Project Number: NC-007

Project Title: Plant Germplasm and Information Management and Utilization

Period Covered: 09/2016 through 8/2017

Date of this Report: Feb 28, 2017

Annual Meeting Dates: August 16-17, 2017

Participants: url needed

Minutes: url needed for the minutes from the meeting

Accomplishments and Impacts:

Plant Introduction Research Unit and the NCRPIS:
Plant genetic resources are acquired from throughout the world to provide valuable sources of genetic diversity for use in scientific research, education, and crop improvement programs in the U.S. and internationally. Curatorial personnel acquire, maintain and conserve, phenotypically evaluate, genetically characterize, document, and distribute plant genetic resources and associated information. They also develop software tools to assist in genebank workflows and public access to the collections and associated information. The project specializes in heterozygous, heterogenous, outcrossing crops and their wild relatives of maize, vegetables, oilseeds, woody and herbaceous ornamentals, and a wide variety of crops such as amaranth, perilla, quinoa and others. The genetic resources conserved and provided by this project enable researchers to access to a broad range of well-characterized crop and crop wild relative genetic diversity for their research, breeding, and educational objectives. Identification of genetic resource collection representation gaps are necessary in order to develop priorities for acquisition to expand the diversity of genetic resources maintained, and are addressed via exploration and/or exchange with other collections. Efforts devoted to characterization and evaluation of the genetic resources for priority traits and genetic profiles, coupled with information access, help researchers better target those accessions which can best address their objectives.

Germplasm Acquisition, Maintenance and Distribution:
North Central Regional Plant Introduction Station (NCRPIS) personnel acquired 194 new accessions (holdings total 54,412 accessions), and distributed nearly 55,500 units of seed to fulfill 1,411 orders from 1,020 requestors, of 22,811 unique accessions. In other words, 42% of the entire collection holdings were distributed, reflecting unusually high demand. About 62% of the distributions were to US recipients, and 38% to international recipients. Collection availability overall stands at 76.5%. Seed health testing was accomplished for 1,122 seedlots, as required to comply with phytosanitary import requirements associated with international shipments. ELISA testing of all Cucurbita seedlings was done prior to transplanting to the field in order to ensure the seedlings were free of squash mosaic virus. Field and greenhouse inspections were conducted to ensure that all plantings were free of diseases, and samples were cultured for of those specimens with observable symptoms. Approximately 2183 accessions were tested for viability as part of routine maintenance activities in order to ensure the quality of the collections. An additional 995 accessions were tested for viability as newly regenerated seedlots were submitted for storage. Backup seed lots were sent of 571 accessions to the National Laboratory for Genetic Resource Preservation (NLGRP) in Ft. Collins, CO and 2043 accessions were sent to the Global Seed Vault in Svalbard, Norway.

Approximately 1,500 accessions were grown for seed increase in Ames across all taxa, including 76 perennials that will be maintained until seed increase goals are achieved. Sunflower increases were planted for 515 accessions, including 57 UGA-SAM1 lines and 360 pre-breeding lines, and 38 other accessions were grown for regeneration at the Parlier, CA repository site. Approximately 200 accessions of tropical maize accessions were sent to a commercial nursery provider in Mexico in August for increase, as they are not adapted in Ames, and 387 more of temperate adaptation were grown in Ames. Vegetable regenerations were attempted for 158 accessions; 396 were grown for amaranth, quinoa, panicum and accessions of other taxa; and 49 ornamentals.

Facility security was enhanced, and equipment storage improvements made in the outbuilding via addition of steel shelving that increased vertical storage space substantially.

Evaluation and Characterization:
More than 1000 accessions were grown for various observation trials in 2017. The final year of a comprehensive phenotypic evaluation of *Daucus* crop wild relatives was completed by vegetable curatorial personnel, in collaboration with researchers who are revising the taxonomy of *Daucus* and allied Apiaceae. The oilseeds project grew a unique collection of *Helianthus* genetic resources, 360 pre-breeding lines from the University of British Columbia developed with funding from the Global Crop Diversity Trust. These lines were developed from crosses between a standard cultivated parent (the majority are with HA 89) and a wild annual species. Basic phenotypic descriptors are captured in conjunction with seed increase.

Software Development:
Our development staff created a new software, a wizard designed to capture raw data from viability tests and load it directly into GRIN-Global database, along with summary statistics on the test and methodology used. Labels were also designed to print viability test method along with viability information and date of test on seed packets for distribution to germplasm requestors. Another wizard is under development to load attachments (images, pdf and other files) and associate them with accession and or inventory records in the database.

State Reports:

Illinois:
Sacks lab: *Miscanthus* is a promising biomass crop and an important ornamental grass. However, only one sterile triploid genotype of *M ×giganteus (M. sacchariflorus × M. sinensis)* is currently available to U.S. farmers for biomass production. Additionally, as a close relative of *Saccharum*, *Miscanthus* is also a valuable source of novel alleles for improving sugarcane. With collaborators in Asia, where Miscanthus is native, we have developed large germplasm collections of *M. sinensis* and *M. sacchariflorus* (n>600 per species) from throughout most of each species native range. Using tens of thousands of SNP markers obtained via high-throughput sequencing, we have identified seven distinct genetic groups of *M. sinensis* (all diploid) and six genetic groups of *M. sacchariflorus* (three diploid and three tetraploid). These germplasm panels have been phenotyped for yield, flowering time and other key traits in replicated field trials in IL and other locations. By combining the genotype and phenotype data, we have conducted genome-wide association analyses and identified marker-trait associations that will be useful in breeding. Through breeding, we have developed new, promising triploid *M ×giganteus* genotypes that are in testing and when released will broaden the germplasm base of this crop. Moreover, we have found that *Miscanthus* is an excellent source of resistance to four key diseases of sugarcane (orange rust, smut, ratoon stunt, and sugarcane yellow leaf virus) and have identified Miscane hybrids that have strong resistance to all four diseases.

In addition, we also conducted phenotypic screens of Rice (*Oryza sativa*) Diversity Panel 1, for seedling stage tolerance to low temperature and for flowering stage tolerance to high temperature. Significant differences were observed for each trait, including individuals with exceptional stress tolerance. For high temperature stress, we have already identified significant SNP-trait-trait associations. These findings will enable researchers to improve rice, one of the most important staple crops for humanity.

Juvik lab: Evaluation of germplasm with for nutraceutical potential has been a central focus. The processed food and beverage industries are keen to replace artificial colorants with natural pigments, responding to consumer demands for healthier more sustainably sourced foods. To meet this demand, a panel of 356 maize accessions that have pigmented kernels were obtained from NPGS and CIMMYT and evaluated for pericarp and aleurone anthocyanin concentrations. Accessions with high concentrations were identified as promising sources of stable pigments that can be produced inexpensively for use in processed foods and beverages. This research was supported by large processed food companies.

Glucosinolates are antioxidants that have anti-cancer properties. We screened a panel of *Brassica* crop accessions (*B. juncea, B. napus, B. nigra, B. oleracea,* and *B. rapa*) and arugula (*Eruca sativa* Mill.) from the NPGS for glucosinolate and glucosinolate hydrolysis product concentrations. Substantial differences among accessions in glucosinolate concentration were observed. A predictive model was developed to estimate anticancer bioactivity with quinone reductase induction as a biomarker.

Hymowitz lab: Using NPGS soybean germplasm, elite breeding lines have been bred that have greatly reduced levels of antinutritional proteins, making the new soybeans easier for people and animals to digest. Soybean is a major source of protein for people and domesticated animals, and a major export crop for the U.S. However, standard soybean cultivars produce some proteins in the seed that reduces their digestibility and causes allergic reactions in some people. The new soybean will improve human nutrition and eliminate a potentially dangerous allergen from the food supply.

Kling lab: Trees have long been an important source of biomass for fuel and bioproducts. As part of a long-term study, twenty-one species of perennial woody species were evaluated for their potential as short rotation biomass feedstocks in IL. The best performing species was observed to be Black Locust, *Robinia pseudoacacia*, with an average annualized dry biomass yield of 12.2 Mg/ha/yr. Subsequent evaluation of additional Black Locust germplasm identified 11 plants (out of 159) that produced more than 18 Mg/ha/yr and 3 other plants that produced more than 20 Mg/ha/yr. High yields such as these, make production of woody biomass more economically advantageous.

Iowa:
Lübberstedt lab: Improving nitrogen use efficiency (NUE) in maize is one of the approaches to reduce N losses to the environment, as well as improve productivity in nutrient-depleted areas. Doubled haploid lines derived from exotic landraces from the Germplasm Improvement of Maize (GEM) program, backcrossed with ex-PVP inbreds PHB47 and PHZ51, were used. Root system architecture traits at seedling stage, and agronomic traits of the GEM-DH lines grown under high and low nitrogen conditions were investigated, and SNPs associated with these traits were identified. Seedling root traits were examined because of the root system's major role in the water and nutrient acquisition important for the plant's survival and growth. Lines were genotyped based genotyping by sequencing.

GEM-DH lines and their testcrosses were grown under high and low N in three environments in order to determine the extent of variation of agronomic traits of the GEM-DH panel grown under high and low nitrogen conditions, identify associations between SNP markers and agronomic traits grown under high and low N, determine consistency of SNPs between per se and testcross trials, and investigate associated SNP markers for candidate genes responsible for agronomic traits under high and low N. There was considerable variation in agronomic traits at the *per se* level, and most traits at the testcross level except for grain yield in Nashua; therefore, in the Nashua trials, repeatability estimates were low for testcross yield in that environment. We were able to identify SNPs associated with anthesis to silking interval, plant height, and grain yield under high and low nitrogen levels. Some of the SNPs were linked to or in linkage disequilibrium (LD) with known genes, while some of these gene models have not yet been identified as known genes; these could be novel genes useful for improving NUE in maize.

Candidate SNPs were identified that were associated with seedling root system architecture and adult agronomic traits under high and low N conditions. Weak positive correlations were found between seedling root traits and per se yield under high and low nitrogen conditions. Since there is a difference between the growing conditions in the growth chamber for the root experiment, and the field conditions in the yield trials, correlation between the traits from these two experiments need to be validated. It would be recommended, for future studies, to phenotype roots in the field in order to confirm if the SNPs identified in the growth chamber experiment would be consistent with those identified in the field. It is also further recommended to look into other traits, such as harvest index, and grain protein content under high and low N to better identify donors to improve NUE in maize.

Kansas:
Germplasm stored at the NCRPIS is of critical importance to the winter canola breeding program. The overall goals are to enhance performance of new cultivars and improve adaptability to the challenging southern Great Plains environment. Breeding efforts focus largely on winter hardiness, disease resistance (primarily *Leptosphaeria maculans* or blackleg), grain yield, and oil content and quality. Accessions are continually sought out and introgressed into diverse germplasm and adapted breeding lines. Five accessions possessing canola-quality oil and meal and winter growth habit were utilized in the 2017 crossing block. Roughly 25 additional accessions that were tested for field (multi-gene) resistance to blackleg have been incorporated over the three previous reporting periods.

During the current reporting period, the program released two new winter canola cultivars. The first cultivar, 'HyCLASS320W', is tolerant to glyphosate herbicide and is licensed to WinField United. It is being characterized as the most winter hardy Roundup Ready® winter canola cultivar available to farmers in the southern Great Plains. The second cultivar, 'Surefire', is tolerant to sulfonylurea (SU) herbicide residual in the soil and it will replace the first SU residual tolerant cultivar, 'Sumner', which was released by the Kansas Agricultural Experiment Station in 2003. Surefire is much later maturing and higher yielding than Sumner. Two Plant Variety Protection (PVP) applications were submitted for the winter canola cultivars 'Torrington' and 'Star 930W'. Another PVP application for 'Griffin' winter canola was resubmitted after a request for additional information. Seed sales for Kansas Agricultural Experiment Station licensed canola products exceeded the equivalent of 50,000 planted acres during the 2016-2017 growing season.

Michigan:
Germplasm evaluation and use in breeding is an important part of the plant breeding and genetics programs at Michigan State University. Goals of the breeding programs include improving disease, insect and stress resistance, to improving yield and market and consumer driven quality traits. Examples of germplasm use are listed below:

Tart cherry: Cherry leaf spot caused by the fungus *Blumeriella jaapii* is the most devastating disease affecting this crop in the major temperate growing regions. Up to nine sprays can be required each growing season to prevent early defoliation and no resistant cultivars are available. The MSU tart cherry breeding program is using sources of cherry leaf spot resistance and tolerance that would significantly reduce the number of sprays required to control this disease. The donor germplasm used includes both a wild and progenitor species.

Cucumber: Fruit rot caused by the soilborne oomycete pathogen, *Phytophthora capsici* is a major disease threat to cucumber production in the eastern and midwestern United States with severe losses occurring in some years. Currently there are no resistant commercial cucumber cultivars with resistance to this disease. After screening fruit from 1076 accessions from the U.S. cucumber PI collection identified three PIs with potential resistance: PI 109483 and PI 178884 from Turkey and PI 214049 from India. Using this germplasm a PI 109483-derived S_6 progeny line with young fruit resistant to infection by *P. capsici* was developed and released as a resistant donor source to be used in disease resistance breeding.

Potato: The potato breeding program utilizes and has access to germplasm from wild *Solanum* species native to South America and representing more than 100 tuber-bearing relatives. Using a diversity panel of wild species, landraces, and cultivars, DNA sequence analysis revealed the historic role of wild *Solanum* species in the diversification of long-day–adapted tetraploid cultivated potatoes, along with the identification of populations that represent sources of germplasm previously untapped that would have potential to contribute to potato improvement.

Soybean: The soybean aphid, *Aphis glycines*, is a highly destructive invasive pest for soybean production in North America since 2000. Developing new cultivars with host-plant resistance is one of the goals for the soybean breeding program. Two aphid-resistance loci, *Rag6* and *Rag3c*, were identified from the wild speices *Glycine soja* 85-32, and mapped to intervals on chromosomes 8 and 16, respectively. A soybean advanced breeding line, E08932, was developed using marker-assisted backcrossing from the wild soybean *Glycine soja* 85-32. This breeding line shows strong resistance to aphids and therefore can be used to more efficiently breed resistant cultivar.

Minnesota:
The University of Minnesota Soybean Breeding Program makes extensive use of the USDA Soybean Germplasm Collection. In 2017, 34 crosses were successfully made between lines with diverse ancestry (i.e., PIs from the collection or direct descendants from PIs) and other parental lines carrying desirable traits such as yield, SCN resistance and protein concentration. All F1 seeds were sent to Chile for generational advancement. All breeding populations made for reasons related to diversity were harvested and advanced to winter nursery. 376 plant rows created for reasons related to diversity were evaluated and 203 were selected based on visual characteristics. Seed was scanned with the NIR for measuring protein and oil. Plant rows were advanced to 2018 PYTs based on protein and oil concentrations. In 2017, multi-location trials of 234 lines with diverse ancestry were conducted in Minnesota. Breeder's seed was made for three breeding lines that had a PI as a parent. These lines were high enough yielding to make it to this advanced stage but not high enough for variety release. Data will be examined more closely over the coming months to determine if a germplasm release would be appropriate.

In addition to these breeding activities, the University of Minnesota Soybean Breeding Program screened a diverse panel of 400 MG I PIs obtained from the Collection for canopy architecture and seed composition traits. A genome-wide association study found strong QTL for branch angle and seed sucrose content. Diversity for branch angle in soybean is being studied for canopy architecture design, and seed sucrose content is being studied for enhanced soybean meal potential.

Missouri:

Maize: The Flint-Garcia lab (ARS in Columbia MO) continues to investigate teosinte (*Zea mays* ssp *parviglumis*) as a source of novel and useful alleles to improve maize for a number of agronomic traits.

Ten parviglumis accession were used as donors to create a collection of over 800 near isogenic lines (NILs) in the B73 background, each carrying a different genomic region of teosinte (Liu et al, 2016). The teosinte NILs have been evaluated for disease resistance by the Balint-Kurti lab (ARS in Raleigh, NC). Four quantitative trait loci (QTL) were identified for southern leaf blight (SLB; Lennon et al, 2017) and six QTL were identified for gray leaf spot (GLS; Lennon et al, 2016). Of these QTL, one GLS QTL appears to be a novel resistance QTL originating from teosinte. In addition, one QTL appears to confer multiple disease resistance to both GLS and SLB. The Missouri group has evaluated these NILs for multiple kernel traits including kernel weight and kernel composition traits. When comparing the QTL profiles between the nested association mapping (NAM) population and the teosinte NILs, Karn et al, 2017 identified two novel QTLs in the teosinte NILs for kernel starch, protein, and oil content that do not segregate in NAM. In addition, several QTLs were identified in NAM that do not segregate in teosinte. These subspecies-specific QTLs provide insight into likely selection for kernel composition traits during domestication and modern breeding, resulting in the loss of variation (teosinte-specific QTL) during domestication or the rise of new variation (maize-specific QTL) since domestication. The NILs are available through the Maize Genetic Stock Center.

A second project involves the development of the Zea Synthetic population, created by random mating the 25 NAM inbred founders, along with eleven geographically distinct outbred teosinte individuals to create a population of alleles with differing inbreeding histories. This population is being used to study the effect of alleles after mild inbreeding (one generation of self-pollination) and after severe inbreeding (doubled haploid production). A stable resource of 2000 DH lines was created and will be released to the public in 2018.

Soybean: The Gillman lab grew two field studies in 2015-2017 related to Genome Wide Association Studies (GWAS) using 380 entries from the NPGS soybean collection: 1) Seed composition; and 2) reproductive success traits under high ambient temperatures. Another 200 NPGS entries are being evaluated via GWAS and Genomic Selection in 2018-2019 for water use efficiency related traits as part of a multi-collaborator and location study.

New York:
Gore lab: The development of affordable high-throughput plant phenotyping methods is necessary to allow for the genetic characterization of agronomical and physiological important phenotypes in sweet corn breeding populations at multiple time points over the growing season. However, this need is further complicated by the complex interaction between the plants, the field environment, and agricultural management practices. The collection of high-dimensional phenotypic, genotypic, and environmental data sets would allow for the genetic basis of phenotypes related to plant productivity to be better understood and such phenotypes more effectively selected upon by breeders in sweet corn breeding programs.

In summer 2017, the Gore lab at Cornell University grew a diverse population of ~500 sweet corn inbred lines in replication at Cornell University's Musgrave Research Farm. The population contained sweet corn inbred lines provided by the USDA-ARS North Central Regional Plant Introduction Station in Ames, IA. The sweet corn population was genotyped with nearly a quarter of a million genetic markers at genome-wide coverage. In the field, a handheld instrument having the dual functionality of a fluorometer and chlorophyll meter was used to simultaneously measure leaf photosynthetic and pigment phenotypes on the sweet corn population at reproductive maturity. The instrument also recorded environmental variables such as light intensity, ambient temperature, and humidity at the time of plant measurement. Additionally a small unmanned aerial vehicle (sUAV) with RGB and

multispectral cameras was flown at several time points over the field season to collect plant image data for the phenotyping of canopy height and normalized difference vegetation index (NDVI) on a plot-level basis.

The photosynthetic and pigment phenotypes were found to be under moderately strong genetic control, suggesting that these phenotypes would be responsive to selection in a breeding program. We are now striving to reveal the genetic basis of these phenotypes via a genome-wide association study. This work is poised to provide a deeper understanding of the genetic basis of natural variation for important phenotypes related to plant productivity, providing novel findings that sweet corn breeders can leverage for the genetic improvement of this important crop.

Doyle lab: This project aims to characterize genetic variation and species relationships in perennial wild relatives of soybean. This group of 30 or more species has levels of genetic variation considerably higher than soybean or its annual progenitor. Perennial species harbor resistance to numerous plant pathogens and are adapted to diverse climatic niches. We seek a better understanding of this largely untapped resource for soybean.

Preliminary analysis were completed of our first full scale genotyping by sequencing (GBS) library including 20 different species, 115 accessions including 48 allopolyploid accessions. Thirteen accessions belong in species different from what was expected and we have confirmed that one putative species would best be treated as two separate species. This is similar to a small scale preliminary project in which we uncovered five mis-identified accessions and levels of within species population subdivision that were unexpected in both diploid and allopolyploid species. We have completed sequencing of seven additional libraries with 406 accessions; read mapping and SNP calling continues. This brings the total number of accessions sequenced to 621 from eight libraries.

Herbarium vouchers have been made for each accession used for GBS. Morphological analyses to support updated species descriptions are continuing. Our results provide valuable information for identifying sources of potentially useful genetic variation that can be integrated into soybean. It is important that the units ("species") in which genetic variation is organized be defined using rigorous, objective genetic criteria. It is also important that this genetic variation be associated with morphological variation ("phenotypes") in a way that will enable the formal naming of species. The coherent combination of genetic variation and formal names will enhance the utility of existing germplasm collections for identifying and mobilizing variation in improvement of soybean.

ME Smith lab: For objective 1 (encourage the use of a broad diversity of germplasm to reduce crop genetic vulnerability), our project used USDA collections of public corn inbreds from historic breeding programs and exotic corn germplasm as genetic resources to build disease and insect resistance into U.S.-adapted sweet corn backgrounds. From these breeding efforts, we have developed several finished inbreds that are candidates for release based on resistance to northern leaf blight (one inbred with promising resistance), anthracnose leaf blight (comparing nine inbreds to choose those for release), and insect resistance (comparing 12 inbreds for European corn borer and resistance to choose the top candidates for release).

For objective 2 (educate students, scientists and the general public regarding plant germplasm issues), talks to graduate students from Cornell University and exchanges with hundreds of visitors viewing a display at New York's Empire Farm Days all emphasized the importance of genetic resources to continue addressing the needs and issues arising in our agricultural and food system via plant breeding. In addition, educational talks and media outreach addressed issues and concerns about genetically engineered crop varieties. Over the five years of this project, 57 talks were presented on this topic, which reached over 5,200 listeners in person, over 400 on one tele-town hall meeting, and

many additional people via video recordings, news articles, and web-based media. These presentations emphasized education and explicitly did not promote a particular viewpoint on this very polarizing topic. Targeted learning outcomes included what genetic engineering is, how it fits within the context of long-term crop selection/improvement and conservation efforts, how extensively genetically engineered varieties are being grown, where they make their way into our food, and what our scientific understanding is to date regarding the risks and benefits of genetically engineered crops.

North Dakota:
Screening of agronomic and horticultural germplasm is ongoing and important in identifying potential new crops for North Dakota and the surrounding region. New crops often have greater production risk and low producer appeal, but when major crop commodity prices are poor producers are more receptive to growing a new crop that could provide greater profits. Faba bean (*Vicia faba* L.), industrial hemp (*Cannabis sativa* L.), Ethiopian mustard (*Brassica carinata* L.), and grain sorghum (*Sorghum bicolor* (L.) Moench) are currently being evaluated as potential new crops in the region. Grain sorghum screening included 106 and 101 genotypes, sourced from the NPGS, that were evaluated in 2015 and 2016, respectively, at Prosper and Carrington, ND. From these initial adaptation screenings 22 genotypes were selected for replicated yield performance and seed increase trials in 2017. Four to six of the higher yielding genotypes are slated for inclusion in a RCBD seeding date and genotype field study in the 2018 growing season at several locations. Adapted sorghum genotypes primarily based on yield and seed quality could provide an alternative grain crop for drier regions of North Dakota where corn performance is poor. Ethiopian mustard yield response across planting dates over a three to four week period resulted in reduced yield as planting date became delayed from mid-April at the north/central and early-May at the east/central North Dakota locations. Yield reduction was 80% and 40% at the north/central and east/central locations, respectively, compare to the early planting dates. *B. carinata* yield performance appears higher than camelina (*Camelina sativa* L.), but lower than canola (*Brassica napus* L.) based on previous camelina and canola performance trials in the state.

Ohio:
The Ornamental Plant Germplasm Center continues to acquire, maintain, and develop germplasm of herbaceous ornamental plants. As part of these activities in 2016 and 2017 we have made available for distribution the *in vitro* cultures of *Phlox*; they were sent out to fulfill germplasm requests for the first time in 2016. We have also expanded our in vitro back-up activities of the *Chrysanthemum, Leucanthemum, and Dianthus* clonal accessions.

The discovery of GMO markers within cultivars of *Petunia* in the trade made it imperative that we suspend distribution of the *Petunia* germplasm collection at the OPGC until it could be tested for the presence of such makers. All *Petunia* accessions were tested in collaboration with the USDA-ARS, and all lacked those markers, so the collection can again be distributed without restriction.

In the area of seed biology, we've continued work on defining the quality parameters in seeds of perennial species of *Phlox* which have proven difficult to germinate. We have identified the fungi typically associated with the seed. We have optimized the Tetrazolium test for rapid assessment of seed lot viability, and we have examined the germination potential of isolated embryos. The results point to a typical non-deep physiological dormancy imposed by the residual endosperm as isolated embryos readily germinate. However, Gibberellic acid treatment is not an effective substitute for the moist chilling requirement.

We have also been examining the seeds of various *Begonia* accessions that were produced locally, to insure that the populations are consistent with those of the parents and that there is no contamination with other *Begonia* species. These seeds were obtained from accessions that had been maintained

clonally. The extremely small size of *Begonia* seeds require very careful assessment of individual seed lots for consistency.

Our germplasm enhancement activities have focused on interspecific hybridization in *Phlox*. Progeny of the reciprocal cross between *P. roemeriana and P. drummondii* have been grown to determine the potential for novel flower color combinations. The hybrids were highly sterile, but some backcross progeny with *P. drummondii* have been obtained for further evaluation.

During this reporting period, we trained 4 undergraduate students (as student workers) in the greenhouse and field operations associated with germplasm management and 2 undergraduate students learned tissue culture procedures for germplasm conservation.   One visiting scholar from Brazil has been involved in the seed biology studies in *Phlox*. One undergraduate student pursued a research internship exploring seed biology of *Phlox*. Our facilities and programs are regular components (tours, demonstrations, labs) of various undergraduate courses at The Ohio State University. The OPGC maintains a website dedicated to sharing information about our germplasm and research/development associated with it. We participate in the annual Cultivate'17 activity, which is the largest horticultural trade show and educational program in North America.

Texas:
Dr. Wenwei Zhou and Dr. Seth Murray continue to focus on development of aflatoxin and fumonisin resistant corn based on subtropical germplasm. Several releases are pending seed increase from these efforts. These programs have progressed to the stage that heat tolerance, yield and other pest resistance characteristics are competitive with elite germplasm from other sources while maintaining the enhanced resistance for aflatoxin and fusarium.

Amaranth molecular work has been initiated by Dr. Okumoto and will continue for the next year. Continue educational outreach on the role of germplasm collection, preservation, increase and evaluation in Texas breeding programs for all crops.

Wisconsin:
The sweet corn breeding project used germplasm from NCRPIS in at least four different projects described below.

Finding new flavor with culinary corn.
Modern varieties of sweet corn are prized by consumers for their sweetness and tenderness when eaten on the cob. As a result, sweet corn has gotten much sweeter in the past few decades resulting in a gap in the market for savory varieties of vegetable corn. We are working with a number of heirloom varieties from the northern United States as well as Chilean choclos and roasting elotes and pozoleros from Mexico. We hope to improve these heirlooms for both eating quality and field performance in order to bring more and different types of flavor and texture to vegetable corn used in a range of culinary dishes. We are using both tasting techniques in addition to sugar and carbohydrate analysis in the lab. In the lab, we have begun testing the starch, total polysaccharides and water-soluble polysaccharide concentrations in order to assess why eaters might enjoy certain varieties over others and use the quantitative data to inform selection.

Chefs in the Madison area have become more involved in tasting and evaluating vegetables, creating a regional cuisine and contributing the local economy and environment. We are working with local chefs to create flavor profiles and use their expertise to better advise our selection for flavor and texture in the heirloom populations. From tastings this summer we have learned that important characteristics for culinary corn include the ability for kernels to hold their shape, surprising colors and stronger corn

flavor. As the culinary corn program progresses we hope to work more with farmers, chefs, and eaters to breed for corn that is both suited for organic production and the dinner plate.

Complexity in Plant Disease Resistance: Association analysis and genomic selection for resistance to Sugarcane Mosaic Virus and other maize diseases.

In maize (*Zea mays* L.), sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) are important pathogens worldwide causing yield losses. Resistance to SCMV and MDMV in adapted maize is abundant, and a large fraction of the genetic contribution to resistance has been associated with two loci, *Scmv1/Mdm1* and *Scmv2*. However, these loci do not count for 100 percent of genetic variation, and other loci add to the complexity of resistance by modifying the efficacy of *Scmv1/Mdm1* and *Scmv2*. This study utilized a diverse maize panel, the Wisconsin Diversity Panel, consisting of over 600 inbreds adapted to a northern Corn Belt environment to evaluate variation for SCMV and MDMV resistance. In a genome-wide association study (GWAS) of the panel inoculated with SCMV in an open field trial at the three-leaf growth stage, *Scmv1* accounted for much of the genetic variation, especially early after inoculation, and *Scmv2* and other loci contributed to resistance several weeks after inoculation. A large presence/absence variation encompasses *Scmv1*, and 44 percent of the lines contained the absence state and were fully susceptible to SCMV. Inoculation with SCMV was subsequently preformed at two different growth stages to evaluate the efficacy of *Scmv1*. Symptom development was greater with inoculation at three-leaf than six-leaf stage, but some lines, with at least 99 percent sequence homology in *Scmv1* with resistant lines, became fully symptomatic after 35 days regardless of growth stage at inoculation. Greenhouse inoculation was conducted to evaluate genetic differences in resistance to SCMV and MDMV. GWAS based on the greenhouse study determined that *Scmv1/Mdm1* contributed a greater amount to resistance for both SCMV and MDMV in the greenhouse compared to the SCMV field study. Finally, genomic selection (GS) for SCMV resistance was coupled with GS to three other maize diseases. Prediction accuracy for SCMV resistance based on cross-validation was as high as 0.75, and prediction accuracies for other diseases ranged from 0.45 to 0.69. GS prediction accuracies for multi-disease selection based on a selection index varied from 0.39 to 0.66 suggesting index-based GS could be effective for selection of lines resistant to all four diseases.

Sweet Corn Husk Length and Corn Earworm Resistance

Corn earworm is certainly the most destructive pest of sweet corn and one of the costliest of *all* crop pests in North America. The migratory nature of corn earworm makes it unpredictable and challenging to manage on a farm scale, and climate change is likely to expand its overwintering range, increase the number of generations per year, and cause earlier infestations. Corn earworm is ubiquitous; it feeds on over 200 host plants, including important crop plants (e.g. maize, soybean, cotton, snap bean, tomato) as well as weedy and uncultivated plants. This ubiquity limits the ability to use cultural controls such as crop rotation and increases the pest's adaptability and probability of successful development. The oviposition and feeding habits also limit the efficacy of chemical controls. Plant breeding remains one of the few promising tools for reducing the economic damage caused by corn earworm.

Corn earworm is an especially challenging pest for organic sweet corn growers, since few effective management strategies are permissible under the National Organic Program (NOP). Infestations are particularly pernicious in the southern U.S., where temperatures permit overwintering and multiple reproductive cycles. The severity of corn earworm infestations is such that, before the widespread use of pesticides, the distribution of sweet corn production was limited to northern states, and the geographic range of *organic* sweet corn production is still limited today.

Organic producers are in particular need of breeding for resistance to corn earworm since the advent of transgenic *Bt* varieties have supplanted much of the classical breeding for earworm resistance. However, novel forms of corn earworm resistance are of interest to conventional farmers as well, since

increasing reports of *Bt* resistance indicate a limited lifespan for one of their main management tools. Corn earworm (*Helicoverpa zea)* is a major insect pest in maize and is especially problematic in processing corn and organic cropping systems, which have a limited set of available management options. We are examining morphological and biochemical traits for potential sources of corn earworm resistance. In 2015, NC Design II mating design was created, crossing long- and short-husked inbred maternal parents with inbreds putatively containing maysin, a flavonoid that delays earworm development, and non-maysin inbreds. In 2016, we evaluated the progeny of these crosses for husk extension past the tip of the ear and for earworm damage. We found significant interaction effects between the male and female parents for husk extension, with the long-husked females producing long-husked progeny with only some male parents. The long-husked females (A684, A685, A686) and a longer-husked male also produced progeny with lower damage ratings, while the maysin-containing parents did not consistently produce progeny with lower damage ratings.

Genetics and biochemistry of native su1 alleles and modifiers of the sugary1 phenotype.
The development of wild type starch in maize (*Zea mays* L.) endosperm is contingent on debranching enzymes (DBE) in starch biosynthesis. Two types of DBE are conserved in maize endosperm, pullulanase-type and isoamylase-type. Pullulanase is encoded by *Zpu1* and uses the substrate pullulan. Three isoforms of isoamylase exist in maize endosperm and include *Su1* (*Sugary1= Isoamylase1*), *Isa2* (I*soamylase2*), and *Isa3* (*Isoamylase 3*). *Su1* is associated with three enzymatic protein complexes. One complex is homomeric and contains exclusively ISA1, and two complexes are heteromeric ISA1/ISA2. None of the three complexes are active in the presence of *su1-ref* thus resulting in significantly reduced starch concentrations and greater quantities of water-soluble polysaccharides (WSP) in the developing endosperm. While *su1-ref* does not form a protein complex with *Zpu1*, the presence of *su1-ref* significantly reduces pullulanase activity.

  Five recessive *su1* alleles were characterized as having differences in starch and WSP compositions in endosperm. Each allele was backcrossed into a common inbred background and immature endosperms were evaluated for differences in isoamylase and pullulanase DBE activity. No significant differences in DBE activity were found. Of the five alleles, the northeastern allele accumulated the greatest quantities of WSP and the least starch while the Peruvian allele was indifferent from wild type. The northeastern allele is used in commercial sweet maize production and is referred to as *su1-ref*.

  Pseudostarchy maize inbreds (*su1-ref/su1-ref*) were selected to accumulate wild type starch concentrations despite the *su1-ref* allele. Evaluation of starch modifying enzymes via zymography determined that the pseudostarchy inbreds have partially restored activity in the ISA1/ISA2 heteromeric complexes was well as fully restored pullulanase activity. Additionally, the amylopectin structure associated with the pseudostarchy inbreds is indifferent from the structure associated with *Su1*. A recombinant inbred line (RIL) population derived from a biparental cross between a pseudostarchy inbred and an extreme sugary inbred reveal that five quantitative trait loci (QTL) have significant associations with the pseudostarchy phenotype.

  To further explore the role of *Isa2* in the presence of *su1-ref*, immature endosperm from isogenic double mutant *isa2-339, su1-ref* and single mutant *Isa2, su1-ref* inbred lines were evaluated for carbohydrate composition and glucan structure. The *isa2-339, su1-ref* endosperms had significantly less starch and a greater abundance of WSP compared to the *Isa2, su1-ref* suggesting that *Isa2* may play a prominent role in starch biosynthesis. Additionally, the *isa2-339, su1-ref* endosperms had a greater abundance of short chain lengths in both amylopectin and WSP structure compared to *Isa2, su1-ref* suggesting *Isa2* plays a significant role in glucan structure. Lastly, to differentiate the role of *Isa2* and the presence of starch modifying loci present in a population, immature endosperms containing the *isa2-339* allele were evaluated in differing genetic backgrounds fixed for *su1-ref*. No background effects were observed suggesting *Isa2* may play significant role in starch biosynthesis independent of ISA1 enzymatic activity.

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Outcomes/Impacts:
- Access to well-documented, high quality plant genetic resources enables the basic and applied research applications necessary to sustain agricultural productivity and to achieve needed advances in nutrition, health, bioenergy, and economic growth.
- Germplasm evaluation results, use of new DNA markers, new and improved genetic linkage maps, identification of QTL for important agronomic traits and their flanking markers are useful for future genetic improvement of cultivars using both conventional and modern breeding approaches, and for further genetic analysis of important traits, and map-based cloning of genes. Use of molecular markers as a quality assurance tool to ensure genetic purity of genebank collections is advancing. Examples: identification of genetic marker and trait associations for yield, flowering time and other traits of *Miscanthus* will support breeding advances; new triploid *Miscanthus* genotypes have been developed to broaden the crop's germplasm base; germplasm resources of teosinte, a wild relative of maize, have proved to be novel sources of kernel composition traits and their corresponding alleles; traits contributing to soybean reproductive success under high temperature stress are being determined; agronomic studies provide guidance to growers on best management practices, critical for new crops or those new to a region.
- Development of new cultivars provides solutions for production challenges as well as introducing novel variation. Examples: introduction of two new winter canola cultivars with herbicide resistance that are adapted to the southern Great Plains, providing growers with options for cropping systems, important in as climate variability impacts agronomic production; improved disease resistant rootstocks of fruit trees, and improved tolerance during flowering to temperate extremes help reduce production risks and financial risks to growers; sources of germplasm that provide resistance to cherry leaf spot disease will result in cultivars that require fewer chemical sprays to control disease; maize inbreds for release with disease resistance to anthracnose leaf blight or northern leaf blight, or to production of aflatoxin or fumonisin in grain while maintaining heat tolerance and yield capacity.
- Development of new markets for crops provides value to growers and society. Examples: use of heirloom maize varieties in breeding programs for improvement of eating quality and field performance to bring more and different types of flavor and texture to corn used in a culinary dishes, and development of flavor profiles; maize accessions that have high levels of pigments produced in the pericarp and aleurone, of interest for processed foods and beverages; elite soybean lines that have greatly reduced levels of anti-nutritional proteins, which improve digestibility and eliminate a potentially dangerous allergen from the food supply; and identifying sorghum genotypes that can provide an alternative grain crop for drier regions of the northern Great Plains;

- Training of undergraduate and graduate students, postdoctoral candidates, and visiting scientists provides valuable experience with use of plant genetic resources for a wide range of objectives, and provides for the next generation of US scientific leadership in agricultural and allied sciences. Incorporation of genetic resource investigations in development predictive analytic tools provides a valuable opportunity to develop scientists who can capitalize on technical and analytical advances to realize the inherent value of the germplasm collections. Plant genetic resource use will continue to support agronomic, genetic, molecular biology, plant pathology, entomological, horticultural, ecological, biochemical, industrial, anthropological, medical and pharmaceutical, animal nutrition, and bioenergy research advances.
- Germplasm use will continue to contribute to the aesthetics and the sustainable management of the world we live in, and the health, welfare and security of the world's peoples.