance our understanding and selection of the glandless trait in cotton, ultimately allowing cottonseed to be produced for feed or food uses through traditional breeding methods. For research support we thank Cotton Incorporated, Cary, NC.

An investigation of Canadian bread wheat diversity.

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Understanding the structure of genetic diversity among Canadian bread wheat varieties will facilitate germplasm management, understanding the genetic bases of different market classes, and identifying the past targets of selection. We used the Wheat 90K SNP iSelect assay to genotype 388 bread wheat cultivars grown in Canada. We mapped probe sequences using blast+ to the draft Chinese Spring bread wheat reference genome giving us genomic positions for 13,192 polymorphic markers. Variety relationships according to known pedigrees are frequently, but not always, reflected as expected at the molecular level. Based on grain attributes and growth habit major groups of varieties in Canada are Hard Red Spring (HRS), Hard Red Winter (HRW), or Soft White Spring (SWS). Clustering divides these into six groups: one dominated by HRW varieties and one by SWS, HRS varieties are split across the remaining four groups with one containing all those with abundant semi-dwarf ancestors. The six clusters explain 27.7% of the allelic variation by AMOVA with the division between non-semi-dwarf HRS varieties and all others alone explaining 19%. Expected heterozygosity and linkage disequilibrium amongst all varieties revealed very large haplotypes on chromosomes 1A, 2A, 4A, 6A, 7A, 5B, and 6B segregating in each of the six clusters. Locus-by-locus AMOVA identified markers with different allele frequencies between the HRS, HRW, and SWS classes. Marker alleles linked to a vernalization gene differed between HRS and HRW, but markers linked to those controlling grain colour and kernel hardness have similar allelic frequencies among phenotypically distinct populations. We suspect that poor marker specificity of the array and the polyploid nature of the wheat genome could be reducing the signal to noise ratio making associations difficult to find. This study contributes to the growing body of evidence of the distinctness of Canadian HRS wheat while identifying new structures of diversity.

Comparison of DSSAT CERES-Wheat Simulation Results from Limited Dataset calibration with Platinum Dataset calibration

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Spring wheat is currently a crop of limited importance for Alaska due to its long growing season. However as climate change continues and the number of growing degree days received per season increases, spring wheat could become a crop of importance. The objective of this presentation is to compare the DSSAT CERES-Wheat crop simulation model calibrated with historically collected limited dataset of Ingal, a short growing spring wheat from Alaska with a calibration using a platinum dataset of Ingal, AC Intrepid, and Ingal x AC Intrepid. The purpose is to examine the accuracy of historical datasets from the field variety trials for future projection under climate change scenarios. An Ingal dataset from a three year study of phenology and yield components during 1989-1991 in Palmer was used with variety trial Ingal datasets from Fairbanks during 2012-2016 to calibrate the DSSAT CERES-Wheat. In Fairbanks 2012-2016, average observed days after planting for anthesis was 48 in contrast to 52 days simulated. For maturity, the average observed days after planting was 79 in comparison with 80 days simulated. For yield, the average observed grain was 2246 kg/ha, higher than simulated yield of 2157 kg/ha. To simulate future climate impact on wheat growth during the years 2040-2049, climate projections from Scenarios Network for Alaska + Arctic Planning were input, and average simulated values for Ingal were 50 dap for anthesis, 76 dap for maturity, and a yield of 2311 kg/ha. In the 2018, we intend to collect a platinum dataset for Ingal, AC Intrepid, and Ingal x AC Intrepidd, at two sites, Palmer and Fairbanks. This dataset will be used to separately calibrate DSSAT. Results will be compared with the limited dataset calibration. Yield and maturity are the targeted characteristics for evaluation.

Prospector: An Android application for NIRS-based phenotyping of cassava quality traits

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More than 500 million people across Latin America and Africa rely on cassava as a major source of calories, many of them smallholder farmers. The dry matter content of the starchy root is an important quality trait for both growers and consumers. Breeders routinely phenotype this trait using timeconsuming and laborious methods such as estimation with specific gravity or by transporting samples to another location for oven-drying. Near infra-red spectroscopy (NIRS) has been shown to be highly predictive of dry matter in cassava roots, but most spectrometers are prohibitively expensive and/or immobile, requiring the same amount of effort as the oven-drying method. We investigated the use of the SCiO, a handheld, Bluetooth-connected NIR spectrometer, for field-based dry matter prediction in cassava root. Calibration, validation, and test sets were assembled using oven-dried measurements paired with SCiO scans of roots of diverse clones from two major cassava breeding programs, IITA in Nigeria and NaCRRI in Uganda. Spectral data were preprocessed for smoothing and noise reduction, and prediction models were developed using partial least squares regression. The presented preliminary results suggest that mobility and connectedness of this spectrometer allow for field-based collection of spectral data with a smartphone for accurate dry matter content prediction, a step that can be easily integrated into the existing harvesting workflow of cassava breeding programs. Further work will involve the addition of samples to calibration sets to improve dry matter prediction models. These and other models developed with the SCiO will be packaged into an app and incorporated into the PhenoApps suite of Android applications for plant phenotyping.

Fine Mapping of the Hazelnut S-Locus

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The European hazelnut (*Corylus avellana L*.) is a diploid (2n = 2x = 22) tree crop important to the economy of Oregon's Willamette Valley, where 99% of hazelnut production in the United States is located. C. avellana exhibits sporophytic self-incompatibility (SSI), controlled by a single S-locus with at least 33 unique alleles. The alleles exhibit codominance in the stigma and dominance or codominance in the pollen. SSI is present in many families and is understood best at the molecular level in Brassica. However, Brassica gene sequences have not proven useful for investigations in Corylus. With new genomic tools available for the study of hazelnut, including a new Pacific Biosciences reference genome for 'Jefferson', more progress can be made toward the goal of the identification of the genetic determinants of SSI. A search of the PacBio contigs in the S-locus region identified 2,722 di-nucleotide repeats, excluding those that only contained A's and T's. When sequences of seven cultivars were aligned with the 'Jefferson' reference, 708 showed clear polymorphism in number of repeats and had conserved flanking regions. 161 primer pairs were designed, and 52 were polymorphic when DNA of 24 cultivars was amplified and separated on agarose gels. These markers were characterized and 19 were discarded as being monomorphic or not useful for mapping, leaving 33 markers to be screened against a mapping population (OSU 252.146 x OSU 414.062) of 138 individuals and added to the linkage map. Further steps will involve screening these markers on a population of 150 S-locus recombinants with known S-alleles and the incorporation of SNPs from GBS and ddRAD-Seq to the linkage map.

# Results from the Peanut Genome Initiative and the Impacts on Cultivar Development

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The Peanut Genome Initiative (PGI) was a five year international effort which began in 2012. The objectives were to sequence the peanut genome and develop and apply new genomic technologies to peanut science. A large part of the funding came from the U.S. peanut industry. Their primary goal was to develop marker-assisted selection (MAS) methodologies that lead to improved cultivars. Peanut is an allotetraploid with a very large genome. One of the first accomplishment of the PGI was to sequence the genomes of the two progenitor diploid species of peanut. Recently, the sequence of the cultivated species was completed. To develop genetic markers for MAS, several structured populations were developed, genotyped, and phenotyped. Molecular markers have been developed for several economically important traits and are being implemented in breeding programs. This is having a great impact on the efficiency and effectiveness of peanut cultivar development.

# GENETIC DISSECTION OF FORAGE QUALITY IN A WORLD CORE POPULATION OF SPRING 2-ROW BARLEY

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Forage quality is where plant breeding meets animal nutrition. Even a 1% increase in forage digestibility substantially increases the average daily weight gain of livestock and results in reduced inputs for the farmer and rancher alike. Despite its importance, much remains unclear about the genetics controlling forage nutritional quality in barley or the quality of publically available germplasm resources.

A germplasm resource of particular interest is the Barley World Core (BWC) held by the National Small Grains Collection (NSGC). As part of the Triticeae CAP, the BWC population was created to capture the full genetic diversity of the NSGC and was genotyped with a genome-wide, 9k SNP-chip panel.

A set of the most genetically diverse, spring 2-row lines were selected from the BWC for a genome-wide association study to map forage quality and to assess the BWC's potential to contribute novel positive alleles.

The selected lines were phenotyped in Bozeman, MT, USA to measure economically important forage quality traits under both irrigated and dryland conditions. Based on the forage quality field results, an association analysis was performed and lines with positive characteristics selected for introgression into the barley forage breeding program.

From the association analysis, novel QTLs for forage digestibility, forage yield, grain yield and forage harvest date were discovered and the genotype by environment interactions explored with a mixed linear model.

Evaluation of PD resistant wild grapevine accessions against a hypervirulent Xylella fastidosa strain

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Pierce's Disease (PD), a disease caused by the xylem-limited bacteria *Xylella fastidiosa* (*Xf*), has been an ongoing problem for the past few decades in viticulture, causing an economic loss of \$100 million every year just in California. The PD Resistance Grapevine Breeding Program at UC Davis discovered *PdR1*, a single dominant locus found in a resistant accession from Monterrey, Mexico (Krivanek et al. 2006. Theor Appl Genet. DOI: 10.1007/s00122-006-0214-5). The *PdR1* locus has been successfully introgressed into different *Vitis vinifera* varieties while still holding strong resistance to common wildtype *Xf*. Moreover, different PD resistant accessions have been identified to have different sources of resistance from *PdR1*.

A mutated strain of Xf was recently developed for research purposes. The Xf mutant, deficient in a secreted protease PrtA required for biofilm formation, was recently shown to be hypervirulent (Gouran et al. 2017. Nat Sci Rep. DOI:10.1038/srep31098). This hypervirulent Xf strain (prtA<sup>-</sup>) exhibits reduced cell length and hypermobility in grapevines, leading to an early onset of PD symptoms. However, it is currently unknown how resistant grapevines will respond to this strain.

In this work, we investigate the effects of the *prtA*<sup>-</sup> strain in PD resistant accessions. We are phenotyping at four different time points using the Cane Maturation Index (CMI) and Leaf Scorch-Leaf Loss (LS-LL) Index. We are also quantifying bacterial titers using ELISA and quantitative-PCR from cane tissue. These results will provide insight into the mechanism of action of *PdR1* and other possible sources of resistance as well as the resistance robustness under a the *Xf prtA*<sup>-</sup> strain.

## A Spinach Genome for the Next Generation of Breeders

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Spinach is a leafy vegetable which is a rich source of vitamins and micronutrients which has become an increasingly important part of many diets as shown by the increased world-wide production over the past couple decades. Next generation sequencing technologies have exponentially increased the number of species for which genome sequence is available. While technologies with long-read sequencing has become increasingly utilized to develop high-quality genome sequences, most publicly available genome sequences are still predominately in draft stage composed of a high-number of contigs and many of which are not anchored into chromosome scale assemblies. Here we report the completion of the first publicly available spinach genome using long-read sequencing. Sequencing-based maps generated from a genetic mapping population were utilized along with mate pair sequencing to generate pseudomolecules in which the N80 falls into the six spinach chromosomes. Annotation produced 34,878 gene models of which 1,004 are annotated as resistance genes. A highly contiguous and anchored genome will allow for enhanced capabilities of future studies for the next generation of spinach breeders.

# Breeding *Euphorbia lagascae* for large-scale production of vernolic acid, an important industrial epoxidized ester

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Development of plant based sources of vernolic acid would provide Ontario farmers with a new market diversification opportunity and industrial manufacturers with a source of renewable, environmentally friendly chemical feedstock. Previous research focused on developing Euphorbia lagascae as a source of vernolic acid, a naturally occurring epoxidized ester that can directly substitute for the artificially epoxidized esters derived from petroleum, soy or linseed oil. The seeds oil of Euphorbia lagascae contains substantial amounts of vernolic acid, which has a number of industrial uses. Recent agronomic research by our group demonstrated that E.Lagascae can grow well in southern Ontario and that the seeds can be harvested using conventional equipments. Average seed yields for Euphorbia grown in 2015 at the Simcoe Research Station were 30g/plant at a stand density of 20000 plants/ha.With an average oil content of 50%, of which 60% is vernolic acid, this translates into a theoretical yield of 180 kg ha-1 of pure vernolic acid. The first objective of this study is to improve the germination rate of field sown seed of Euphorbia lagascae through conventional breeding techniques. The second objective is to determine the genetic control and physiological basis of germination ability in E. lagascae including dormancy factors. To break seed dormancy, seeds will be subjected to eight treatments. Five wild type Plant Introductions (PI) with good germination ability but with a pod shattering phenotype, will be crossed with a non-shattering EMS generated mutant line (EU006). The resulting F1 plants will be selfed to develop five F2 populations in which inheritance of the germination ability and level of pod shattering will be studied. Further development of this crop through plant breeding to improve germination rate may provide substantial economic benefit to Ontario farmers by offering them a new oilseed crop as a source of renewable epoxidized ester for industrial markets.

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Genomic selection for nutritional traits and cooking time in common bean (*Phaseolus vulgaris*L.) using Genotyping by Sequencing

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Common bean (*Phaseolus vulgarisL.*) is an important source of minerals and protein in the human diet. However, breeding for seed compositional traits is costly and labor intensive. Another trait important to consumers is cooking time (CT). Nutritional composition and cooking time may be good candidate traits for genomic selection as they are quantitatively inherited and difficult to measure.. In the present study, we describe the initial assessment of the predictive ability of a genomic selection model for cooking time, protein, and zinc concentration across 206 common bean lines of the Andean diversity panel using single nucleotide polymorphism (SNP) markers identified by Genotyping by Sequencing. Overall, prediction accuracy increased when the training population size was also increased. The prediction accuracy ranged from 0.31 (CT) to 0.55 (seed Zn concentration). Although the accuracies were not high, it is important to note that these accuracies were higher for the extreme phenotypes (top and bottom 10%), reaching prediction accuracies of 0.76 for seed zinc concentration. The above results indicate that genomic selection has potential use for traits that are difficult to routinely integrate in common bean breeding programs due to the costs and/or efforts required to quantify them.

Genetic Architecture Within the Elite Cornbelt Dent Germplasm Pool

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Additive genetic variation is key for plant breeders to continue to make genetic progress. The elite Cornbelt Dent germplasm pool traces back to 7 key founder lines, is highly stratified into 3 main heterotic patterns, and has generally remained closed to influxes of new genetic diversity. As part of the Genomes to Fields Initiative, this project examines the consequences that over 70 years of genetic improvement have had on the elite Cornbelt Dent germplasm pool. Specifically, to dissect genetic architecture underlying agronomic performance in maize across climatically and geographically diverse environments over 2 growing seasons, 2016 and 2017. Using important off-PVP inbred lines and secondgeneration lines derived from the off-PVP lines, three subsets of lines were identified based on maturity: early maturity set, intermediate maturity set, and late maturity set. Each set consisted of 5-6 Stiff Stalk inbred lines and 16-17 non-Stiff Stalk inbred lines, that were mated using a North Carolina (NC) mating Design II to generate approximately 96 hybrids for each maturity set. The combined analyses suggest that the elite Cornbelt germplasm pool still has additive genetic variation present for grain yield, but that it is only present in the non-Stiff Stalk portion of the germplasm pool. We found no evidence in any of the maturity sets of the presence of additive genetic variation in the Stiff Stalk germplasm pool for grain yield.

Genetic basis of synergistic and antagonistic interactions between qDTY<sub>12.1</sub> and genetic background of IR64 as inferred from transcriptomic data

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qDTY<sub>12.1</sub> is a major drought QTL in rice, responsible for significant gains in yield under drought stress. Differential gain in yield under drought stress was reported by IRRI in near isogenic lines of IR64, introgressed with  $qDTY_{12.1}$ . Our hypothesis is that genes of  $qDTY_{12.1}$  interacts synergistically or antagonistically with genes of genetic backgrounds or unwanted introgressions in IR64 resulting in an enhancing or dragging effect of qDTY<sub>12.1</sub>. To understand genetic basis of these positive or negative interactions, we compared transcriptomic profiles of four lines; recipient parent IR64, donor of qDTY<sub>12.1</sub> Way Rarem and two introgression lines of qDTY12.1 in IR64 background with contrasting yield under drought; high yielding (HY) and low yielding (LY), generated at vegetative, booting and grain filling stage in control and drought condition. Preliminary results show that 18 out of 33 genes within the boundaries of gDTY<sub>12.1</sub> are expressed in all four lines. Scattered distribution of 18 genes over whole region of QTL indicates requirement of whole qDTY<sub>12.1</sub> for explaining its genetic variation. One candidate gene out of 18 genes has been selected for detailed analysis. It's highly induced expression only at the booting stage only in HY line under both non-stress and stress, in contrast to high expression in LY line only in drought stress, suggests its vital role in HY line even before drought hits the plant. Literature as well as co-expression database analysis showed that the gene of interest interact with genes related to flower development and cytokinin pathway. Hierarchical clustering of these genes based on expression data showed that gene of interest tightly co-cluster with genes in HY line possessing contrasting expression in LY line. Biological function analysis of these co-clustered genes shows their key role in improving plant efficiency through enhanced plant growth and development. Further experimentation needed to validate these results.

# Genome Wide Association Studies of Root System Architecture in Barley

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Plant root system is responsible for anchoring of plant in the soil as well as acquisition and absorption of water and plant nutrients for productivity. Genetic studies into barley root system architecture traits using seedling roots are few. Identification of SNP associated with root system architecture traits would enable the selection of barley genotypes with better root architecture that might help to improve barley productivity. In the present study, an association study panel of 284 barley genotypes was phenotyped for seedling root architecture traits using the germination paper-based moisture replacement system, image capture units, and root-image processing software. We have identified several significant SNP associated with root system architecture traits. The result will greatly improve our understanding of the genetics of root system architecture traits in barley. In addition, SNPs associated with these traits could be applied in breeding programs for marker assisted and genomic selection of seedling root traits to improve barley.

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# DISCOVERY OF MAJOR QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO*FUSARIUM OXYSPORUM f.sp.* BATATAS IN SWEETPOTATO USING GENOTYPING BY SEQUENCING

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Breeding for disease resistance in sweetpotato (*Ipomoea batatas*) has traditionally been performed using conventional assays to determine susceptibility or resistance to various pathogens. Though the crop is generally known for its robustness against many abiotic and biotic stresses, these pressures maintain a steady foothold in the minds of breeders and farmers. As climate change threatens to radically shift the intensity of these different forces, sweetpotato breeders must develop quicker and more efficient means to evaluate upcoming varieties to keep pace.

One of the biotic pressures new lines are tested against is *Fusarium oxysporum* f.sp. *batatas*, a wilt inducing fungus capable of drastically reducing yields in highly susceptible varieties. While the current impact of *F.* oxysporum f.sp. *batatas* has been relegated to a small nuisance via development of resistant varieties, this scenario could quickly change. To prepare for this possible future we seek to develop methods through this study to rapidly detect resistance to *F. oxysporum* f.sp. *batatas*.

This research builds upon our previous work to detect single nucleotide polymorphisms (SNPs) associated with quantitative trait loci (QTL) for key processing traits in our DC mapping population. The mapping population began as a cross between 'DM04-0001' and 'Covington,' resulting in 454 genotypes. The progeny from these parents, chosen for their significantly different processing characteristics, has exhibited a normal distribution between the parents. We believe this will aid us in detecting QTLs through the association of SNPs generated via genotyping by sequencing runs we have already performed. During Summer 2017 we began the first three reps, set up using randomized complete block design. During Summer 2018 an additional four reps will be completed in the same manner for further statistical integrity. Using the information gathered here we hope to apply what we learn to aid in the development of future breeding populations.

The Effectiveness of Per Se Selection for Plant and Ear Height in Maize

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Constant improvement of yield potential in staple food crops, such as maize (Zea mays) is critical to meeting the food demands of a growing worldwide population. Corn is most commonly bred using double haploids. This technology allows for the rapid creation of completely homozygous inbred breeding populations in a short time frame. Double haploids are grown and corn breeders select inbreds to be tested as hybrids based on the phenotypes expressed in that growing season, therefore many inbreds are discarded before they are ever tested as a hybrid. This research focused on the effectiveness of per se selection of double haploids compared to the hybrid phenotypic expression of random breeding populations. The primary traits of interest were plant and ear height. Approximately 2,500 double haploids from 36 random breeding populations were measured for per se plant and ear height at 6 locations in 2017. In addition these double haploids were also tested in 3 hybrid combinations (7,500 hybrids) with the same testers used on all double haploids. Hybrid plant and ear height data was collected at 6 locations for all 7,500 hybrids. These data were used to discard hybrids for unacceptable plant and ear height. The hybrid selections were compared to selections made on the random double haploid breeding populations. Breeder comments/discards were also compiled for the inbred appearance related to stature. This research determined how effective it is for breeders to discard double haploids based on the per se traits such as plant and ear height.

Identification of elite sources of fire blight resistance in apple (Malus × domestica Borkh.)

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Fire blight, a bacterial disease caused by Erwinia amylovora Burrill, can cause structural damage and tree death potentially resulting in substantial economic losses in apple. Many commercial cultivars are susceptible to fire blight and current management practices are unsustainable and/or not effective against all stages of the disease. Fire blight resistance is difficult to phenotype due to the erratic nature of the disease, the quantitative nature of resistance, and the impacts of tree vigor and environment on susceptibility. Breeding for resistance is potentially a sustainable and effective long-term solution. This study's objective was to determine levels of resistance to fire blight of cultivars and other important breeding parents of a pedigree-connected apple reference germplasm set. Multiple actively growing shoots of each tree (~3 trees/individuals) were inoculated with E. amylovora Ea153/na in 2016 (5 ×  $10^{8}$  CFU mL<sup>-1</sup>) and 2017 (1×  $10^{9}$  CFU mL<sup>-1</sup>) using a cut-leaf inoculation method. For each inoculated shoot, shoot length, healthy tissue length (in 2016), and lesion length (in 2017) were measured. From these measurements, the proportion of current season's shoot growth that was blighted (i.e. SLB) was calculated for each shoot. Disease severity for each shoot was rated based on the age of wood infected. In both years, wide variation in fire blight susceptibility was observed among individuals with responses ranging from resistant to highly susceptible. Individual means ranged from 0-100% SLB. Although some individuals appeared resistant in one year and susceptible in the other, most demonstrated similar levels of susceptibility/resistance in both years with a correlation between years for replication means of r=0.57 (p<0.001). Resistance and susceptibility information gained in this study will inform parental selection in the WSU apple breeding program and provide an updated comparison of fire blight susceptibility among cultivars to inform apple producers.

Endophytes in Mediterranean summer dormant tall fescue: determining what role they play <u>William Krogman<sup>1</sup></u>, Michael A. Trammell<sup>1</sup>, Erin Hubbard<sup>1</sup>, Carolyn A. Young<sup>1</sup>, E. Charles Brummer<sup>2</sup> <sup>1</sup>Noble Research Institute, Ardmore, USA. <sup>2</sup>University of California, Davis, USA

Tall fescue (Festuca arundinacea) is a successful forage grass that is prevalent throughout the eastern United States where its persistence is attributed to a symbiotic endophytic fungus (Epichloë spp.). However, much of this region is planted with endophyte-infected tall fescue that is toxic to grazing livestock. A recent release of summer active Continental tall fescue now includes naturally occurring endophyte strains (selected endophytes) as a value-added trait that retains plant persistence without the toxicity to grazing livestock. Mediterranean tall fescue persist in the hot and dry summers typical of the southern Great Plains utilizing summer dormancy as a survival mechanism, but the advantage of endophyte infection is unknown. We introduced various endophyte strains to Mediterranean tall fescue to evaluate plant persistence under grazing, harvesting, and drought conditions. Eight tall fescue cultivars were selected, six Mediterranean and two Continental lines, which were planted in isolations as endophyte-free (E-) and endophyte-infected (E+). In the fall of 2015, seed was produced and harvested from each set of clonal pairs to provide material for evaluation in the field. Seed was harvested from 2015 to 2017, with bulk seed weight showing no differences for seed produced between E+ and E- clonal pairs. Seeded sward plots established in 2015, 2016, and 2017 in Ardmore, OK, Lane, OK, and Vashti, TX and in several locations in California using clonal pair seed are currently under evaluation to determine the benefit of endophyte in Mediterranean tall fescue on persistence under both grazing and harvesting conditions. Preliminary data from Oklahoma and Texas from both grazing and harvesting trials showed a general decrease in overall stand percentage after two years and a low cold tolerance during establishment for Mediterranean tall fescue regardless of infection. Data will be collected from these trials for several more years in all locations.

Evaluating Marker Assisted Selection of a Quantitative Trait: Snow Mold Tolerance in Winter Wheat

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Plant breeding develops new cultivars by assessing performance in target environments and selecting plants with the greatest potential. Selecting for highly quantitative traits is complicated by the influence of environmental conditions, numerous genetic loci, and genotype-by-environment interactions. Using snow mold tolerance in winter wheat as a model, this study aims to determine the effectiveness of marker-assisted selection for quantitative traits by comparing selected and unselected populations of recombinant inbred lines derived from crosses between susceptible and tolerant parents. Genotype-byenvironment interactions were investigated in the unselected populations using the Finlay-Wilkinson regression, and quantitative trait loci (QTL) were detected and evaluated for impact singly and in combination. Additionally, a marker-selected sub-population was compared to its unselected counterpart and genomic prediction was evaluated as an alternative to marker-assisted selection for QTL. Finally, the populations were compared to investigate whether marker-assisted selection can be used for improvement of snow mold tolerance. Results indicated, however, that selection was unsuccessful, which is likely due to the challenges of rating such a highly quantitative trait that also requires highly specific environmental conditions for phenotype development. This knowledge can be used to improve breeding for snow mold tolerance and other quantitative traits by better leveraging methods of selection.
Peanut on the wild side - surprises of introgression with wild relatives

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The peanut crop suffers from numerous pests and diseases. Development and adoption of resistant varieties is the most cost efficient and effective way to control the spread of the disease and reduce yield losses. Wild species form a secondary gene pool, and provide a source of strong resistance alleles, but they have undesirable agronomic traits, such as small seeds and spreading habit, that are a disincentive to their use in breeding. The identification of genomic regions that harbor disease resistances in wild species is the first step in the implementation of marker assisted selection that can speed the introgression of wild disease resistances and the elimination of linkage drag. We have identified genome regions that control different components of rust, Late leaf spot and nematode resistances in populations developed using various *Arachis*species. In breeding, in some cases, desirable traits were quickly recovered with a few cycles of backcrosses and selection. However, using new, higher resolution genotyping methods uncovered unexpected genomic instability. These findings highlight new mechanisms of introduction of diversity to the peanut crop.

QTL analysis for improvement of seed quality characteristics in adzuki bean (*Vigna angularis* Willd.)*Christine Lee and K.P PaulsDepartment of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1* 

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Adzuki bean [Vigna angularis (Willd.)] is a high-value Asian legume crop which is grown in Ontario for export to Japan, primarily for the manufacture of red bean paste. Profit per acre for adzuki can be as high as \$790, compared to \$465 for identity preserved soybean. However, production of premium quality beans is vital for Ontario growers to remain competitive in the export market. Current Ontario production of adzuki relies mainly on one variety, 'Erimo', which has the appropriate starch particle size to impart the appropriate texture for high-quality bean paste, however has a small seed size and an undesirable dark seed coat colour. Improvements to these seed quality traits are needed to help increase the marketability of Ontario adzuki in the Japanese market. The objective of this research is to utilize single nucleotide polymorphism (SNP) markers to create a linkage map for traits related to the improvement of adzuki bean quality. A bi-parental  $F_3:F_4$  mapping population of 84 genotypes was created from a Erimo x Baoquin cross. Phenotype data will be collected from the F3:F4 population grown in Elora and Woodstock (2018). Seed size, seed coat colour, and starch particle size will be analyzed using a novel high-throughput 1KK imaging software, colourimetric, and scanning electron microscope methods, respectively. Genotyping by sequencing will be used to genotype the  $F_3$  individuals. SNP analysis will be conducted using the Fast-GBS pipeline and QTL analysis and mapping will be completed using MapQTL 6 and JoinMap 4. Preliminary results show segregation for both seed size and seed colour traits in addition to agronomic traits such as plant height, yield, and maturity in the population. Through the determination of the genetic contributions for important seed quality traits this research aims to help breeders to develop high-quality seed through marker-assisted selection.

Potato tuber composition: associating SNPs with metabolic features

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Potato (Solanum tuberosum) is a widely consumed crop where tuber composition is vitally important for producers and consumers alike. Potato tubers are rich sources of amino acids, vitamin C, and potassium but also contain toxic glycoalkaloids (herbivory deterrents) and other as yet unknown compounds. With so many potential breeding objectives, we used a non-targeted metabolomic profiling approach to examine water/methanol extracts of cooked potato tuber cores to maximize information recovered per assay. With this strategy, we identified 981 features that represent a mixture of primary metabolites, specialized metabolites and hydrolyzed fragments of abundant proteins. We chose to profile the SolCAP potato diversity panel so that we could apply GWAS to map loci associated with individual compounds. Using the GWASpoly package, we were able to associate 472 features with at least one SNP. An additive genetic model detected the most marker/trait associations, with 1009 SNPs and 301 features, while a duplex-dominant model detected the fewest (230 SNPs, 160 features). This dramatically increases the number of traits mapped by GWAS in the SolCAP panel. SNP markers were not uniformly distributed throughout the genome, instead being clustered on chromosomes 3, 7 and 8, with dozens of features associated with SNPs in several 2 Mbp hotspots. Of the 981 features we measured, 99 have some structural information. Of these, 67 were mapped by GWAS. Although ANOVA revealed that SNPs adjacent to the known GAME7 and GAME12 glycolakaloid biosynthetic genes explained some variation for levels of three glycoalkaloids (alpha-chaconine, beta-chaconine, and alpha-solamarine), the most significant SNPs detected by GWASpoly are on chromosomes 2, 7 and 8 and are not linked to any known glycolalkaloid genes. These SNPs offer new opportunities to reduce glycoalkaloids in breeding populations.

Development of newly synthesized amphidiploids and their genome composition

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Cultivar improvement for peanut is limited by the narrow genetic base of this species. In order to introduce new genetic resources into peanut breeding programs, crosses were made among A-genome wild peanut diploids species (male) with several B genome species (female). Nine new amphidiploids were established by colchicine treatment producing a total of 115 S0 seeds. Four out of the nine new amphidipoids were advanced to S1 generation yielding 824 seeds. The most productive amphidiploid hybrid was [A. ipaënsis KG37006 x A. correntina 9530]4x. Genotyping of the amphidiploids and their respective parental lines revealed frequent gene conversion between the parental alleles. Evidence suggests that most of the conversion occurred at the diploid stage. However, further gene conversion was observed in both the S0 and S1 generations of the new amphidiploids suggesting genome instability of these new materials. Therefore, these new genetic materials should not be treated as a homogenous bulk for breeding programs. However, crossing the new amphidiploids with cultivated peanut yielded viable F1 hybrid seeds which have the potential for introducing additional genetic variation into breeding populations and lead to improved lines for agronomic traits.

Rapeseed root architectures: from phenotypes to candidate genes

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Rapeseed/canola (Brassica napus L.) root system varied widely among the winter and spring growth habit types. A significant variation of various root trait between winter and spring types occurred between 40-60 days after seeding. Under drought, the root traits of winter type affected more compared to spring type. Genetic study on root vigor identified three major dominant genes with unknown many minor genes control the vigorous root system in rapeseed/canola. A genome-wide association study was conducted using 224 diversified B. napus germplasm accessions. The accessions were phenotyped for different root architectural traits both in greenhouse and field conditions during 2015 and 2016. A total of 37,500 and 30,262 SNP markers were used to detect marker trait association in the greenhouse and in the field studies, respectively. Fifty-two and thirty-one significant markers were identified at 0.01 percentile tail *P*-value cutoff for different root traits in the greenhouse and field, respectively. Multiple markers associated with different root traits were detected within a close physical distances on chromosome A01, A02, A04 and C03 referring a possible co-localization of the loci for different root traits. A total of 37 candidate genes related to root architectural traits were identified within the close proximity of different significant markers. Three of these markers, chrC03 12098594 (RL), chrA01\_8813067 (PRB), chrA04\_rand\_54410 (R1Dia), were identified within the candidate genes, P*alycoprotein 6 (PGP6)* for root hair elongation, *Tetraspanin 7 (TET7)* for root morphogenesis, ARABIDILLO-2 for lateral root development, respectively. Cryptochrome 2 (CRY2) for root development, Cyclin-dependent kinase 2;3 (CYCA2:3) for lateral root formation, and several other genes related to auxin activated signaling pathway, gibberellic acid mediated signaling pathway, cytokinin-activated signaling pathway, ethylene-activated signaling pathway, root meristem specification were identified. This is the first report on understanding the genetics and molecular basis of root system architecture in B. napus.

### Jeong Hyun LIM

maturation and grain size in rice

National Research Foundation of Korea(NRF), seoul, Korea, Republic of. CJ cheiljedang, seoul, Korea, Republic of

Functional analysis of Oryza sativa LATE ELONGATED HYPOCOTYL (OsLHY) in controlling of seed

In higher plants, a timing of flowering has important role for grain production, in which the optimal timing of flowering leads to increased grain yield. In rice, many flowering time-associated genes and circadian rhythm associated genes have been identified by plant scientists, but those functions are not clear yet. We have been screened to a various mutants of the flowering associated genes and the circadian rhythm associated genes, and we found an abnormal seed phenotype in oslhy mutant. OsLHY is a homologue gene of arabidopsis LHY and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), in which LHY and CCA1 has functional redundancy in arabidopsis, but OsLHY (OsLHY/CCA1) is just one gene in rice. OsLHY is circadian rhythme gene (highly expressed at ZT 0), but the function of OsLHY has not been reported yet. In field condition, oslhy mutant shows a various defected phenotype as semi-dwraf phenotype, low number of tillers, rolling leaf, and abnormal seed maturation phenotype. Interestingly, abnormal seed development phenotype of oslhy mutants can find from milky stage (about 10 days after heading), and a matured seed shows a brown color, small size, and low maturation rate as sug-1, sug-h, and pul mutant which are involved in starch bio-synthesis pathway. OsLHY is MYB-like transcription factor, and locates in nuclei. In micro-array analysis, transcripts level of various starch biosynthesis associated genes is decreased in oslhy mutant than WT, in which ISA1 (sug-1) and PUL expression level are significantly decreased in oslhy mutant. Taken together, we suggest that OsLHY positively regulates a transcriptional level of starch biosyntetic enzyme, and these regulation is closely involved in seed maturation mechanism in rice.

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Genetic analysis of young fruit resistance to Phytophthora capsici in cucumber

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Phytophthora fruit rot caused by Phytophthora capsici, a soil-borne oomycete pathogen, can be a devastating disease for cucumber production. As young fruit are especially susceptible, the objective of this work is to identify quantitative trait loci (QTLs) associated with young fruit resistance. A cucumber accession, PI 109483, was previously identified as a source of young fruit resistance and a resistant breeding line, MSU109483-53, was developed. Crosses were made between the susceptible pickling cucumber inbred line, Gy14, and two resistant MSU109483-53-derived lines: S₀ generation, B5, and doubled haploid, DH A4-3. In Summer 2017, F<sub>2</sub> progeny of Gy14 x B5 (n=400) along with parent lines and F<sub>1</sub> were screened in the field. To facilitate accurate phenotyping, plants were trellised to reduce contact with the fruit and soil. This reduced wounding due to removal of soil during the cleaning process and lessened possibility of contamination from other pathogens. Harvested fruits were sanitized with 1% bleach and placed in sealed trays to maintain high humidity and inoculated with P. capsici isolate Bartley's 1 ( $1 \times 10^4$  zoospores/mL). The normal distribution of disease scores for the F<sub>2</sub> population indicated that young fruit resistance is a quantitative trait. Based on the initial result, the top and bottom 12% of plants were selected. Selected plants were harvested three additional times and inoculated with an elevated pathogen concentration ( $5 \times 10^4$  zoospores/mL). Replicate harvests providing 5-52 fruits for each plant showed the reproducibility of the disease scoring and the accuracy of individual selection. The second population, 222  $F_2$  progeny of Gy14 x DH A4-3 along with the parents and F1, was screened in the greenhouse in Spring 2018; 7-45 fruit were tested per plant. For bulk segregant QTL-seq analysis, 10-15 resistant and susceptible individuals were selected from each F<sub>2</sub> population for DNA extraction and sequencing.

Quantitative Trait Loci Associated with Resistance to Soybean Cyst Nematode HG Type 2.5.7

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Soybean cyst nematode (SCN) is one of the most damaging pests of soybean (*Glycine max*) worldwide. Damage by SCN costs North American soybean growers well over \$1 billion each year. More than 95% of SCN-resistant cultivars in North America are derived from a single source, PI 88788, and widespread use of this source has led to the selection of virulent biotypes such as HG (Heterodera glycines) Type 2.5.7 that can overcome PI 88788 resistance. Therefore the main objectives of this study were to (1) identify and validate quantitative trait loci (QTL) that underlie resistance to SCN HG Type 2.5.7 isolate and (2) study the impact of SCN resistance on seed yield. To achieve the goals, two F<sub>4</sub>-derived recombinant inbred lines (RIL) populations were established from crosses between two high yielding Ontario-adapted elite soybeans, OAC Brooke and OAC Calypso, and cultivar LD07-3419, which carries SCN resistance genes derived from both PI 437654 and PI 88788. The RIL populations were screened for resistance to SCN HG Type 2.5.7 under greenhouse conditions and genotyped using genotype by sequencing (GBS) for identifying single nucleotide polymorphism (SNP) markers. In total, five SCN related QTL were identified and validated in the populations using multiple QTL mapping approach. No significant correlation was found between seed yield and the resistance to SCN or identified QTL in the RIL populations evaluated in two non-SCN-infested fields. The identified QTL can be used in marker-assisted selection to accelerate the development of SCN-resistant soybeans with new resistance genes.

Genetic variation for Goss's Wilt resistance in the short-season commercial maize germplasm pool.

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Goss's Wilt (*Clavibacter michiganensis* subsp. *nebraskensis*) is a bacterial disease of maize (*Zea mays*) that manifests itself predominately as a leaf blight. Recently, it has spread from the high plains of the United States and is considered an emerging threat to Ontario. Fungicides are ineffective at controlling Goss's Wilt; however, development of resistant hybrids may be an option for mitigating yield losses. Resistance is thought to be quantitative and controlled by 9-11 QTLs, making it more difficult to select for compared to single gene resistance. During the summer of 2017, 50 inbred lines were screened in Manitoba for resistance to a cocktail of 4 Goss's Wilt isolates. Disease severity ranged from resistant to highly susceptible. The 50 inbred lines represent 20 stiff stalk and 30 non-stiff stalk short-season inbred lines. The 50 inbred lines were mated using a North Carolina Design II to generate 600 hybrids. The 600 hybrids along with the 50 inbred lines are currently being screened in Manitoba for resistance and will be used to identify genomic regions associated with resistance to Goss's Wilt. In addition, we are investigating the possibility that the 50 inbred lines possess both single-gene and quantitative resistance. There are several families of lines that show extreme resistance responses, some members being highly resistance and some members being extremely susceptible. Several  $F_2$  populations derived from these lines are currently being tested to determine the mode of inheritance. Goss's Wilt may soon be in Ontario. Finding QTLs conferring quantitative resistance as well as gene-for-gene resistance will allow corn breeders to develop highly resistant varieties and prevent the disease from becoming prevalent in the region.

Utilizing wild germplasm of rice for increasing variability and carrying out Genome-wide association studies for productivity related traits

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Wild species are widely used to broaden the genetic base of agricultural crops. In this study, a set of 370 different accessions of O. rufipogon was collected from different geographical origins. Lines were genotyped with reduced representation sequencing methodology of Genotyping by Sequencing (GBS) approach. We identified 1.9 million SNPs, out of which ~59,000 SNPs were retained after filtering for missing value <0.10 and MAF < 0.05. Principal Component Analysis (PCA) revealed a weak population structure, with no discrete clusters. Analysis of genome-wide Linkage Disequilibrium was done using PopLDdecay program and LD decays in this population within 10Kb distance. Replicated phenotypic data was recorded in 2014 and 2015 for agronomically significant and productivity related traits, including plant height, culm thickness, panicle length, number of primary branches, grain length, grain width and hundred grain weight. Genome-wide Association Studies (GWAS) were conducted using FarmCPU program in R. GWAS results revealed a total of 41 SNPs to be significant at p-value < 10E-05 and 18 SNPs were significantly associated with more than one trait. Physical locations of 23 SNPs, coincided with previously annotated protein-coding genes. In order to incorporate useful alleles from wild species to elite background, backcrossing was done to generate a population of 10,632 BC<sub>2</sub>F<sub>1</sub>individuals and 128,000 BC<sub>1</sub>F<sub>2</sub>individuals, which belong to 35 different families. A small set of related lines from the backcross population will be used for genomic selection (GS). The genomic estimated breeding values (GEBVs) for each line will be estimated and next cycle of GS will be carried on by crossing the lines with best GEBVs.

Breeding for Fusarium basal rot resistance in short-day onions

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Fusarium basal rot (FBR), caused by a soil-borne fungus Fusarium oxysporum f. sp. cepae (FOC) is one of the most devastating diseases of onion worldwide. FBR disintegrates the compressed stem and is particularly damaging to the bulbs in storage, as the initial decay is difficult to detect. The development of resistant cultivars could be the best possible alternative over the conventional methods to control FBR, viz., crop rotation and soil fumigation, allowing farmers to utilize the same fields for multiple crops without the need for soil fumigation. In this study, a mature bulb screening method was used to evaluate selected populations of seven short-day Grano-type cultivars that originated from two different artificial inoculation mature bulb selection methods. Additionally, we sought to develop an objective method of scoring for more accurate disease quantification. Transversely-cut basal plates were inoculated with PDA plugs containing a uniform spore suspension of the virulent FOC isolate 'CSC-515'. FBR severity and incidence percentage were recorded for 20 randomly-selected bulbs after 21 days of incubation. Digital images of the infected basal plates were generated both using ambient light and by using an epifluorescent microscope that was fitted with UV light-emitting diodes and four long pass barrier filters. The advantage of using a spore inoculation as compared to a mycelium plug inoculation was realized in 2017, when a reduced disease severity and incidence was observed in FBR3, FBR1-2 and selected populations of the checks, all generated by the former method. A bright autofluorescence of the infected tissue illustrated that UV fluorescence imaging could be used effectively for objective disease quantification of FBR.

### Navigating the Maize of Short-Season Ancestry Laura Manerus, Dr. Elizabeth Lee, Dr. Andy Robinson University of Guelph, Guelph, Canada

Preliminary analyses on maturity stratified maize lines suggest that the germplasm pool which exhibits early flowering has broader ancestry than what was originally proposed. As part of a project done by Genomes to Fields (G2F) a series of yield trials were conducted using lines from recently expired patents (off-PVP) or their second-generation lines. The lines were also stratified for testing in the Early, Intermediate, and the Late G2F testing environments. The Early group is believed to have less genetic diversity due to extensive selection for earliness. However, the Early set exhibited the greatest genetic variation as a percentage of the total variation for grain yield, plant height, and days to silking and was the only set to have exhibited significant additive genetic variation in both the males and females for grain yield. This has led to the hypothesis, the modern early season germplasm has founder lines that are not part of the founders of the corn belt dent germplasm. A sample of short season germplasm inbred lines from North Dakota State University, Agiriculture and Agri-food Canada, early flowering off-PVP lines, and short-season University of Guelph lines derived from commercial hybrids will be used in this analysis. Genotype by sequence data will be filtered and formatted for use in STRUCTURE software which will give further information on the relationship to the 7 proposed founder lines and any other possible founders. Early STRUCTURE results show that the 7 proposed founders are absent in the clusters that make up a large ancestry and suggests that the short season germplasm has different founders.

QTLseqr: An R package for bulk segregant analysis with next-generation sequencing

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Since the early 1990's, Bulk Segregant Analysis (BSA) has been an extremely useful tool for rapidly identifying markers in a genomic region associated with a trait of interest. BSA is amenable to any type of codominant markers, including single nucleotide polymorphism (SNP) markers. This has allowed for the adaptation of this technology for use with next-generation sequencing (NGS) reads. SNPs detected in reads aligning to genomic regions closely linked to the trait should deviate from the expected ~50% representation observed in non-linked regions. In plant breeding research, the main pipeline used for BSA, termed QTL-seq, was developed and has been widely used in several crops for many traits. The QTL-seq pipeline has not been updated in several years and some software and version incompatibility issues have arisen. There has not been an R package released for this type of analysis further limiting widespread utilization of this otherwise well-designed pipeline. An alternate approach for evaluating statistical significance of QTL from NGS-BSA is based on a tricube-smoothed G statistic, however a software implementation was never developed or distributed. We present QTLsegr, an R package for NGS-BSA, that can perform both analysis methods. QTLseqr, can quickly import and filter SNP data from the Genome Analysis Tool Kit (GATK) pipeline, then calculate and plot SNP distributions, relative allele frequencies, the tricube-smoothed G values, as well as log<sub>10</sub>(p-values) allowing for easy identification of QTL regions. QTLseqr is currently available at https://github.com/bmansfeld/QTLseqr

Validation of Multiple Disease resistance loci in Maize using families derived from segment substitution lines

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Southern leaf blight (SLB), northern leaf blight (NLB), and gray leaf spot (GLS) caused by Cochliobolus heterostrophus, Setosphaeria turcica, and Cercospora zeae-maydis and Cercospora zeina respectively, are among the most important corn diseases worldwide. In a previous project, we identified loci underlying disease resistance in multiple disease resistant (MDR) lines by the creation of chromosome segment substitution line (CSSL) (BC3F4:5) populations in multiple disease susceptible (MDS) backgrounds. We were able to detect quantitative trait loci (QTL) for disease resistance for each disease: 36 for SLB; 16 for NLB; and 20 for GLS. Among these, 30 QTLs were associated with variation in resistance to a single disease, 17 to two diseases, and four to all three diseases. In this project, we aim to validate the 9 strongest putative QTLs for MDR discovered in the previous study. To do so, 13 lines that each carried at least one of the 9 strongest resistance alleles were selected to make 13 populations of F2:3 backcross families. Since most of the parent lines contained more than one resistance allele associated with disease resistance, we designed markers to assess the segregating of each of the identified resistance alleles; 30 markers in total. During the summer growing season of 2017, the 13 populations were assessed for resistance to SLB (Clayton NC) and GLS (Blacksburg VA and Andrews NC), on summer 2018 they will be assessed for NLB resistance. Moderate correlations between SLB and GLS were found and heritabilities for each disease were within expected ranges. At NAPB we will be showing our latest results regarding the validation of MDR QTLs.

Common QTLs Suggest that Metabolites Modulate Pest Resistance in Soybean <u>Evan McCoy</u><sup>1</sup>, Maria Ortega<sup>1</sup>, John All<sup>1</sup>, Zenglu Li<sup>1</sup>, Nikola Kovinich<sup>2</sup>, Wayne Parrott<sup>1</sup> <sup>1</sup>University of Georgia, Athens, USA. <sup>2</sup>West Virginia University, Morgantown, USA

Caterpillars are the major pest of soybean in many parts of the world. Therefore, soybean insect resistance QTLs (SIR-QTLs) from unadapted, but highly resistant plant introductions (PI) are being introgressed into elite soybean lines. The frequently used resistance sources, PI 229358 and PI 227687, carry four validated SIR-QTLs known as M, H, G, and E. However, additional sources are necessary for use in resistance management strategies. Towards that end, greenhouse assays were conducted on an unrelated but resistant soybean line, G00-3213, and its parents and grandparents. One parent, Boggs, which had not previously been tested for resistance, is the most likely source of the resistance found in G00-3213. Mapping in a population of Boggs x PI 494851 (susceptible) RILs identified SIR-QTLs N, O, and F. SIR-QTLs N and O were validated in an F<sub>2:3</sub> population of Boggs x Benning. With multiple SIR-QTLs now available, and QTL M already cloned, it is possible to formulate hypotheses on their mode of action. QTL M alters isoflavone metabolism, and most of the SIR-QTLs co-map with major enzymes in the phenylpropanoid pathway. Isoflavones have also been implicated in pathogen resistance, and the SIR-QTLs co-map with known QTLs for resistance to white mold and Phytophthora sojae. The possible role of isoflavones is therefore being tested using SIR-QTL isolines to look for differences in metabolite pools between infested and insect-free conditions. If the same compounds are responsible for insect and pathogen resistance, it may be possible to select for broad-spectrum resistance via leaf metabolite profiles.

Evaluating soybean ancestral cultivars for resistance to Southern Stem Canker (Diaporthe Aspalathi).

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Diaporthe aspalathi is the causative agent of Southern Soybean Stem Canker, which is a major disease in soybean production in the Southern United States. The disease can cause up to 80% yield loss. Soybean (Glycine Max) has a limited genetic base with 35 ancestral cultivars providing 95% of the genes found in modern soybeans. Evaluating these 35 cultivars for resistance to Southern Stem Canker could help understand the resistance that is seen in modern cultivates. The 51 genotypes, including the 35 ancestors, 6 known resistant cultivars, 4 susceptible checks, and 6 parents of mapping populations were evaluated in Griffin, GA using two strains of D. aspalathi (BL 3-15 and BL22). Genotypes were inoculated with *D. aspalathi* infected toothpicks method 4 weeks after planting. Plants were scored on scale of 1 to 5 with 1= no symptoms and 5 = premature plant death. The internodes of infected plants were counted to evaluate the spread up the stem of the plant. Thirty-three genotypes were identified as being resistant to southern stem canker, which included 6 known resistant lines, 25 ancestral cultivars, and 3 of the parents of the mapping populations. 18 genotypes were identified as susceptible, including the 4 susceptible checks and 3 parents of the mapping population. Resistance to southern stem canker was not significantly correlated to the maturity of the genotypes. Eighteen resistant and 7 susceptible genotypes were Northern germplasm (MG000 to IV), while 15 resistant and 11 susceptible genotypes were Southern germplasm (MGV to X). The evaluation of these genotypes provides understanding of the genetic diversity of soybean ancestors and selection of populations for fine mapping work.

Genomic prediction strategies for evaluating trait stability in a multi-parent hexaploid wheat population

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Genomic prediction in plants, animals, and humans is driven by an assortment of academic curiosity, entrepreneurial incentive, and altruistic ambition. Yet, the resulting collective advancements in biotechnology and statistics are limited by the polygenic, epigenetic, and epistatic factors that cause traits to express differentially in each unique environment and genetic background. Harnessing genomic prediction to realize genetic gain in agriculture will require approaches that are tailored to commercially relevant breeding populations, trait architectures and target environments. To explore new applications for genomic prediction in hexaploid wheat, we used a multi-parent inbred population with the genetic structure of a pre-breeding population where one elite cultivar was crossed to 26 diverse founder parents. The population was evaluated in three environments to collect phenotypes including yield, spectral reflectance indices, end-use-quality traits and stripe rust resistance. In addition to using Finlay-Wilkinson regression to detect environmentally stable and plastic QTL, we used genomic prediction to study genetic influence on trait behavior. Multiple cross-validation strategies were explored including a new approach that acts as a method for comparing trait penetrance in each half-sib family. The conclusions drawn from these experiments demonstrate the fluidity of phenotypic expression in populations with the familial structure of applied wheat breeding programs.

Identification of quantitative trait loci associated with cold tolerance in an interspecific chickpea recombinant inbred line population.

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Fall planted chickpea (Cicer arietinum L.) yields are often double that of spring sown chickpea in regions with Mediterranean climates that have mild winters. However, winter kill can limit the productivity of fall-sown chickpea. Developing cold tolerant chickpea would allow expansion of the current geographic range where chickpea is grown, and also improve productivity. The objective of this study was to map the quantitative trait loci (QTL) associated with cold tolerance in chickpea. An interspecific recombinant inbred line (RIL) population of 129 lines derived from a cross between ICC 4958, a cold sensitive desi type (C. arietinum) and PI 489777, a cold tolerant wild relative (C. reticulatum) was used for this study. The RILs were evaluated for cold tolerance in the field for four years and two experiments under controlled conditions. Assessment of cold tolerance in the field was based on leaf damage scores recorded in early-spring and assessment under controlled conditions was based on leaf damage recorded seven days after freezing tests. The population was genotyped using genotyping-bysequencing (GBS). Three significant QTL were found on linkage group (LG) 1B, 3 and 8. QTL on LG 3 and 8 were consistent in six environments with LOD score ranges of 5.16 – 15.11 and 5.68 – 23.96 respectively. The QTL on LG 3 explained 7.17 – 34.6% of the variance across all environments, while the LG 8 QTL explained 11.5 – 48.4% phenotypic variance in all environments. The QTL associated SNP markers have potential for breeding increased cold tolerance into cultivated chickpea.

#### Assessing Genome Size in Abelia by Flow Cytometry Indicates Ploidy Differences Among Species

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*Abelia* is genus of flowering woody shrubs with high ornamental and landscape potential. Despite the diversity of the genus and its 174 years of use as an ornamental, only a few species and cultivars are available commercially and these are mostly sports of *Abelia xgrandiflora*. Also, no genetic information is available and ploidy levels are unknown. Genome sizes were estimated in Abelia as a first approach to understand *Abelia* ploidy levels. Genome sizes were estimated in the species *Abelia chinensis* (two accessions), *Abelia engleriana*, *Abelia xgrandiflora*, and *Abelia serrata*, the hybrids 99-1-1 (*A. chinensis* x *A.* 'Edward Goucher', 99-6-7 (*A.* 'Edward Goucher' x *A. chinensis*), and 99-6-11 (*A.* 'Edward Goucher' x *A. chinensis*), and 99-6-11 (*A.* 'Edward Goucher' x *A. chinensis*), and the cultivars *A.* 'Edward Goucher' (*A. chinensis* x *A. schumannii*), *A.* 'Francis Mason' (sport of *A. xgrandiflora*) and *A.* 'Raspberry Profusion' (*A.* 'Edward Goucher' x *A. chinensis*). A CyFlow © Ploidy Analyser (Sysmex <sup>TM</sup>) was used with *Rapanus sativus* 'Saxa' and *Glycine max* 'Midori giant' as internal standards. *A. engleriana*, *A. xgrandiflora*, *A. serrata*, *A.* 'Francis Mason' and one accession of *A. chinensis* and the hybrids 99-1-1, 99-6-7, and 99-6-11, as well as the cultivars *A.* 'Edward Goucher' and *A.* 'Raspberry Profusion' have a genome size two times larger, approximately 1.92 pg of DNA. This may indicate the presence of polyploidy in the genus.

# QTL Discovery for Resistance to Charcoal Rot Caused by *Macrophomina phaseolina* in Strawberry *Fragaria x ananassa*

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Since the phase-out of the soil fumigant methyl bromide in 2005, soil-borne pathogens have risen on strawberry farms throughout the U.S. Of these, Macrophomina phaseolina, causal agent of charcoal rot, is currently the most problematic. Symptoms become apparent when strawberry plants are stressed and temperatures are warm. These criteria describe a typical strawberry growing season both in Florida and California - the two leading strawberry producing states in the U.S. In fact, M. phaseolina has been described as the most important disease concern for the strawberry industry due to its rapid spread throughout the major strawberry growing regions of the U.S. As yet, no post-fumigation chemical controls are effective for managing the spread of *M. phaseolina*. The preferred strategy for control would be the introgression of genetic resistance. No fully-resistant cultivars have been released to date, though some show moderate resistance. The present study investigates the genetic architecture of resistance to charcoal rot in strawberry (Fragaria x ananassa), using the University of Florida's elite strawberry breeding germplasm. Clonal replicates of more than 1,100 seedlings from 73 full-sib families obtained from crosses made among 62 parents were inoculated in two consecutive growing seasons in central Florida. Plant collapse was recorded weekly throughout each growing season. Sub-genome specific single nucleotide polymorphism markers were mapped in the octoploid strawberry genome, and FlexQTL<sup>™</sup> software was used to perform genome-wide, pedigree-based QTL analyses. Two large-effect quantitative trait loci, one on linkage group 2A and a second on linkage group 4B, together accounted for most of the genetic variation for resistance. We investigated the genetic mode of action for these two loci separately and together, and identified haplotypes associated with resistance. The consistent phenotypic effects of these loci across trials and numerous genetic backgrounds make them highly desirable targets for genetic improvement of resistance against charcoal rot.

Grapevine Fanleaf Virus Resistance Screening in a 101–14 x Rotundifolia Population

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Grapevine fanleaf virus (GFLV) causes fanleaf degeneration, one of the most economically severe diseases affecting grapevines worldwide. The disease can result in crop losses of up to 80% by greatly reducing fruit set and causing formation of 'shot berries,' or very small seedless berries. *Muscadinia rotundifolia*, a North American grape species, has previously been shown as a valuable source of GFLV resistance. The objective of this work is to quantify GFLV resistance in the progeny from a cross between the susceptible commercial rootstock 101-14 Mgt. and *M. rotundifolia* cv. Trayshed and to study the inheritance of this trait. For GFLV inoculation, 2-node cuttings of GFLV-infected *Vitis vinifera* cv. Cabernet Sauvignon were grafted onto hardwood cuttings from 32 individuals of the 101-14 x Trayshed population. Five months after grafting and growing in the greenhouse, the roots of the surviving plants were assayed for GFLV using RT-qPCR. Here we present preliminary results of GFLV concentrations in different genotypes. This work provides insight into the inheritance of GFLV resistance from *M. rotundifolia* and continues our progress into developing new rootstocks to ameliorate the effects of GFLV.

Genes showing distinct haplotypes and differential gene expression response to *Sclerotinia sclerotiorum* in partially resistant and susceptible soybean cultivars.

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Sclerotinia stem rot (SSR), also known as white mold (WM) is an important fungal disease that affects soybean (Glycine max [L.] Merr.) production in cool and moist environments. The Northern United States and Canada are regions where the climate is conducive to the development of the disease leading to significant damage to yield and grain quality. A number of quantitative trait loci (QTL) have been reported for partial resistance to SSR as complete resistance has not yet been identified. Modern DNA technologies such as high-throughput genotyping (via GBS or SNP arrays) have been used to dissect complex disease resistance traits using genome-wide association studies (GWAS). The objective of our study was to combine two innovative approaches, a genome-wide gene expression technology using RNA sequencing (RNA-Seq) with GWAS and QTL studies to dissect the genetics of resistance to WM as one of the most complex diseases in soybean. Comparative analysis of results from a number of GWAS/QTL studies and RNA-Seq data in 32 soybean genotypes with contrasting SSR resistance phenotypes was conducted. Promising genomic regions that harbor genes showing differential expression and located within previously described GWAS/QTL regions have been identified. Some of the candidate genes and regions exhibited differences in haplotypes and gene expression between the partially resistant and susceptible cultivars. These candidate genes will be useful for improving our understanding of the genetic basis for WM resistance and identifying significant genomic regions for future breeding efforts.

Genome-wide association study for *Exserohilum turcicum* severity using SNP markers in field corn and popcorn tropical inbreed lines

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Several diseases affect maize (Zea mays L.) production around the world. Among those diseases the northern corn leaf blight caused by *Exserohilum turcicum* is one of great importance that can provide losses between 50% in field corn and 90% and popcorn. The quantitative trait loci (QTL) mapping is an excellent tool for breeders on the identification of candidate genes in breeding programs. Genome-wide association study has been used as a powerful tool for identification of candidate genes using single nucleotide polymorphism markers (SNPs). Therefore, the aim of this work was to identify putative candidate genes associated to resistance to northern corn leaf blight in maize tropical inbreed lines from various backgrounds. The research was done in State University of Maringá, Brazil. The panel was assembled with a total of 165 inbreed lines (92 field corn and 73 popcorn genotypes), representing the main hybrids and populations in Brazil. Lines was phenotyped in one environment in a randomized complete block design with two replicates, where the experimental plots consisted of a single row in a 6m with 0,9m between rows, totalized of 28 plants per plot. The severity scale was scores 1-7, with severities of 0.5, 1.0, 2.5, 6.5, 15.5, 30 and 54%. Genome-wide association analysis was performed using the phenotypic data and a set of 267,525 SNPs with minor allele frequency >5% generated using genotyping by sequencing from the panel lines. Two SNPs were significantly associated with Exserohilum turcicum. The first one is located inside of the gene GRMZM2G049141 that is related with ubiquitin ligase complex that is responsible trichome cell morphogenesis and jasmonate signaling. The jasmonate acid is related to defense responses against insects and necrotrophic diseases and several parts of the plant. The second one is located less than 1cM to the gene GRMZM2G179358 that has no protein related.

Characterization of Red Clover Accessions from the National Plant Genetic Resource Center in the Southern Great Plains

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Red clover (*Trifolium pratense* L.) is an important legume species for grazing and hay pastures in the southern USA and is often inter-seeded into grass pastures to improve forage quality and yield. However, the persistence of red clover as a component of many pastures in the southern USA can be reduced due to disease, insect pressure or poor grazing tolerance. During the fall of 2016, the forage breeding group at the Noble Research Institute established a trial near Gene Autrey, OK, USA, in order to evaluate PI accessions from the National Plant Genetic Resource Center (NPGRC) that may have a high breeding value for livestock and hay producers in the southern Great Plains. A total of 30 PI accessions representing 13 originating countries, 20 synthetic populations from the Noble Research Institute's forage breeding program and 6 commercial check cultivars were included in the trial. Red clover plants were transplanted into an established pasture of Texoma MaxQ II tall fescue [*Festuca arundinaceum* (Schreb.) S.J. Darbyshire]. Trial design is a randomized complete block design with 4 replications. Various trait and grazing data were collected in 2017. Data collection will continue into the 2018 field season. Material selected will be integrated into our red clover breeding program for the development of a persistent cultivar for the southern USA.

Identification and characterization of fast-neutron induced mutations underlying altered seed composition phenotypes for improvement of soybean seed composition

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Soybean is a major component of feed, food, and fuel. Protein, oil, amino acids, fatty acids, and sugars in seeds affect the efficacy of soybean. Improving soybean seed composition is an essential goal for soybean breeders. However, negative relationships between protein content and yield as well as protein and oil contents present challenges for soybean breeders improving both seed quality and yield. Fast neutron radiation introduces genomic deletions, duplications, and translocations resulting in altered plant phenotypes. Two elite soybean lines were irradiated with fast neutrons and screened for altered seed composition using near-infrared (NIR) spectroscopy. Twenty-three lines with altered seed composition were selected based on NIR data from five environments over two years and yield tested at five locations. Protein contents of these mutants were 0.6 to 2.9% higher than those of the parents, and high sucrose mutants had 0.6 to 3.7% higher sucrose than the parents across environments. Comparative genomic hybridization (CGH) was performed on four of these mutants and identified putative mutation regions resulting in these phenotypes. Whole genome sequencing (WGS) of two mutants was conducted to assist in marker development at genomic mutation regions. Two F<sub>2:3</sub> populations were developed from two seed composition mutants to determine association between genomic changes and altered phenotypes. Bulked segregant analysis of these populations using the SoySNP50K Infinium BeadChip identified deletions on chromosomes 12 and 16 putatively responsible for elevated protein and sucrose contents, respectively. Deletion regions were confirmed with both CGH and WGS. Mutants with altered seed composition are a new resource for gene characterization in soybean and provide elite breeding materials for development of varieties with improved seed composition.

Application of Bayesian Statistics to Breeding of Drought Tolerant Tall Fescue

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Population mean and variance are important parameters in the study of plant breeding. The comparison of these parameters is a question that plant breeders faced with all the time. There are various approaches for estimation and inference for population variances. However, these approaches are either sensitive to violation of assumptions, or involve too much of calculation; therefore, are not widely adopted by the plant breeding community. A widespread practice for estimation and inference of population mean is analysis of variance(ANOVA). Despite of popularity, this procedure is not necessarily the best approach, and requires certain assumptions that tend to be overlooked. This paper briefly discussed the principle of this traditional approach and that of Bayesian statistics, with the intention to promote the application of a more flexible and realistic model for analysis of population phenotypic data. A comparison of the two approaches was provided using a sample dataset collected from a drought tolerance study on tall fescue (*Festuca arundinacea*).

Identifying the genetic bases for wheat improvement with RNA-Seq SNP discovery and genotyping

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The ability to analyze whole transcriptome has opened new horizons for understanding crop improvement. Wheat yields, plant architecture, and other traits have improved due to breeding, and a central objective is to identify the genetic bases of these improvements. Here, we used novel RNA-Seq analyses to assist with this objective. First, we identify single nucleotide polymorphisms (SNPs) between the modern Canadian wheat cultivar, Stettler, and its progenitor, Red Fife, from RNA-Seq reads. Although the cultivars are closely related, SNP discovery identifies more than 22,000 SNPs. Many SNPs are between homologous loci, and few SNPs are between homoeologous or paralogous loci. Second, we use RNA-Seq data to genotype a population of 156 doubled haploid (DH) lines. The unambiguous SNP genotyping allows precise mapping of trait-contributing loci to the complex wheat genome. Major, pleiotropic loci as well as many small effect loci have driven wheat improvement. Although plant height has a continuous distribution within the DH population, a dwarfing gene, originating from Stettler, has a strong effect on plant height and several other traits. Many other favorable alleles that originate from Stettler have smaller effects and contribute to leaf area, spikelet length, and protein concentration.

### RNA-Seq Analysis of Floral Bud Response to Freeze Treatments in Southern Highbush Blueberry CV. 'O'Neal'

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Blueberry production encompasses wide geographic and climatic ranges, spanning Canada to Mexico. Grown in warmer climates, southern highbush blueberries (SHB) are less cold tolerant and more precocious compared to northern highbush blueberries. The native germplasm of SHB have decreased cold hardiness and chill hour requirement. While these traits expand the seasonal window of blueberry production, SHB are also more susceptible spring frosts. Our objective is to determine differentially expressed genes involved in cold tolerance in relation to bud development and recovery from freeze events. Buds of a prominent cultivar in North Carolina, 'O'Neal' (SHB), were collected prior to and following budbreak, treated with either freezing (-12 °C) or nonfreezing (+4 °C) conditions, and recovered at +4 °C for varying durations (1-day and 1-week). RNA was extracted and mRNA-Seq libraries were paired-end sequenced. Reads were trimmed and aligned to a transcriptome assembly of 'O'Neal'. A total of 180,487 contigs were functionally annotated with BLAST2GO (V5) software package. Bioconductor package DeSeq2 was implemented in Trinity (V2.5.1) to identify differentially expressed unigenes between tissue, freeze treatment, and recovery. DESeq2 identified 8,611 DEGs of which 2,482 (43%) had BLAST hits and 1,900 (33%) were annotated. DESeq2 ( $\log_2$  fold change >|2|;  $p \le 0.05$ ) determined 1,913 DEGs related to tissue, 3,810 DEGs related to temperature, and 4,440 DEGs related to recovery. In a pairwise comparison of DEGs between treatments, there was greater upregulation in tissue post-budbreak compared to tissue prior to budbreak; freeze-treated buds had greater upregulation compared to non-freeze treated buds; and buds with 1-day recovery had greater upregulation compared to those with 1-week recovery. Understanding cold-related DEGs will aid in the development of a marker assisted breeding program for cold resistant high yielding blueberry selections.

#### Effect of Cultivar Mixtures on Yield in Common Bean

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A number of studies have demonstrated that cultivar mixture cropping has benefits over monoculture cropping in controlling disease, increasing water use efficiency and increasing yield stability. The mixture effects are hypothesized to be driven by beneficial interactions between the plants and their environments that are enhanced by diversity in the genetic, physiological, structural and developmental characteristics of the component varieties. The objective of this study is to determine the agronomic (including crop yield) and ecosystem effects of increasing crop diversity by using common bean cultivar mixtures instead of monocultures in cropping systems. To meet this objective, matched replicated trials were established at Elora (ERS) and Woodstock (WRS) with four diverse bean cultivars (two white, black and light red kidney), in pure stands and binary mixtures (alternate rows and random mixtures). Crop data were collected for the conventional above ground traits including: plant density, flowering, maturity, plant height, SPAD, harvestability, seed weight, yield, anthracnose resistance and nitrogen content in mature seeds. Based on the positive experience with intercropping systems in other crops, we were expecting yield increase, greater yield stability and better disease resistance in bean cultivar mixtures. Significant differences among four genotypes and their mixtures were identified in both locations for all analyzed traits. Beans evaluated at the WRS matured earlier and had higher yields compared to the mixtures grown at the ERS. The results from the first year showed that there is no yield penalty to using bean cultivar mixtures instead of monocultures. The research has the potential to provide a theoretical basis for the use of precision agriculture tools to plant fields with mixtures instead of monocultures. It could lead to greater in-field diversity in the crop and in the above and below ground ecosystems that might provide greater buffering capacity and resiliency to the cropping system.

Understanding Compositional Changes During the Alkaline Cooking of Maize (*Zea mays* L.) in chip production to Mitigate Acrylamide Formation

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Acrylamide is a carcinogen that was discovered in cooked starchy foods in 2002. The discovery of acrylamide led to significant efforts to reduce its content in foods due to consumer health concerns. Acrylamide is a by-product formed from the Maillard reaction when reducing sugars and free amino acids react at high temperatures. The high temperature processing of maize during chip production results in the formation of acrylamide, which has traditionally been reduced with asparaginase. However, the high cost of asparaginase coupled with the growing consumer demand for processed foods with natural ingredients has necessitated the need for alternative acrylamide reduction strategies. One strategy is to breed for maize with low free amino acids and reducing sugars. It is also critical to understand how the maize chip processing influences the formation of free amino acids and reducing sugars. The goal of this project is to understand how levels of reducing sugars and free amino acids change throughout the cooking process in maize chip production. To meet this goal, 120 inbred lines with extreme values based on NIR equations for total sugars, starch, and nitrogen content were selected for this research. Compositional attributes including starch, reducing sugar, protein, and amino acid content are being measured on raw kernels from the selected lines. Additionally, these samples are being processed through a small-scale bench top cooking protocol with subsamples taken at key steps during cooking. Quantitative measurements of reducing sugars and free amino acids for each subsample will be obtained for these genotypes using ion exchange chromatography, while total starch and total protein will be analyzed with the Megazyme total starch kit and LECO nitrogen analyzer. Elucidating how levels of these substrates change throughout the cooking process will provide valuable information for future breeding efforts of food grade maize.

#### Onekk: A High Throughput Seed Phenotyping Android Application

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Seed morphology includes important characteristics that affect the end uses of crops. Rapidly measuring morphological phenotypes and utilizing this information for indirect selection within breeding programs could lead to increased yields, improved end use quality, and cultivars targeted for specific uses. A high throughput phenotyping app to measure and count seeds, such as OneKK, could be useful for multiple crops only if it provides prompt and accurate measurements. By utilizing a smartphone or tablet camera, OneKK makes rapid seed phenotyping easily accessible, portable, and cost effective. OneKK uses an established algorithm to calculate length and width and a novel watershed algorithmic approach to estimate the number of seeds within the image - even when seeds are immediately adjacent. To validate the accuracy of OneKK, a subset of seeds from common crops were manually measured for length and width. The same samples were processed using OneKK to measure the average length, average width, and sample count. A high correlation between both morphological measurements and seed counts was observed, and measurements from OneKK were collected considerably faster. To validate the utility of OneKK for genomic research, the Synthetic/Opata doubled haploid wheat population was utilized for QTL mapping. Seed measurements taken with OneKK were successfully used to map a QTL using Composite Interval Mapping. A newly-updated version of OneKK is publicly available and will provide plant breeding and genetics research programs with a powerful tool for both selection and genomic analysis.

## Mapping race-specific black spot disease resistance in tetraploid landscape garden roses (Rosa x hybrida).

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Black spot is one of the most serious foliar diseases of landscape roses. The leaf defoliation arising from black spot reduces the ornamental value and marketability of the plants. It also severely weakens the plants, resulting in a greater likelihood of loss over winter. Genetic resistance to black spot is present in the germplasm, but is in the form of gene-for-gene resistance. With at least 11 prevalent races of black spot present, black spot resistance is a challenge to breed for making it a good candidate for markerassisted selection. The world's first high-density, integrated SNP map of roses of all 56 rose chromosomes was created using GBS on 300 progeny from the cross '564' x 'Gentle Giant'. Resistance to black spot races 10 and 8 was mapped in this population. Recently we have created a second SNP-based map using 250 progeny from the cross 'CA60' x 'Singing in The Rain' where we again mapped black spot race 10 resistance, but have also mapped resistance to race 5 and resistance to three additional newly identified races (isolates VSK04, Hy-12.4, VOTB17-1). Phenotyping used an improved detached leaf assay and race-specific resistance genes mapped to the same location. Research is on-going to generate a consensus map in order to identify markers associated with black spot resistance that would be widely applicable in rose breeding programs. A whole genome sequence of diploid Rosa multiflora was generated to assist the rose breeding program with developing markers that are diagnostic across multiple breeding populations.

Targeted recombination to increase genetic gain in self-pollinated species

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Targeted recombination refers to inducing or selecting for a recombination event at genomic positions that maximize genetic gain. A previous study indicated that targeted recombination could double the rate of genetic gain for yield in maize (Zea mays L.), a cross-pollinated crop for which historical genetic gains have been large. Our objectives were to determine: 1) if targeted recombination can sufficiently improve predicted gains in self-pollinated species, and 2) if prospective gains from targeted recombination vary across crops, populations and traits. Genomewide marker effects were estimated from previously published genotypic and phenotypic data on 21 biparental populations of barley (Hordeum vulgare L.), pea (Pisum sativum L.), soybean [Glycine max (L.) Merr.], and wheat (Triticum *aestivum* L.). The predicted performance of a doubled haploid with one or two targeted recombinations per chromosome was calculated. Our results showed that having one or two targeted recombinations per chromosome doubled (on average) predicted gains for all traits in 22 populations of the four selfpollinated crops. The predicted gains varied among traits and populations. For most traits and populations, having targeted recombination on less than half of all the chromosomes led to the same or higher predicted gains than nontargeted recombination. Together with previous findings in maize, our results suggested that targeted recombination could double genetic gains in both self- and crosspollinated crops.

Genome-wide association studies (GWAS) for grain number per unit area, kernel weight, and grain yield in soft red winter wheat germplasm

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Characterizing traits beyond grain yield (GY) per se will enable the understanding a framework of yieldrelated traits that mechanistically explain grain yield. A diverse panel of 270 soft red winter wheat (SRWW) elite lines, adapted to Eastern US and Canada, from the Triticeae Coordinated Agricultural Project (TCAP) initiative were planted in an incomplete randomized block design in the fall 2016 and again in fall 2017. The TCAP panel has 37K genomic-scale single nucleotide polymorphism (SNP) data aligned with the current wheat reference genome (IWGSC\_WGAv1.0) to perform genome-wide association studies (GWAS). Our goal is to mechanistically explain grain yield by its yield component traits and identify loci and candidate genes involved in grain number per unit area (GN) and kernel weight (KW). For GWAS, we use 37K genome SNP markers that were produced using alignment of next generation sequencing reads to the current wheat reference genome (IWGSC WGAv1.0). The data from the first year of study revealed wide ranges for GY, GN, and KW, with heritability of 0.82, 0.49, and 0.72, respectively. GWAS revealed marker-trait associations (MTAs) for GN, KW, and GY. We identified loci on chromosome 7A, 7B, and 7D for GN and GY. Many MTAs for GN and GY were co-localized on a region on chromosome 7D, with favorable alleles present only in 21 elite lines. An MTA was identified for KW on chromosome 4B. The genomic regions around the identified sites were searched for candidate genes. Potential candidate genes such as remorin, a gene on chromosome 7D that influences carbohydrate transport and grain setting, or a gene from small auxin upregulated RNA (SAUR) family on chromosome 4B that regulates plant growth and development, are two examples of promising genes identified from the first year analysis of this study. Currently, we are phenotyping the germplasm for the second year.

Chromosome substitution line: useful genetic resource for targeted exploitation of the beneficial alleles from wild and unadapted germplasm in cotton

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The germplasm of Upland cotton (Gossypim hirsutum) has been eroded seriously during the domestication process by exploitation of a few elite lines over time. Previous attempts by cotton breeders documented that gene retention and genetic recombinations are very difficult to broaden the germplasm base of Upland cotton from alien species. To overcome barriers for effective introgression, we have developed a number of alien chromosome substitution (CS) lines from G. barbadense (CS-B), G. mustelinum (CS-M) and G. tomentosum (CS-T). Most of the CS lines are nearly isogenic to the inbred 'Texas Marker-1' (TM-1, G. hirsutum). Comparative analysis of the CS lines have provided means to identify and associate important traits with specific substituted chromosome or chromosome segments. We released a set of 17 disomic CS-B lines through hypoaneuploid-based backcrossing in a near-isogenic genetic background of TM-1 line. We have also released near-isogenic chromosomespecific recombinant inbred lines (CS-RILs) by crossing specific CS lines with the common recurrent, inbred TM-1. Analysis of CS-RILs for traits and SSR and/or SNP markers enable markers to be associated with genes with marked effects on important fiber traits. By creating and analyzing various types of CSderived near isogenic families, the chromosome-specific genetic effects were determined for various agronomic and fiber properties. Results have validated CS-B11sh, CS-B16, and CS-B17 harboring RKN, FOV1 and FOV4 resistance genes associated with SSR markers, respectively. Preliminary results based on various morpho-physiological traits suggest that CS-T04 has higher tolerance against drought and low temperature. CS-B25 was associated with improved fiber traits including high fiber strength, length and low micronaire. The results of gene expression data from CS-B25 have provided new insight into the molecular mechanisms of fiber development during the fiber elongation stage and detected several novel candidate genes that may be utilized in cotton fiber quality improvement.

FaRCa1: A major locus for resistance to anthracnose fruit rot in strawberry

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The cultivated strawberry (*Fragaria*  $\times$  *ananassa*) is an allo-octoploid species ( $2n=8\times=56$ ) that belongs to the Rosaceae family. Warm and wet conditions in central Florida provide the fungus Colletotrichum acutatum with the ideal environment to cause considerable economic losses in commercial strawberry production. Plants can be contaminated in nurseries where symptoms are not visible. In the field, C. acutatum spores are dispersed by irrigation, rain, insects, and workers. In ripening fruit, lesions are firm, dark brown and sunken and are known as anthracnose fruit rot (AFR). Genetic resistance to AFR is a very desirable control strategy. Previous European research showed that a single dominant gene (Rca2) controlled high levels of resistance and minor genes control intermediate levels of resistance to C. acutatum pathogenicity group 2. The objective of this research is to examine the genetic architecture of resistance to Florida AFR isolates in University of Florida breeding germplasm. In the first year, 33 full-sib families resulting from crosses between parents with different levels of resistance to AFR were generated. Offspring, parents and control cultivars were planted in a randomized complete block design with four clonal replications. Inoculation was conducted using a  $5 \times 10^3$  conidia/ml solution of three isolates of C. acutatum using a hand-pump sprayer. Phenotypes were scored weekly as incidence of AFR, and genotyping was performed using the IStraw35 Affymetrix Axiom single-nucleotide polymorphism array. A pedigree-based QTL analysis was performed using FlexQTL software. A major locus (FaRCa1) was discovered that was distinct from the previously described *Rca2*. The locus *FaRCa1* explains 50.7% of the total phenotypic variation in 2016-17. The same locus was detected in 2017-18. These results will form the basis for new DNA tests to aid the development of new cultivars combining improved resistance with commercial yield and quality.
Genetic Architecture And Relationship Between Seed Free And Protein Bound Amino Acid Pools In Maize

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Seeds are a major source of protein in human and livestock diets. However, the seeds of major staple crops such as maize, soybeans, and rice are deficient in several essential amino acids (EAA). Failure to consume sufficient levels of EAA per day leads to severe malnutrition, even if one's calories requirements are met. So far, limited successes have been achieved in improving the seeds amino acids in either classical or transgenic approaches since these traits are tightly regulated. In fact, seeds actively rebalance their composition even when severe perturbation to the seed protein content and composition is introduced by transgenic measured. However, the genetic identity of this mechanism is not clear. Nevertheless, seed amino acid composition displays extensive natural variation which can be exploited to uncover how mother nature genetically regulates these essential traits. To this end, we characterize the natural variation of these traits across 282 genetically diverse lines of maize using advance high-throughput analytical methods and associate it with the natural genetic variation using genome wide association study (GWAS). More specifically, we focus on the characterization of two functional pools of amino acid and their potential interplay: the free amino acid pool, which comprises  $\sim$ 5% of the total amino acid in seeds and the protein bound amino acid pool, which comprises  $\sim$  95% of the total amino acid in seeds. Our results show that the two functional pools have distinct genetic basis and that their interplay is genetically driven as well. Uncovering the genetic basis of both amino acid functional pools as well as their interplay may open new avenues to seeds biofortification.

Genotyping-by-sequencing and its application to asparagus breeding program

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Objectives of asparagus breeding programs worldwide include improved yield, quality, longevity and disease resistance. Understanding the genetic architecture of these traits as well as those that support the development of hybrids, such as tissue culture response and andromonoecy, the production of berries on male plants, would facilitate cultivar development. Limited mapping studies have been published in asparagus, however, methodologies such as genotyping-by-sequencing (GBS) can accelerate genetic analyses and cultivar development. GBS is a simple system for developing molecular marker maps and quantitative trait loci identification in a range of plant species including those with complex genomes such as asparagus. It also allows diversity analysis at a depth not possible with previous methods. This poster will present some preliminary data for association mapping and diversity analysis in asparagus.

#### A Genome-Wide Association Study of Fusarium Head Blight Resistance in Winter Wheat

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Fusarium head blight (FHB) caused by Fusarium graminearum is one of the most detrimental diseases of wheat (Triticum aestivum L.). Fusarium damaged kernels and mycotoxin contamination in yield causes significant economic losses. Inadequate disease control strategies render breeding for FHB resistant wheat varieties as a favorable approach. The objective of this research is to identify genomic regions associated with FHB resistance in a Canadian Winter Wheat Diversity Panel (n=450) and to develop genomic selection models for FHB resistance breeding. The diversity panel was phenotyped in two FHB nurseries in Ontario in 2017. The disease incidence ranged from 10% to 100% with an average of 65%. At 21days after inoculation, disease severity ranged from 7% to 100%, with an average of 25%. The diversity panel was genotyped using Illumina iSelect wheat 90K SNP beadchip, which revealed >50K polymorphic markers providing dense coverage across all chromosomes. Phylogenetic trees, Principal Component Analysis and STRUCTURE analysis eluded to the presence of population structure in the panel. After correcting for population structure, genome-wide association studies identified regions associated with FHB incidence on chromosome 5A, with FHB severity on chromosomes 1A, 2B, and 5A, and with FHB Index on chromosomes 2A, 2B, 5A, and 6A. This research is expected to further develop a source of wheat germplasm, as well as optimise a genomic selection breeding strategy to support the development of varieties with enhanced FHB resistance.

Detecting alien introgressions and chromosomal aberrations using genotyping-by-sequencing <u>Narinder Singh</u>, John Raupp, Dal-Hoe Koo, Bernd Friebe, Bikram Gill, Jesse Poland Wheat Genetics Resource Center, Kansas State University, Manhattan, KS, USA

Genotyping-by-sequencing (GBS) has been utilized in, but not limited to, genetic mapping, genome-wide association studies, population genomic studies, and genomic selection. High-throughput nature and multiplexing capability, coupled with low cost of GBS make it an appropriate tool for research. We present a different array of applications of GBS to detect chromosomal aberrations and alien introgressions in wheat. Because of the reducing genetic diversity in the cultivated crop species, using their wild relatives and progenitors presents a great opportunity for crop improvement. In wide hybridization, traditionally, alien introgressions are detected in the progeny using fluorescent-in-situhybridization (FISH) or genomic-in-situ-hybridization (GISH) under the microscope. Although effective, FISH and GISH are low throughput and tedious, which discourage researchers from assaying the germplasm on a large scale. SSRs, ESTs and STSs have been used to make this process a little easier, however, the use of these markers systems depend on their pre-existing knowledge and availability, therefore, rendering them useless for many species. Implementing GBS to detect alien introgressions is easy and effective and does not require prior marker knowledge, and in contrast to traditional methods, can allow the researchers to visualize and estimate introgression sizes with accuracy. We implemented GBS to detect Aegilops geniculata and Rye (Secale cerealeL.) introgressions in the wheat background. Utilizing GBS for detecting alien introgressions will facilitate the use of alien germplasm for crop breeding and ultimately result in faster improvement. Extending its applications, we also applied GBS to detect chromosomal aberrations, such as deletions, monosomic, ditelo, nullisomic and multisomic wheat lines. We were not only able to confirm the known deletions in wheat deletion stocks but also detected new uncharacterized deletions. This shows the flexibility of GBS in a variety of applications, which allow the researchers to achieve faster results than established traditional methods with comparable or even better accuracy.

High-throughput phenotyping enabled genetic dissection of ground cover in wheat.

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Early establishment of ground cover is a highly desired selection target in wheat breeding. The genetic variation underpinning ground cover should be extensively explored to develop stress adapted cultivars. The next-generation phenotyping can provide the required scale and throughput to screen a large number of lines necessary to dissect the genetic architecture of ground cover trait in wheat. We performed phenotypic evaluation for pre-booting ground-cover of CIMMYT elite spring wheat panel with unmanned aerial system (UAS) or drone. The visual ratings and aerial imagery were collected throughout the growing season at three diverse agroecological environments in India. The digital ground cover was estimated through the supervised image classification procedures on multi-spectral images. The image-derived estimate of ground cover was compared with the visual ratings. The repeatability/broad-sense heritability of the digital ratings showed significant variation across locations and time-points ( $H^2$ =0.54-0.84). On average, the digital estimate of ground-cover showed 20% higher repeatability than visual ratings at all environments and time-points. Significant genetic correlations between visual and digital ground-cover further established the strong association of both measurement ratings. Through the strong validation experiments, we demonstrate the considerable power and scalable potential of HTP in dissecting the genetic basis of ground cover in wheat breeding and genetic studies. This information is being integrated with environmental data and dynamic phenological traits to gain insights into the genetic underpinnings of this important growth trait.

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### Targeted improvement of canola meal protein quality <u>Kenny So</u>, Mohamed Elhiti, Ralph Kowatsch, Robert Duncan University of Manitoba, Winnipeg, Canada

The adoption of plant proteins as a sustainable alternative to animal protein may be a partial solution to food security. Large quantities of canola seed are crushed for oil annually, resulting in equally large quantities of protein-rich canola meal. Canola meal protein primarily consists of the seed storage proteins cruciferin and napin, each of which possesses unique functional properties for food processing. Breeding efforts in canola have traditionally focused on the improvement of oil content and oil quality. As canola is primarily an oilseed crop, improvements to meal protein cannot be made at the expense of oil. Equilibrium between seed storage proteins has been reported in crop species whereby the total seed protein content remains static, but the composition of the protein pool can fluctuate. We hypothesize that a reduction in cruciferin accumulation may result in the improvement of napin content. To knockdown cruciferin expression, we simultaneous target two cruciferin genes for editing using the CRISPR/Cas9 system. Preliminary results suggest a reduction of cruciferin content within the total seed protein of transgenic lines. No obvious morphological phenotypes were observed across the different lines and their progeny. The mutation arising from CRISPR/Cas9 editing will be identified and plants will be functionally characterized. Vector-free plants may then be incorporated into canola breeding programs. The successful manipulation of seed storage proteins by genome editing enables the improvement of canola meal protein quality in a targeted manner without reducing the value of the crop as an oilseed.

Zea mays spectral reflectances at broad wavebands are heritable and predict offspring biomass

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Maize genetic variation for spectral indices has been correlated with genetic variation for biomass, and various spectral profiles and biomass have shown differential contributions from parents towards offspring. Although traditional spectral indices, such as NDRE, are widely used to estimate traits, recent work indicates that visible and far-red regions across the spectra also correlate with traits. Here, we evaluate the utility of partial least squares regression (PLSR) models using leaf spectral reflectance first derivatives (SR; 400-800nm) to predict early seedling biomass (DWPP) from a set of maize hybrids planted in six different environments under early and late planting conditions. Within environments, PLSR models for the estimation of DWPP explained a greater proportion of genetic variation on average (30%) when compared to NDRE (4%). Heavily weighted wavelengths involved in predicting DWPP were within the red edge, a region associated with genetic differences for chlorophyll, and ~400nm, a region associated with carotenoids. We evaluated hybrids in half-sib families produced by a NCII mating design and found parental inbred GCA effects on red edge reflectances were moderate and significantly correlated with hybrid DWPP. We conclude that although reflectance wavelengths within known indices most strongly contribute to PLSR estimates of DWPP, the integration of various, diverse wavelengths across the spectra is more informative for predicting DWPP. Furthermore, inbred spectral GCA effects correlate with hybrid DWPP, enabling the use of inbred SR attributes to predict hybrid DWPP.

QTL mapping of PI 494182, a new source of resistance to soybean cyst nematode

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Soybean cyst nematode (SCN), *Heterodera glycines*, is the most devastating pest of soybean (*Glycine max*) globally. Plant resistance is the most durable and yield-efficient strategy to fight this pathogen. Currently, the parental line PI 88788 is widely used and nearly the sole source of resistance against SCN in commercialized cultivars. Unfortunately, field reports show that this source of resistance is being overcome in many areas. With the goal of diversifying the sources of resistance to SCN, we studied PI 494182. This soybean introduction from Japan has previously shown good resistance to HG-type 2.5.7, the most prevalent virulent type of SCN in North America. Using Genotyping by Sequencing (GBS) on a RIL population of 150 lines (PI 494182 x Costaud), we produced a genetic map that allowed us to identify QTLs associated with resistance to SCN. We report five QTLs that were previously identified in different resistant lines, such as Peking and Hartwig. The higher resolution conferred by GBS has allowed the accurate positioning of these QTLs on the physical map. Furthermore, re-sequencing data will provide additional information to identify candidate genes putatively involved in resistance to SCN in PI 494182. Overall, this work will give breeders new tools, genetic markers, to diversify sources of resistance against SCN.

Evaluating the radish-derived Rfo introgression in *Brassica napus* L. following recurrent full-sib selection.

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The Ogu-INRA CMS system is a cytological variant of the radish- (Raphanus sativus L.) derived ogu CMS system introduced through interspecific introgression into Brassica napus L. In this system, there are three different lines: A-line (male-sterile), B-line (male-fertile maintainer line for the respective A-line) and R-line (male-fertile restorer line). An A-line and an R-line are crossed to obtain fertile F1 seed used in commercial production. The restorers (R-lines) contain an introgression that is associated with poor agronomic performance due to a large undesired segment of the radish chromosome that was introgressed along with the *Rfo* gene. The objective of this research was to test the hypothesis that multiple cycles of intermating will result in R-lines with improved agronomic performance and a shorter radish introgression. A base population was developed by designing R-line by R-line crosses. Twelve plants from each initial cross were grown and chain-crossed at random, without selection, other than the presence of the *Rfo* SCAR marker. Three intermating crossing cycles ( $C_0$ ,  $C_1$  and  $C_2$ ) were completed and each was selfed twice in order to compare all populations at the C<sub>0</sub>S<sub>2</sub>, C<sub>1</sub>S<sub>2</sub> and C<sub>2</sub>S<sub>2</sub>. Total pod number, seeds per pod, a visual pod rating, thousand seed weight and yield were evaluated. Improvements for all traits were found at  $C_0$  and  $C_1$  when compared to the best parent. Individual families from two of the crosses showed a yield increase of over 78 % from the best parent. This suggests that improvements in yield components can be obtained from restorer by restorer crosses. Improved genotypes will be tested using radish gene-based markers to track the introgression size.

Analysis of training population for genomic selection in *Brassica napusL*.

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Genomic selection (GS) has become a promising tool in plant breeding. Genomic selection can be considered an advanced form of marker-assisted selection (MAS) and was first used for improving the rate of genetic gain in animal breeding. However, MAS focuses on major marker effects, while GS attempts to evaluate all gene effects along the entire genome on traits of interest for each genotype. Using GS, breeders can make selections with predicted performance of a breeding population instead of the observed performance. This can aid in shortening the breeding cycle, reduce field evaluation costs and ultimately lead to rapid cultivar release. This research attempted to examine different factors that affect the prediction accuracy in GS, such as the training population (TP) component and size, model choice, marker density, gene/marker effects and trait heritability. The TP consisted of 92 parents, including 31 B lines (maintainers) and 61 R lines (restorers) within the oquINRA CMS pollination control system. The TP was phenotyped in the field in 2016 and 2017 field seasons at multiple locations for both agronomic traits and seed quality traits. The TP was sequenced using the *Brassica*60K Illumina Infinium™ SNP genotyping array at Agriculture and Agri-Food Canada in Saskatoon. Following genotyping, 3,480 markers identified the relatedness amongst the TP. Preliminary genomic selection was conducted using Tassel 5.0 based on 2016 field trial at Glenlea Manitoba as well as the selected 3,480 markers, which revealed that different traits impacted prediction accuracy. Seed yield had the highest prediction accuracy of 0.21, followed by flowering time (0.12), plant height (0.07) and glucosinolates (-0.04). This study will offer a solid reference for identifying the appropriate method to evaluate TP effects on prediction accuracy.

### Genetic Improvement of Sorghum Biomass Composition <u>Patrick Sweet</u>, Mitch Tuinstra, Cliff Weil Purdue University, West Lafayette, USA

Plant biomass is an abundant source of renewable energy, but the efficiency of its conversion into liquid fuels is low. One reason for this inefficiency is the recalcitrance of biomass. This recalcitrance is due to the complex and rigid structure of the plant cell wall. A better understanding of the genes effecting cell wall composition in bioenergy crops could improve feedstock quality and increase conversion efficiency. To identify genetic loci associated with biomass quality traits we utilized genome-wide association studies (GWAS) in an 840-line *Sorghum* diversity panel. We identified several QTL from these GWAS including those for lignin and polysaccharide composition. As a follow up to GWAS, a causative polymorphism for lignin monomer composition was identified within the coding region of a homolog of phenylalanine ammonia-lyase (PAL). This SNP turns out to induce a premature stop codon in PAL resulting in an increased S/G ratio. As an additional follow up to GWAS we demonstrate how these QTL can be used to improve genomic selection models for biomass quality.

Taking the Trial to the People: A New Approach for Vegetable Variety Evaluation in Tennessee

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Variety trials are important as a means to assess regional adaptation of pre-release and released material and as an opportunity to increase consumer brand awareness. While statewide variety testing programs for agronomic crops are ubiquitous, trialing of vegetable lines is often limited in geographic scope by labor intensive management requirements. A collaborative approach between researchers and citizens may provide an opportunity to address this challenge. In 2017, a home garden variety trial program was established which used a citizen scientist approach to evaluate vegetable variety performance in Tennessee. In the pilot year, the trial program was limited to 15 counties. Forty-eight participants put out 247 trials across the following categories: beans, carrots, sweet corn, cucumber, melon, pumpkin, and summer squash. Varieties were selected from sources available to home gardeners and included both tried and true varieties and promising new varieties. Each trial consisted of a paired comparison and participation was limited to seven trials per person. Participants grew and evaluated trials in their own garden, noting which variety had better germination, plant health, first fruit, yield, attractiveness, and flavor. They also gave a performance rating on a scale of 1 to 10 and selected which variety they preferred and which they would recommend to other gardeners. Results were compiled in an annual report available at tiny.utk.edu/hgvt. For each trial, the following data were presented: percentage of participants who preferred or recommended each variety, mean rating that variety received across all evaluations, traits identified as significantly superior in one variety versus the other variety. Significant differences were calculated using Fisher's exact test with an alpha level of 0.1. These trials provide a unique opportunity for breeders to assess released and pre-release varieties for performance over a broad geographical range, evaluate flavor preference across a diverse audience, and build brand awareness.

Subgenome-specific mapping of QTL for powdery mildew resistance in cultivated strawberry, *Fragaria ×ananassa*.

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Powdery mildew (PM) caused by Podosphaera aphanis is one of the major fungal diseases of cultivated strawberry worldwide. Commercial strawberry cultivars have widely variable resistance to PM, and very few have high levels of resistance. Resistance to PM is considered a complex trait influenced by both genetic and environmental factors that present challenges to phenotypic evaluation in the field. To precisely detect loci associated with PM resistance, six connected full-sib families from crosses among nine parents ranging from highly resistant to susceptible were evaluated in a field trial at the University of Florida GCREC during 2017-2018 season. The study relied on natural inoculum and was evaluated four times at one-week intervals beginning at the earliest symptoms of disease. Each parental genotype and seedling was clonally replicated three times and arranged in a RCB design. Severity of PM was evaluated based on mycelial leaf coverage rated from zero to six, corresponding to 0-1% and 95-100% coverage, respectively. Genotyping was performed with the IStraw35k Axiom® SNP array. The mean disease scores for ratings one through four were 2.11, 3.49, 3.12 and 2.14, respectively. Simultaneous pedigree-based QTL detection in all families using FlexQTL<sup>™</sup> and 5,336 polymorphic and genetically mapped SNPs, revealed a major QTL located on linkage group 7C that was consistently observed across rating dates. The second disease rating showed the highest probability for the QTL, with interval length of 19 cM. Estimated narrow sense heritability was 0.30, with 22.5% of phenotypic variance explained by the LG 7C QTL. In summary, an apparent major QTL associated with PM resistance was discovered and appears to be associated with the highest levels of resistance present in University of Florida strawberry germplasm. If repeatable in future studies, the moderate effects of this QTL would provide substantial utility in resistance breeding.

Downregulation of DGAT1Genes in Soybean Increases Seed Protein Concentration

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Triacylglycerols (TAGs) are the main constituents of storage oil in plant seeds, including soybean (Glycine max). TAGs are mainly produced through three sequential acylation of glycerol backbone in the Kennedy pathway. In this pathway, acyl-CoA: diacylglycerol acyltransferase (DGAT) is a key enzyme that regulates the final step of TAG biosynthesis by catalyzing the esterification process of diacylglycerol (DAG) to TAG. Among the three DGAT gene families identified in plant species, the DGAT1 family has the important role in the biosynthesis of TAG in the seeds. To study the importance of DGAT1genes on soybean seed composition traits, including oil and protein contents and the fatty acids profile, three DGAT1 isoforms were simultaneously knocked down in soybeans cv. "Jack" using trans-acting siRNA technology. The results of studying 18 transgenic T<sub>1</sub> and T<sub>2</sub> lines grown in controlled greenhouse environments showed a significant (P<0.05) reduction in the seed oil concentration, when compared to the control plants. The seed protein concentration was significantly (P<0.01) higher in transgenic lines, with reduced mRNA levels, than in control lines. There is no significant correlation has been detected between the level of DGAT1genes expression and the level of seed fatty acid compositions. The transgenic plants did not display apparent phenotypic changes when compared to the control plants, under controlled conditions in the greenhouse environment. The current study demonstrates the significant influence of DGAT1genes on oil and protein concentrations in soybean seeds. This information may contribute to the development of new cultivars with altered seed protein and oil compositions that will best fit industrial applications and end-user's needs.

Loss-of-Function Mutations: A Resource for Functional Genomics in Soybean

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In the last decades, a large amount of genetic variability has been induced by various mutagens (physical and chemical) to create a resource for functional genomics in soybean. However, these approaches often altered large genomic regions with a very limited precision. In this study, using the GmHapMap dataset, a subset of variants was predicted to have a large functional impact in soybean. Of these variants, we observed 18,031 putative loss-of-function (LOF) mutations that are predicted to severely impair protein synthesis or function through disruption of splicing, introduction of a premature stop codon, shifts in the coding frame and alterations to the start/stop codons in 10,663 genes (19.3% of all soybean genes). To assess the quality of this catalogue of mutations, we investigated the phenotypic impact of one of these LOF mutations. In this case a LOF mutation resulting in a frameshift (2-bp insertion, allele frequency of 0.003) was identified in microsomal omega-3 fatty acid desaturase (FAD3A), a key gene for linolenic acid synthesis in soybean seeds. Near-infrared spectroscopy (NIRS) analysis on two soybean lines (with and without this LOF mutation) showed a significant (P < 0.01) decrease in linolenic acid level in the mutant line (4%) compared to the wild type (10%). This development of a catalogue of LOF mutations has numerous implications for soybean geneticists and breeders: (i) it will be a large catalogue of functional mutants for nearly 11K genes, with a high level of quality and precision; (ii) sometimes offer multiple independent mutations in the same gene, and (iii) hold potential for application in breeding programs as genetic makers.

Genome-wide association study for productive traits in cassava

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Cassava (Manihot esculenta) is an important starchy root crop, source of energy for millions of people as well as raw material for industrial processing. The advances of the genotyping methods have been propitiating the usage of powerful tools to understand the genetic architecture of important traits for breeding purpose. In this study a genome-wide association analysis (GWAS) was undertaken to identify genetic markers associated with fresh root yield (FRY, in t ha<sup>-1</sup>), dry matter content (DMC, %), starch yield (SY, in t ha<sup>-1</sup>) and aboveground biomass (AGB, in t ha<sup>-1</sup>). A genome mapping by genotyping by sequencing (GBS) using 51,513 single nucleotide polymorphisms (SNP) was carried out. The cassava clones (N=290) were field-evaluated in different stages of a typical evaluation process in cassava breeding program from 2013 to 2016 in four locations of northeast Brazil. The GWAS was performed using a mixed linear model processing SNP by SNP in a single analysis, a genetic marker-based kinship matrix was accounted. SNP markers with  $a -\log_{10}(p - value)$  which exceeded the Bonferroni threshold >5.71 were considered to be statistically significant. The GWAS analysis identified two, five, two and five SNP loci associated with FRY, DMC, SY and AGB, respectively. The markers identified as significant for SY were the same for FRY (phenotypic correlation between the traits FRY and SY=0.98), both in chromosome 9. One of the associated SNP in chromosome 9 hit directly on a candidate protein coding gene for an ethylene-responsive transcription factor. Previous studies indicate that ethylene may play significant roles in seasonal acclimation of cassava root development and also in plants response to abiotic stress. These findings provide an opportunity to refine the breeding strategy, as the significant SNPs can be potential candidates for molecular assisted selection.

Characterization of QTL controlling spontaneous haploid genome doubling in maize <u>Benjamin Trampe</u>, Thomas Lübberstedt Iowa State University, Ames, USA

Maize (Zea mays L.) is a globally important crop for food, feed, and fuel. The use of doubled haploids has dramatically reduced the time needed to produce inbred lines. Doubling agents (i.e., colchicine, oryzalin) are used to double the haploid genome to produce doubled haploid lines that are 100% homozygous. Maize haploids are typically treated in the greenhouse during the 2-leaf stage and then transplanted by hand into the field. Both steps are extremely labor intensive. Spontaneous haploid genome doubling (SHGD) does occurring naturally, though at low levels, and circumvents the need for these steps. This study was conducted to identify QTL that influence SHGD. Progeny from a biparental mapping population (210 F2:3 families) were evaluated for spontaneous haploid genome doubling in a replicated, multi-location experiment in the summer of 2017. Reproductive capacity was determined for each family based on anther formation, pollen production, ear formation, tassel size, and seed set. A major QTL was found on chromosome 5. This will enable development of lines with SHGD and fine mapping to identify candidate genes.

An evaluation of diversity and the genomic contribution of *Phaseolus acutifolius* introgression within Ontario's *P. vulgaris* breeding program

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Resistance to common bacterial blight (CBB), caused by Xanthomonas axonopodis pv. Phasoli, has been a focus of the Ontario Phaseolus vulgaris (navy bean) breeding program, resulting in the release of several successful CBB resistant varieties. This resistance was derived from two interspecific crosses with P. acutifolius (tepary bean) and is conditioned by several loci on different chromosomes. Nineteen genotypes from Ontario's bean breeding program, including the two tepary bean introductions, were subjected to a genotyping by sequencing analysis (Pstl + Mspl digestion) and a total of 37 million 50-135 bp single-end reads (~1.9M reads/line) were obtained. The reads were processed on the Fast-GBS pipeline to identify nucleotide variants using the *Pvulgaris* 442 v2 reference genome. After filtration for variant quality, 5560 polymorphic, high quality single-nucleotide polymorphisms (SNPs) were identified. Population structure was estimated using a variational Bayesian inference, implemented in fastSTRUCTURE. Based upon the log probability of the rate of change in LnP(D) between successive K values, four primary genetic groups were determined. The evolutionary history was inferred using the Neighbor-Joining method and a bootstrap test of 1000, with the evolutionary distances computed using the Maximum Composite Likelihood method, to assess admixture. An identity by decent analysis was conducted using BEAGLE v4.1, which identified regions which tepary bean introgression may have contributed to genomes within the breeding program, based upon a LOD probability score, in addition to the known regions of conferred CBB resistance. Furthermore, nucleotide diversity ( $\pi$ ) was assessed in sliding windows of 1000-bp across the genome using VCFtools. Compared to a similar analysis performed on the middle American diversity panel of common bean (Moghaddam et al. 2016), this collection of 19 individuals from the Ontario navy bean breeding program is more than two fold less diverse ( $\pi$  = 3.4 x 10<sup>-4</sup> and 8.8 x 10<sup>-4</sup>, respectively).

Linkage mapping of Ug99 stem rust, stripe rust, and powdery mildew in double haploid winter wheat.

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With the world population set to reach over 9 billion by 2050, improving food security is extremely important for staple crops such as wheat, rice, and corn. Puccina graminis f.sp. tritici (stem rust), P. striiformis f.sp. tritici (stripe rust) and Blumeria graminis f.sp. tritici (powdery mildew) are important pathogens of wheat which threaten production worldwide. New attention has focused on the recently emerged stem rust Ug99-race group in Africa and the Middle East, virulent to over 75% of the world's breeding material. A double haploid population of 248 lines from MD01W28-08-11/Coker9553 was used to dissect adult plant resistance to Ug99 stem rust and also evaluated for stripe rust and powdery mildew resistance. Genotyping-by-Sequencing produced a 2428cM map with 1249 unique SNP loci, and 6 additional KASP markers incorporated, which was used for multiple disease resistance linkage mapping. Two QTL were consistently identified which provided stem rust resistance from the MD01W28-08-11. QSr.nc-6D on the short arm of chromosome 6D explained 7-13% of variation and QSr.nc-4B explained 6-7% of variation. The distal region of 6D is of interest because there are several previously reported stem rust resistance genes in the region that may be closely linked or allelic variants, including with QSr.nc-6D. These APR loci represent an important source for quantitative Ug99 resistance which may extend the life span of resistance to the race group. QTL mapping also identified a single stripe rust resistance locus (QYr.nc-4B) from Coker9553 as well as two powdery mildew resistance loci, QPm.nc-2B and QPm.nc-6B, from Coker9553 and MD01W28-08-11, respectively. These newly described loci represent a valuable source of disease resistance from two soft red winter wheat cultivars which are prevalent in the breeding programs of the eastern United States.

109 Characterization of Nested Association Mapping Population in Dry Bean

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Understanding the genetic bases of different traits agronomic and quality traits are important for efforts to breed improved lines in common bean. The Nested Association Mapping (NAM) population of F4:5 recombinant inbred lines (RILs) was created with the cultivar Ex Rico 23, and 10 founder lines that span the genetic diversity of Ontario Mesoamerican germplasm. The NAM population was evaluated for different agronomic traits in the field in four environments and will be genotyped using Genotyping by Sequencing (GBS). Results of the phenotypic characterization will be presented.

Using spontaneous haploid genome doubling to access favorable alleles in exotic maize germplasm <u>Anderson Verzegnazzi</u>, Ursula Frei, Elizabeth Bovenmyer, Thomas Lubberstedt Iowa State University, Ames, USA

Exotic maize (Zea mays L.) germplasm, usually open-pollinated varieties (OPV), represent a massive pool of genetic diversity largely untapped. The high genetic load and heterozygosity of OPV individuals hampers their use in breeding process. Doubled haploid (DH) has been shown an effective method for maize line development and alternative for exotic germplasm exploitation, where rapid homozygous fixation could capture most the variation present in the original source. A major bottleneck on DH process is the restoration of male/female fertility through artificial genome doubling (AGD). Spontaneous haploid genome doubling (SHGD) would eliminate the need of artificial chromosome doubling, allowing direct seeding of haploids, simplifying the production steps, reducing time and costs. The main objective of this study is to quantify the effect SHGD on germplasm exploitation and on selection of superior maize inbred lines. Testcross performance evaluation will be conducted for estimate the genetic variance among progenies derived from two breeding methods (DH and SSD) and the two chromosomal duplication methods (artificial and spontaneous). Results will be comparing with modern hybrids to determine whether lines with SHGD genes have the same breeding potential as lines without those genes. Genotype-by-sequencing data will be used for the investigation of a possible bottleneck associated with the haploidization process, and identification of possible segregation distortion related with SHGD genes.

Meloidogyne incognita Resistance Impacts on Soybean Yield

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Southern Root-knot nematode [Meloidogyne incognita] (RKN) causes major damage in soybean in southern United States. Occurring mainly in sandy soils, RKN can cause yield losses up to 75%. Besides crop rotation, management relies on the use of resistant cultivars. Few resistance QTLs were found in PI 96354, PI 408088, PI 417444, and PI 438489B. PI96354 represents the most popular source of resistance used in breeding programs. In this experiment, 100 high-yielding lines from the advanced yield trial (AYT) of the University of Missouri - Fisher Delta Research Center Soybean Breeding program were tested in 4 different environments in Clarkton and Portageville, Missouri. Clarkton is on sandy soil with high RKN pressure, and Portageville consists of 2 on loam soil and 1 on clay soil, both without RKN pressure. The maturity groups of selected lines ranged from 4-early to 5-early. They were previously phenotyped for RKN resistance at the University of Georgia based on number of galls, and resistance was reported in a scale from 1 (resistant) to 5 (susceptible). Yield (bu/ac) of RKN resistant lines across all maturity groups was 14.75 bu/ac higher than susceptible lines under RKN pressure, while no significant difference was observed between resistant and susceptible lines without RKN pressure (2.14 bu/ac difference). Further analysis showed significant difference among resistant lines under RKN pressure, but no significant difference was observed without RKN pressure. This possibly indicates that different genetic backgrounds for RKN resistance may have an impact on the level of resistance. The identification and implementation of resistance genes in breeding programs stands as a powerful tool to manage RKN. Potential new sources of resistance may contribute to superior levels of resistance and lower yield suppression under RKN pressure.

#### GBS MAPPING OF LOCI IMPACTING MALIC AND CITRIC ACID CONTENT IN CRANBERRY

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Malic and citric are the main acids contributing to high acidity, quantified as titratable acidity (TA - citric acid equivalents), in American cranberry (Vaccinium macrocarpon) fruit. Commercially grown cranberries have a TA of 2.3-3.0%, requiring high 'added-sugar' for palatability of juice and other products. Typically, malic acid levels range 6 to 8 mg/g FW and citric acid 8 to 11 mg/g FW in commercial cultivars. We identified two independently segregating Mendelian loci with low acid alleles, providing for low citric ( $\approx 1 \text{ mg/g}$ ) and low malic acid ( $\approx 3 \text{ mg/g}$ ) phenotypes, respectively. Homozygous genotypes for the respective loci exhibited TA ranging from 0.5% (malic acid) to 1.0% (citric acid). Multiple SSR marker alleles in a biparental cross revealed the citric acid locus has multiallelic 'normal' alleles with partial dominance. An association analysis, utilizing segregating progenies and GBS data anchored to a reference genome, defined the QTL region for each locus. SNPs that segregated closely with the phenotypes enabled development of KASP markers within 1 cM of each locus. KASP markers for the citric acid locus are located at the distal end of chromosome 1 spanning 63.3kb. KASP markers for malic acid locus are located at the distal end of chromosome 4 spanning 67.6kb. Transcriptome analysis of high and low citric acid phenotypes identified 92 differentially expressed genes. A BLAST search of all transcripts identified 89 potential genes inside or proximal to the QTL region. Malate dehydrogenase was identified as a candidate gene underlying the malic acid phenotypes. This gene is located on chromosome 4 at an approximate position of 34,437,172, within 734kb upstream of the KASP marker for malic acid production. Markers linked to these traits will facilitate marker assisted selection in breeding for cranberry fruit with reduced acidity.

Quantitative Trait Loci Associated with Value-Added Traits in Food-Grade Soybeans

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Soybean (Glycine max (L.) Merrill) is a predominant global source of plant-based dietary protein for human and livestock consumption. Western society has increasingly embraced soy as a more healthful substitute for meat and dairy products. Increasing seed sucrose concentration can improve the palatability of soy-based products, while manipulating the quantity and quality of protein in soybean seeds can alter the nutritional value of soy-based food products. Protein concentration, however, is a complex trait that is negatively associated with yield, which discourages the production of high-protein soybean cultivars through classical phenotypic selections. Quantitative trait loci (QTL) can be used to expedite the improvement of complex value-added seed composition traits, while mitigating yield loss. The objective of this study is to identify QTL associated with seed protein and sucrose concentrations as well as seed size and yield in recombinant inbred line (RIL) populations that are segregating for the target traits and derived from crosses between high-protein elite cultivars. The RILs were evaluated for the above and other important agronomic traits at three locations for two years, comprising five environments, across southwestern Ontario, Canada. The populations were genotyped using genotyping-by-sequencing to identify single nucleotide polymorphism (SNP) markers. Several QTL of interest ( $R^2 > 10\%$ ) were identified and validated in the RIL populations using multiple QTL mapping and single marker analyses. The identified QTL associated with seed protein and sucrose concentrations would be of immense value to the development of new high-yielding soybean cultivars with improved nutritional value and consumer palatability using marker-assisted selection.

A Genome Wide Association Study of Symbiotic Nitrogen Fixation in the Middle American Genepool of Common Bean (*Phaseolus vulgaris* L.)

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Common bean (Phaseolus vulgaris L.) is grown world-wide and comprises a significant source of human nutrition and household income in many regions. When nitrogen-fixing legumes such as beans are grown, the need for added nitrogen fertilizer is reduced, however beans have long been considered poor nitrogen fixers. The present study aims to measure nitrogen fixation in a large number of smallseeded bean genotypes and to identify genomic regions associated with symbiotic nitrogen fixation (SNF) using a genome wide association study (GWAS) approach. A panel of 319 bean genotypes from the Mesoamerican genepool was assembled and included the Mesoamerican Diversity Panel (MDP; BeanCAP) and modern Agriculture Agrifood Canada-University of Guelph cultivars. This panel was grown at three low-nitrogen field sites in Ontario, Canada and Puerto Rico from 2014-2016. Various agronomic and symbiotic nitrogen fixation (SNF)-related parameters were measured in the field and post-harvest. Isotope analyses were performed on seed samples and results ( $\Delta N$  values) were used to quantify nitrogen-fixing capacity using the natural abundance method (Shearer & Kohl, 1986). Analysis of variance and multi-variate analyses were performed on phenotypic data using SAS 9.3 (SAS Institute). Genotyping-by-sequencing (GBS) and two Illumina iSelect 6K Gene Chips (BARCBEAN6K 1 and BARCBEAN6K 2) were used to identify SNPs in 280 genotypes of the MDP. The 6k Illumina Infinium iSelect Custom Genotyping BeadChip (BARCBEAN6K\_3) was used to identify SNPs in the Canadian genotypes. The genotypic datasets were combined based on position in the second build of the Phaseolus vulgaris v2.1 reference genome. GWAS analyses were performed using the Genomic Association and Prediction Integrated Tool (GAPIT) to identify genomic regions associated with SNF in common bean. Significant variation for nitrogen fixing capacity and other traits was found. GWAS revealed regions of the bean genome associated with nitrogen fixation. Genotypes with superior nitrogen fixing capacity have been identified.

# Genetic Dissection of Seed Yield and its Components in Full Season Canadian Soybeans Using Genetic and Genomic tools

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Improving yield potential in major crops such as soybean (*Glycine max* L.), which is considered as the predominant source of plant-based protein and oil across the world, is the most sustainable way to address global food security. Soybean yield is a quantitative trait under controlled by several genes and strongly influenced by environmental factors. Despite the fact that soybean yield is a trait with low heritability, most of the breeding programs have been mainly focused on improving the trait through selecting for yield per se, but not selecting for morphological or physiological yield-related traits with high heritability. Several studies on major crops such as wheat, corn, and rice showed significant genetic relationships between yield potential and photosynthetic parameters, normalized difference vegetation index (NDVI), and photochemical reflectance index (PRI). However, there has not been a comprehensive study in soybean that studies the genetic control of yield through its components. Therefore, one of the objectives of this study is to investigate the relationship between soybean yield and important morphological (e.g., harvest index, leaf area) and physiological (e.g., gas exchanges, Non-Photochemical Quenching, NPQ) traits, as well as reflectance indices (e.g., NDVI, PRI) using high-throughput phenotyping (HTP) approaches. The second objective is to identify genomic regions associated with the target traits and create accurate genomic prediction models suitable for marker-assisted selections. To achieve the goals, a population of 250 soybean genotypes will be evaluated for the yield and its related morphological and physiological traits in several environments across Southwestern Ontario, Canada. The population will be also genotyped using genotyping-by-sequencing (GBS) approach. The current study will provide a better understanding of the genetic control of yield in soybean through studying its components to develop reliable genetic and genomic tools, which in tune may facilitate the development of cultivars with improved yield potential.

Identification of candidate genes for root-growth rate in *Brachypodium distachyon* <u>Zara York<sup>1</sup></u>, Rhoda Brew-Appiah<sup>1</sup>, Jayfred Godoy<sup>1</sup>, Ying Wu<sup>2</sup>, Dorrie Main<sup>1</sup>, Karen Sanguinet<sup>1</sup> <sup>1</sup>Washington State University, Pullman, USA. <sup>2</sup>Northeast Normal University, Changchun, China

Water and nutrient acquisition are among the most important root functions for enabling tolerance to abiotic stresses such as drought, heat, and salinity. Previous research in crops such as wheat has shown that deeper rooting is associated with increased drought tolerance; yet there is limited information regarding the genes that impact below-ground traits due to difficulties in assessing root phenotypes and the developmental plasticity of root systems. This study identified candidate genes associated with seedling root-growth rate in Brachypodium distachyon. We performed a quantitative trait loci (QTL) analysis for root-growth rate in a population of B. distachyon recombinant inbred lines (RILs). The RILs were generated from two natural accessions with contrasting root-growth rates; Bd 3-1 has rapid root-growth and Bd21 has a slower growth rate. QTLs were found on chromosomes one, three, four, and five. The molecular basis of the QTLs was assessed using whole genome sequencing of a bulk segregant population to identify single nucleotide polymorphisms (SNPs) in candidate genes that impact root-growth rate. SNPs were identified in five candidate genes that then underwent quantitative RT-PCR to analyze transcript expression patterns. Further analysis of putative genes that underlie root growth will increase the understanding of grass root development, which can ultimately be used to improve breeding of major cereal crops such as wheat and barley.

### FRUIT QUALITY RELATED TRAIT EVALUATION IN A SEGREGATING F1 POPULATION OF BLUEBERRY FROM A CROSS BETWEEN 'REVEILLE' AND 'ARLEN' CULTIVARS

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Successful breeders accomplish the task of releasing cultivars of superior quality that heighten commercial consumption. Blueberry breeders at NC State University have released several elite cultivars that have contributed to the estimated \$70 M statewide farm-gate value. Blueberries belong to the Ericaceae family and the genus Vaccinium. Many cultivars released today including the parents of the population in the current study are derived from the section Cyanococcus. However, they may include introgressed genetic materials from other sections yet to be discovered. Traditionally, selection for desirable traits is accomplished using recurrent selection through subjective field evaluations. Although a successful means of cultivar development, statistically only one in 10,000 seedlings is chosen as a cultivar which requires significant time, land, and labor resources. The task is made more difficult with increased ploidy levels. As such, there is growing interest in the development of genomic tools that blueberry breeders can use to make selections for fruit quality attributes more efficiently. Recently, genetic linkage maps and QTL makers for a diploid population segregating for chilling requirements and cold-hardiness have been developed using molecular markers. However, little is known about the genetic mechanisms responsible for QTLs that control fruit quality traits like firmness, sugar content, acidity, and berry size in a tetraploid population of blueberries. Our research involves the genotyping and phenotyping of segregating fruit quality related traits believed to be controlled by QTL in a tetraploid F<sub>1</sub> population (n=344) of a cross between cv. 'Reveille' and cv. 'Arlen' (RA) using sequence capture combined with Illumina sequencing technology. Our results suggest that the majority of fruit quality traits are segregating in the RA population in a quantitative manner. The collected phenotypic data and SNP data generated from sequence capture technology will be used for future genetic marker development, genetic map construction and QTL mapping.

Plant Breeding and Selection Recommendations from the USDA June 2018 Workshop on Innovation and Design in Vertical Agriculture and Sustainable Urban Ecosystems

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As the global population grows, so too does food demand as well as constraints on land and natural resources. By the year 2050, the world's population will approach 10 billion people, and at least 2 out of 3 people will live in urban centers. With this increased urbanization comes the unique opportunity to develop engineering and agricultural innovations within urban systems that sustainably stimulate growth to help meet future needs. Vertical agriculture operations could augment production while offering lower emissions, higher-nutrient produce, and reduced water usage and runoff. The recommendations focusing on cultivar environments, crop types, and breeding goals from the Plant Breeding and Selection breakout session of the June 2018 USDA Vertical Agriculture workshop will be presented.

# New sources of soybean fatty acids composition and meal traits identified through forward and reverse genetics

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Soybean [Glycine max (L.) Merr.] is the world's most widely grown protein/oilseed crop and provides about 68% of global food oil as well as protein meal and renewable fuels. Using mutation breeding, the novel soybean germplasm with modified fatty acids composition have been successfully produced to meet the needs of different users. Soluble carbohydrates present in soybean meal attract more interests as an effort to understand their contribution to livestock metabolizable energy. The Ethyl methanesulfonate (EMS) mutagenized 'Forrest' populations have been extensively employed using both forward and reverse genetic approaches to study the function of economically important genes in soybean. In this study, a subset of 810 Forrest M3 families was forward genetically screened to measure the contents of protein, total oil, carbohydrates, and fatty acids. The M4 families presenting altered fatty acids profile from M3 generation were selected for subsequent forward re-screening, and then mutants showing traits heritability have been chosen for genotyping analysis. One GmSACPD-C and one GmFAD2-1A mutants were identified to have stable high seed stearic acid and oleic acid content between M3 and M4 generations by target sequencing. Correlation analysis of M3 families revealed that sucrose, raffinose, and stachyose content were not statistically correlated with protein content, while the negative correlation between oil and protein content was observed. Within seed carbohydrate profiles, the positive correlations were shown among sucrose, raffinose, and stachyose content. Identification of mutants with altered carbohydrate profiles were ongoing with the advantage of the availability of candidate genes in soybean carbohydrate biosynthesis pathway. The obtained mutants with altered fatty acids and carbohydrate profiles can be used in soybean breeding for the desired seeds phenotypes.