# **United States Department of Agriculture Agricultural Research Service**



National Program 301 PLANT GENETIC RESOURCES, GENOMICS, and GENETIC IMPROVEMENT

#### FY 2021 Annual Report

The USDA-Agricultural Research Service's (ARS) National Program (NP) 301, Plant Genetic Resources, Genomics, and Genetic Improvement, supports research that maintains, protects, enhances, and expands the United States genetic resource and information base, and increases knowledge of the structure and function of plant genes, genomes, and biological and molecular processes. Through innovative research tools and approaches, this National Program manages, integrates, and delivers vast amounts of genetic, molecular, biological, and phenotypic information to a diverse global clientele. The ultimate goals for the preceding efforts are to improve the production efficiency, yield, sustainability, resilience, health, product quality, and value of U.S. crops. This National Program addresses the need to:

- Ensure the long-term safety and integrity of our agriculturally valuable genetic resource collections and associated information
- Develop novel approaches for analyzing complex traits, identifying favorable genes underlying those traits, and deploying them in breeding stocks
- Exploit new technologies that enhance traditional methods of genetic improvement
- Genetically improve a broad spectrum of major, specialty, and new crops
- Increase our knowledge of the structure and function of crop genomes and of plant biological and molecular processes
- Acquire, analyze, and deliver genetic and information resources to research communities and to the public

Genetic resources are the foundation of humanity's agricultural future. ARS genebanks contain sources of resistance, tolerance, and adaptation to biotic and abiotic stresses and new genes to improve the quantity, quality, and production efficiency of our food, feed, bioenergy, fiber, and ornamental crops. To ensure those genes are available for research and breeding, ARS must continue to strategically acquire and conserve germplasm that contains them; develop new screening methods for identifying favorable traits; ensure that germplasm is distributed where and when it is needed; and safeguard these collections for future generations.

ARS has taken a leadership role in developing and curating crop genomic and phenotypic databases, which are expanding now and will continue to do so in the future. New tools are needed to efficiently capture phenotypic data and extract useful information, via innovative data-mining strategies, from the ever-increasing flow of data into these databases. Methods to interconnect databases containing diverse types of information are needed to more efficiently, and effectively, identify important properties of genes and genomes and apply that knowledge to crop improvement. In addition, these interconnected databases will enable researchers to better associate specific genes with agriculturally-important traits and build on genetic advances in one crop to accelerate genetic gain in others.

For major, specialty, and new crops, ARS, in close cooperation with diverse public and private sector collaborators, will improve and broaden the genetic base of U.S. crops to reduce genetic vulnerability. NP 301 scientists will devise and apply new technologies to develop superior new crop varieties and enhanced germplasm and will accelerate the deployment of high-value traits into breeding populations. New genetic sources of key crop traits will be identified and incorporated into crop breeding lines and gene pools. New breeding theories and strategies that exploit the vast amount of available genotypic data will also be developed to effectively capture

the intrinsic genetic potential in germplasm. To do so, innovative tools will be developed for dissecting the architecture of complex crop traits. These tools will be applied to mapping and determining the function of underlying genes so that variation in them, and in linked genomic sequences, can be exploited as more precise genetic markers. This knowledge will also enable molecular and genetic variability to be associated with specific phenotypes to enhance the effectiveness of genomic selection, thereby accelerating and strengthening progress in crop genetic improvement.

Plant breeding has a long history of improving crops through phenotypic or marker-assisted selection, usually in the absence of information regarding underlying genes or gene networks that control key traits, or that determine how plants interact with their environment. Understanding the key biochemical pathways and the underlying controls for plant growth, development, and metabolism has been elusive. However, recent advances in plant molecular biology, genetic engineering, and physiology could create opportunities for improving crops by more precisely and rapidly manipulating the existing variability in plant properties, or by creating new variability when warranted. Knowledge of how to precisely modify biological mechanisms will contribute not only to advances in fundamental plant biology, but will also help identify potential unintended consequences of that manipulation.

In addition to conducting research, ARS and its university cooperators will continue to mentor and train the next generation of crop breeders, geneticists, bioinformaticians, genome researchers, and genetic and genomic resource and information managers. Developing the next cohort of researchers is particularly important, considering the projected strong demands for these scientific personnel.

Many of the NP 301 projects include significant domestic and international collaborations including government, industry and academia. These collaborations provide opportunities to leverage funding and scientific expertise for USDA-ARS research and accelerate dissemination of ARS research results, thus enhancing the impact of ARS research programs. During FY 2021, NP 301 scientists participated in research collaborations with scientists from the following 66 countries:

Finland Argentina Australia France Austria Georgia Bangladesh Germany Belgium Ghana Brazil Greece Canada Guatemala Chile Haiti China Honduras Colombia Hungary Costa Rica India Czech Republic Israel Denmark Italy **Dominican Republic Ivory Coast** Ecuador Japan Egypt Kazakhstan Ethiopia Kenya

Mexico Mozambique Netherlands **New Zealand** Nicaragua Nigeria Norway **Pakistan** Panama Peru **Philippines** Poland Portugal Qatar Romania Saudi Arabia Senegal

South Africa
South Korea
Spain
Sri Lanka
Sweden
Taiwan
Tanzania
Thailand
Turkey
Uganda
United Arab Emirates
United Kingdom
Uzbekistan
Vietnam
Zambia

The quality and impact of NP 301 research was evidenced during FY 2021 by the following:

- → 672 refereed journal articles published
- → 46 cultivars and breeding lines released
- → 11 new invention disclosures submitted
- → 3 new patent applications filed
- → 4 new patents issued

During fiscal year 2021, this National Program was composed of four Research Components (see below) and their constituent Problem Statements. Notably, these categories do not act as barriers or "stovepipes" that impede research across the National Program. Rather, many of the individual research projects and resources contribute to the goals of several NP 301 Research Components, or even several National Programs:

**Component 1.** Crop Genetic Improvement

Component 2. Plant and Microbial Genetic Resource and Information Management

**Component 3.** Crop Biological and Molecular Processes

Component 4. Information Resources and Tools for Crop Genetics, Genomics, and Genetic Improvement

Research in Component 1 lead to improved plant varieties, which were the products of innovative exploitation of genetic resources and efficient plant breeding strategies. High throughput genotyping and phenotyping methods were developed for quantitative analyses of complex traits in diverse crop species, and high-resolution genetic maps and full or partial genome sequences enabled genetic markers to be identified and mapped. NP 301 scientists in Component 1 devised innovative ways to apply genomic information from model plants for developing superior methods that were used to identify and breed exotic alleles into adapted genetic backgrounds, helping to improve the efficiency of plant breeding.

The strength of Component 2 lies in the National Plant Germplasm System (NPGS), which is comprised of one of the most comprehensive plant genetic resource collections in the world. Genebank curators and NP 301 researchers also conserved, characterized, and distributed cultures of pathogenic or beneficial microbes that are important to crop production, industrial processes, food safety, and human health. Information associated with NPGS collections were stored, curated and made publicly available via GRIN-Global, a database application that enables genebanks to store and manage information associated with plant genetic resources and deliver that information globally.

Component 3 research sought to understand the functions of crop genes and devise ways to manipulate gene expression. Research conducted under this component elucidated the biological processes underlying crop productivity and quality, and developed new means for assessing the potential effects and risks associated with plant genetic engineering.

Component 4 rendered improved bioinformatic tools and services built on powerful computational infrastructures that are needed to handle changing data types and increased volumes and scales of datasets that present tremendous opportunities for more rapid crop genetic improvement. ARS led in

developing data exchange protocols that facilitated access, analysis, and integration of these data sources. Building on these principles, NP 301 research delivered bioinformatic solutions for the full range of crops important to U.S. agriculture.

The following sections contain selected NP 301 accomplishments, presented according to the relevant NP 301 Research Components. These are not all of the NP 301 accomplishments for FY 2021, but rather are selected based on impact and level of contribution to meeting the National Program goals.

### **Component 1. Crop Genetic Improvement**

'Yorizane', a new self-fertile almond cultivar. Eighty percent of the world's almonds are grown in California, and the majority of the 1.5 million acres are planted with cultivars that require bees to transfer pollen between different trees to produce nuts. Self-fertile varieties require fewer pollinators in the orchard because the pollen moves only a short distance within the flower or within the tree to produce nuts. The California almond industry wants new self-fertile cultivars to reduce the need for honeybees and pollinators. ARS researchers in Parlier, California, developed the new self-fertile 'Yorizane' cultivar, which yielded well in regional trials throughout the San Joaquin Valley for five commercial harvests. Yorizane nuts have been rated highly by the almond industry in marketing potential and kernel appearance and it has great potential for adoption by almond growers.

Molecular marker developed to select for resistance to low falling numbers in wheat. The falling number test is a measure of wheat grain quality. When wheat grain undergoes preharvest sprouting or germination before harvest, the grain deteriorates and low grain falling numbers occur, leading to major losses to growers. ARS researchers in Pullman, Washington, and collaborators at Washington State University and the John Innes Centre identified a new mutant allele in wheat that has a specific single nucleotide change improving resistance to low falling numbers. Findings about this perfect DNA marker were published in *Theoretical and Applied Genetics*, and it is being adopted by wheat breeders to select for increased preharvest sprouting tolerance to avoid producer losses from low falling number test results.

New and improved potato variety released. The U.S. potato industry, valued at \$4 billion annually, needs improved potato varieties. ARS researchers in Aberdeen, Idaho, and university colleagues in Idaho, Washington, and Oregon recently released 'Rainier Russet', a new potato variety that produces high marketable yields in both early- and full-season harvests, enabling its production in a greater diversity of growing regions. Rainier Russet has cold-induced sweetening resistance and uniform fry color, which enables longer-term storage before they are used to make French fries. This is an improvement over other industry standard varieties, where converting starch to sugar results in a darker processed product. Rainier Russet is also notable for long tuber dormancy, which promotes the retention of tuber quality in storage with reduced sprouting. Rainier Russet has an attractive tuber that also makes it suitable for use for fresh consumption, as well as for processing.

Gene level modeling improves predicting field performance. Maize has 37,000 genes that interact together as the plant grows and responds to the environment. One of the key goals of breeding is predicting these interactions from just the information included in DNA. ARS researchers in Ithaca, New York, and collaborators used novel statistical approaches to study more than 70 million measurements

of how much RNA each of these genes produce under various conditions. Their results have substantially improved predictions of how thousands of varieties of maize will produce more than two dozen traits. Importantly, this approach could be applied for many other crops by enlisting the powerful genomic tools available. Long-term, this will enable the application of advanced genomic models to all crops.

New peanut cultivars with disease resistance. Late leaf spot (LLS) is one of the costliest diseases of U.S.-grown peanut. ARS scientists in Tifton, Georgia, created peanut breeding line IAC 322, which contains three chromosome segments from a wild species that provide a very high level of resistance to LLS. Two lines with very high levels of resistance to LLS were selected from this population, evaluated for other agronomic characters, and released with corresponding genetic markers as a technology package for determining the presence or absence of the introduced chromosome segments. These results will allow breeders to use marker-assisted selection to develop peanut varieties with resistance to LLS more effectively and efficiently, thus reducing management costs of fungicide sprays and increasing sustainability and profitability.

Release of commercial sugarcane variety 'HoCP 14-885'. ARS scientists in Houma, Louisiana, in collaboration with the American Sugar Cane League of the U.S.A., Inc. and Louisiana State University Agricultural Center, developed and released a new sugarcane variety in 2021. The new variety, HoCP 14-885, is resistant to sugarcane smut caused by *Sporisorium scitamineum*, mosaic caused by sorghum mosaic virus (SrMV), and brown rust caused by *Puccinia melanocephala*. It has moderate cold tolerance, early maturity, and high sugar yields through multiple harvests. Early maturity is important in Louisiana because the harvest season can be reduced due to late season freezing temperatures. The release of this cultivar offers growers a well-adapted variety that exceeds the sugar yields of the current leading variety across the harvest cycle. Due to its yield stability and resistance to major diseases, HoCP 14-885 can increase industry profits and expand the genetic variability of sugarcane within the growing region.

Release of upland Cotton lines with superior fiber strength and/or length. High-quality cotton with stronger, longer, and finer fiber commands premium prices and brings extra profit to farmers. High cotton fiber yields and superior quality are negatively corelated and breaking this negative relationship has been a major challenge in cotton breeding programs worldwide. ARS scientists in Starkville, Mississippi, created a random mating cotton population consisting of 550 lines, and ARS scientists in New Orleans, Louisiana, evaluated this population to identify DNA markers associated with fiber quality traits, especially strength and length. The researchers in New Orleans assessed both DNA markers and field data to identify seven cotton lines with improved fiber strength and/or length, acceptable yield, and other agronomic traits. In 2021, these cotton lines were released to the public and are now being incorporated into breeding programs for cotton variety improvement.

Identification of a major-effect quantitative trait locus (QTL) for heat tolerance in soybean. Elevated temperatures during seed maturation can damage soybean seeds by escalating seed wrinkling, green seed, and loss of vigor and viability. Future climate models predict the increased potential for heat stress and damage to food crops, such as soybean. ARS researchers in Stoneville, Mississippi discovered a breeding line with high seed germinability under conditions of elevated temperatures. It was derived from a soybean plant introduction from the USDA National Plant Germplasm System and used by ARS researchers in Columbia, Missouri, to create a 172-member genetic population for mapping traits. They evaluated emergence and germination for this genetic population under heat stress conditions and identified a major novel QTL affecting tolerance to heat-induced degradation of soybean seed. These

findings greatly increase the feasibility of analyzing molecular markers to select for heat tolerance, thereby accelerating the development of heat tolerant soybean cultivars for farmers worldwide. This information was disseminated by peer-reviewed publications, and ARS scientists in Stoneville and Columbia have increased their efforts to fine map the genomic location of this genetic locus that controls the trait.

Field-based high-throughput plant evaluation for chlorophyll fluorescence. Photosynthetic efficiency is important for improving crop yields in hot and dry environments and is very challenging to measure in the field. The LEMNA-TEC Field "Scanalyzer" is the largest field robot in the world. It is operated by the University of Arizona in Maricopa, Arizona, and is equipped with a chlorophyll fluorescence imaging system that needed validation for effective application to large-scale plant phenotyping. ARS researchers in Maricopa and collaborators from the University of Arizona and the Donald Danforth Plant Science Center developed the software and data processing pipeline to extract measurements needed to determine photosynthetic efficiency, thus validating the system for field phenotyping. This work has established a standard that enabled field trials to capture the temporal dynamics of chlorophyll fluorescence for plants grown in a hot and dry environment. To date, it has been applied to research on lettuce, sorghum, and sunflower. This system provides a valuable new tool for plant researchers to develop novel germplasm adaptable to sustainable crop production in hot and dry environments.

<u>Unmanned aerial systems</u> (UAS) effectively evaluate sugarcane aphid damage in sorghum. Since 2013, the invasive sugarcane aphid has become a serious pest on all types of sorghum. Development of cultivars with genetic resistance to sugarcane aphid is one strategy to reduce losses, but evaluating sugarcane aphid populations, plant damage, and other traits in the field is labor-intensive, and visual ratings can be subjective. In collaboration with University of Georgia scientists, ARS scientists in Tifton, Georgia, deployed unmanned aerial systems (UAS) and took ground measurements to evaluate sugarcane aphid damage. The study demonstrated that the spectral data collected by the UAS were strongly correlated with ground-based damage ratings. These results demonstrated that the UAS can quickly and accurately assess sugarcane aphid damage, which could help accelerate developing aphid resistant germplasm and cultivars and evaluate the efficacy of other sugarcane aphid management strategies.

Factors associated with potato tuber greening elucidated. Potato tubers exposed to light turn green and are considered toxic to humans due to an increase in glycoalkaloid content. This can result in significant culls and monetary loss and has caused trade issues. Tuber greening is estimated to cause losses ranging from 14 to 17 percent to the U.S. potato industry annually. ARS researchers in Wapato, Washington, and Washington State University researchers assessed phenotypic differences across five potato cultivars. Results showed that the below ground plant architecture of some cultivars position tubers closer to the soil surface, leading to premature and excessive tuber greening; some varieties were eight times more likely to green because of this root architecture, regardless of grower management. These research findings help farmers know which potato varieties are better for certain situations, reduce economic losses due to greening, and preserve the safety of the food supply.

Genetic characterization of durable resistance to hop powdery mildew. Hop breeding has traditionally focused on implementing types of resistance that pathogens can eventually evolve to overcome. ARS researchers in Corvallis, Oregon, and university collaborators identified and genetically characterized one source of resistance to powdery mildew and identified molecular markers for this trait. This

resistance appears to be under the control of three separate regions of the hop genome, with each region possessing multiple copies of genes similar to plant resistance genes found in other crop plants. Markers for this resistance to powdery mildew in hop will enable breeders to select for and incorporate this trait into new varieties for more durable resistance to hop powdery mildew.

USDA's genetic resources tapped for sources of sugar beet genes conferring disease resistance. Developing new sugar beets lines to defend against emerging diseases is critical to U.S. sugar production, but conventional breeding for resistance can be slow and arduous. ARS scientists in Fort Collins, Colorado, applied novel screening approaches to rapidly identify DNA markers for disease resistance from sugar beet genetic resources within the USDA National Plant Germplasm System. This important scientific advancement sets the stage for a new strategy to exploit available datasets of genomic data from ARS sugar beet population releases to dramatically speed up—from decades to years--the process of public pre-breeding sugar beets for disease resistance.

<u>Sequencing the pecan genome</u>. Traditional breeding efforts in pecans can take many decades because of its long juvenile period. ARS researchers in College Station, Texas, working with university and industry cooperators, sequenced the genomes of four different pecan genetic types that are representative of the genetic diversity of cultivated pecan. The cultivar 'Pawnee' was analyzed in sufficient detail to identify the regions of its genome that were inherited from each parent. This research was published in the journal *Nature Communications*. Characterizing the pecan genome will guide future pecan breeding research for developing better pecan cultivars at an accelerated pace for U.S. farmers.

New barley greenbug resistance gene in wild barley. Greenbug causes barley yield losses and facilitates the spread of several viruses destructive to barley. Planting greenbug-resistant cultivars is the most economical and environment-friendly way to reduce losses. Currently, more than 30 different types of greenbug biotypes have been identified, and two greenbug resistance genes, Rsg1 and Rsg2, are available for barley breeding, with each one conferring resistance to only a few greenbug biotypes. But greenbug biotype H is virulent to both Rsg1 and Rsg2, making it urgent to identify novel greenbug resistance genes. ARS scientists in Stillwater, Oklahoma, and Manhattan, Kansas, discovered a new barley greenbug resistance gene from wild barley collected in Turkmenistan and developed markers for its introgression into elite barley cultivars. The newly-discovered gene confers resistance to economically important greenbug biotypes, and it is the only gene conferring resistance to greenbug biotype H. This resistance makes it feasible to combine it with other resistance genes to develop barley cultivars resistant to all economically important greenbug biotypes.

A barley gene regulating chloroplast development. Plant chloroplasts are miniature factories of photosynthesis and critical for crop biomass and grain yield. Nonetheless, chloroplast development is a complicated process, and the underlying chloroplast formation pathways are not fully understood. ARS researchers in Fargo, North Dakota, genetically characterized a mutated strain of barley known as grandpa1 (gpa1) that is defective in chloroplast formation. They found the defective Gpa1 gene is located on chromosome 2H and provided explanations for its possible function. This research, published in *BMC Plant Biology*, provides knowledge for barley geneticists to potentially manipulate this gene to improve photosynthesis for higher yields.

New environmentally friendly 'slow darkening' pinto bean cultivar released. Pinto beans are the most widely grown dry bean market class in the United States. ARS researchers in Prosser, Washington, released a new pinto bean cultivar, 'USDA-Diamondback', which is characterized by superior seed quality and performance under both low and high input production systems. USDA-Diamondback exhibits unique tolerance to drought and low soil fertility, which allows it to be grown with less water and fertilizer. Unlike most other pinto beans, USDA-Diamondback has the new slow darkening seed coat trait that helps it maintain excellent seed quality despite adverse weather during harvest or prolonged storage. A major seed company is interested in licensing this versatile pinto bean cultivar.

Genetic diversity in strawberry characterized in ARS National Plant Germplasm System. Strawberry, one of the most valuable annual U.S. specialty crops, also consistently ranks as one the five most-frequently purchased fresh-market fruits in the United States. ARS scientists in Corvallis, Oregon, characterized the genetic diversity in more than 500 different varieties of cultivated strawberry in the ARS National Plant Germplasm System (NPGS). Eight genetic groups of varieties, each originating from geographically divergent breeding locations, were identified, and pedigree relationships for the varieties were confirmed. Two core subsets of 100 different varieties were identified from the collection, one with a uniform distribution of the different genes, and the other containing maximum genetic diversity. Genetic resources containing genes for resistance to the strawberry diseases anthracnose fruit rot, *Colletotrichum* crown rot, charcoal rot, and *Phytophthora* crown rot were also identified. This new information about NPGS genetic resources for cultivated strawberry will enable breeders and researchers access the genetic diversity needed to effectively develop new strawberry cultivars with durable disease resistance and other valuable horticultural traits.

Soybean genes involved in drought stress tolerance. Drought causes significant soybean yield losses each year in many rain-fed production systems, so breeding soybeans for drought tolerance is a cost-effective approach to stabilize yield under rain-fed management. Identifying genes controlling drought tolerance traits, such as slow canopy wilting, will increase soybean productivity under drought stress conditions. ARS researchers in Maricopa, Arizona; Stoneville, Mississippi; and Columbia, Missouri worked with University of Arkansas and University of Missouri collaborators on a multi-state trial to evaluate 200 diverse soybean accessions under irrigated and rain-fed conditions to identify and confirm molecular markers and candidate genes associated with slow canopy wilting traits. Among the 183 identified candidate genes, 57 single-nucleotide polymorphic (SNP) markers were co-located within genes coding for proteins with biological functions involved in plant stress responses. These findings were published in *Crop Science Journal*. The SNP markers denoting the confirmed genomic regions can be assayed by soybean breeders to improve drought tolerance in soybean.

New method developed to identify multiple known genes in barley. Barley breeders and geneticists need cost effective and efficient methods to identify known genes in breeding populations. Genotyping methods based on resequencing and individual gene analysis are popular, but there is a need for a cost-effective genotyping platform that can simultaneously identify groups of genes that have already been identified. ARS researchers in Pullman, Washington, in collaboration with U.S. barley breeders, developed and tested a new genotyping by multiplexed sequencing method combined with a data analysis pipeline that can accurately identify alleles in 267 genes of interest to barley breeders. This platform, published in *Euphytica*, is being used by barley breeders to conduct genetic analysis and select for winter tolerance, improved malting quality, and desirable agronomic traits.

Completion of a high-density genetic map of hydrangea. Flowering time and flower type are the most valuable traits in breeding bigleaf hydrangea (*Hydrangea macrophylla*). Marker-assisted selection can greatly reduce the time necessary to breed cultivars with the desired inflorescence characteristics. ARS scientists in McMinnville, Tennessee, developed a genetic map of hydrangea and verified the location of a marker that was highly associated with inflorescence type. The map also enabled the discovery of three regions that are associated with the reblooming trait. This map and associated information will lead to more efficient development of additional markers for high-throughput screening, as well as the discovery of candidate genes to enable genome editing for precision breeding in hydrangea.

Analysis of wild U.S. relatives of cranberries expands access to genetic traits. Cranberries are native to North America, where multiple wild cranberry species grow. They are often fertile if crossed and are widely distributed in the United States, but more information is needed to determine which populations contain traits that could help future breeding efforts. ARS researchers in Madison, Wisconsin, analyzed 21 wild populations of domesticated American cranberry and 24 populations of the closest cranberry relative across much of their native U.S. ranges. Wild relatives were found to contain high levels of genetic diversity both between and within species. The genetic analyses also confirmed the field identification of a native population of cranberries on the Okanogan-Wenatchee National Forest in the state of Washington, far outside the previously reported range for the species. This information can inform effective strategies for cranberry conservation and breeding for increased yield, nutritional value, and climate resilience. These results will also inform efforts by ARS and the U.S. Forest Service to conserve the most diverse and unique wild cranberry populations in designated National Forest sites.

Combined high-yield with high-protein and diverse pedigree. United States soybean growers need cultivars with both high yields and high protein to increase competitiveness in global and domestic soybean markets, but success in this research area has been limited. ARS researchers in Raleigh, North Carolina, developed a MG-V line, known as N16-590, that contains significantly higher protein than high-yield check cultivars. This conventional line was released for commercial planting by soybean growers and/or by private and public soybean breeders as a parent to improve protein without sacrificing yield. Part of its pedigree is derived from an exotic parent, so this line also adds diversity to the soybean breeding gene pool. It is estimated that a 1 percent increase in meal protein without loss in yield may increase the value of United States soybean from \$2 to 3 billion.

Genetic diversity in wheat for major economic traits. Crop breeders need genetic diversity to improve agronomic traits and to select for pest resistance. Long-term selection for high grain yield and adaptation can result in reduced genetic diversity and breeding progress. ARS researchers in Pullman, Washington, examined genome-level and agroecosystem-level genetic diversity of Pacific Northwest (PNW) wheat for the past 120 years to identify significant changes. As they reported in *Crop Science*, long-term shifts in genetic diversity were not detected, but fluctuations were significant within market classes and within a subset of the most widely grown spring and winter varieties. At the agroecosystem level, diversity has been rising since the 1990s as the dominance of a few varieties has declined. Breeding programs that cultivate multiple market classes and periodically incorporate new germplasm have sustained useful levels of genetic diversity in PNW wheat over time, indicating that breeding progress will likely continue.

Genomic regions controlling sex expression in hop characterized. Female cultivars of some *Cannabaceae* crops can be chemically induced to produce male flowers and pollen, which enables breeders to directly combine the characteristics of developed cultivars and obtain only female offspring from the cross. This technique has not been successful in breeding hop, which is related to other *Cannabaceae* crops. Understanding the genetic control of sex in hop would potentially provide breeders tools to perform similar techniques in hop. ARS researchers in Corvallis, Oregon, and university collaborators identified regions of sex chromosomes that do not recombine and contain concentrations of genes controlling flowering or pollen action. This basic information provides a first look at the genetic control of flowering in hop and will ultimately aid breeders to make crosses resulting only in female offspring.

<u>Development of sorghum lines with increased seed number</u>. Sorghum, a "climate-smart" cereal crop, is an essential source of food and feed in numerous nations. Sorghum is often cultivated on marginal soils and harsh environments, and production depends on its tolerance to high temperature and drought stress. ARS researchers developed four new inbred lines that possess increased grain number, resulting in significant increases in the number of seeds per panicle. They reported these results in the *International Journal of Molecular Sciences*. These lines will be made available for public release and will be ultimately useful for breeding sorghum for increased production traits.

Improved understanding of the genetic control of virus resistance in common bean. Bean common mosaic virus (BCMV) reduces common bean yield and quality in the United States and worldwide. The best strategy to control the disease is to develop new varieties with improved resistance, which unfortunately is controlled by complex interactions between multiple resistance genes. The most effective way to bypass this complexity is through marker-assisted breeding, but knowledge about the resistance genes was lacking. ARS researchers at Prosser, Washington, identified resistance genes and developed DNA markers for two different resistance genes, bc-1 and bc-u. They determined that the bc-u gene itself is sufficient to delay symptoms, and resistance is significantly enhanced when it is combined with bc-1. These findings solved a key question about the genetic control of resistance to BCMV that has puzzled bean breeders for more than 40 years. The new DNA markers are being used by bean breeders to accelerate the development of new bean varieties with improved disease resistance.

Analysis of the historical breeding stages of the recently domesticated American cranberry. The American cranberry is a recently domesticated species with less than 200 years of breeding history. ARS researchers in Madison, Wisconsin studied a historical collection composed of 362 accessions, spanning wild germplasm and first-, second-, and third-generation selection cycles, to unravel the breeding and domestication history of cranberry. Genetic analyses revealed differences among the stages of cranberry domestication and subsequent breeding and revealed a progressive loss of diversity when transitioning from early domestication stages to current cranberry forms. Loss of diversity can be detrimental to the resilience of the cranberry crop and future breeding efforts. Genetic analyses identified genetic markers associated with average fruit weight and fruit rot, which are two traits of great agronomic relevance and could be further used to accelerate cranberry genetic improvement and benefit growers and consumers.

<u>Twenty-six new corn reference genome sequences</u>. Although a single reference genome sequence for a species is a valuable scientific tool, it does not capture the full complement of genes and regulatory elements within a species that are accessible to breeders. ARS scientists in Ithaca, New York, worked with academic partners in Cold Spring Harbor, New York; Athens, Georgia; St. Paul, Minnesota; and Ames, Iowa, and used information about gene transcripts and proteins from other species to develop

refined methods for predicting corn genes. The group applied this method to 26 new maize reference genome sequence assemblies that contain a large fraction of the genetic variation in corn and which have been phenotyped by researchers across the globe for agronomic traits. Across the 26 genome sequences, a total of 103,000 pan-genes were identified, an increase of 40,000 genes from previous analyses. Approximately 32,000 genes belong to the core/near-core portion of the pan-genome. The other 71,000 genes, which are associated with agronomic value, are found within only one or only a few of the 26 genome sequences.

Genetic regulation of mineral accumulation in rice grains. There is widespread interest in increasing mineral nutrients in edible grains while limiting the accumulation of toxic elements, such as arsenic or cadmium. ARS researchers in Stuttgart, Arkansas, and collaborators from Nanjing Agricultural University (China), University of Nottingham (U.K.), and Dartmouth College identified and molecularly tagged genes affecting the concentrations of 16 mineral elements in grains, roots, and shoot tissues. They found mineral levels differed more between roots, shoots, and grains than between grains produced under flooded versus unflooded fields. This indicated that tissue-to-tissue transport mechanisms regulate grain element concentrations more strongly than field irrigation practices known to affect element bioavailability and uptake. Individual chromosomal regions were identified that affect uptake of multiple elements. This information provides guidance to breeders about the most effective genetic means for improving the nutritive value of rice.

A new form of a durum wheat gene for controlling stem rust resistance. Stem rust, one of the most damaging diseases in durum and bread wheat, is a threat to wheat production worldwide. New strains of the fungal pathogen, such as Ug99, have caused epidemics in East Africa, Europe, and Central Asia in the last two decades. ARS researchers in Fargo, North Dakota, identified two new forms of Sr13, one of the most important stem rust resistance genes in durum wheat. Stem rust testing with multiple strains of the pathogen showed that one of the forms, Sr13c, provided resistance to all current strains of the pathogen, but the second new form, Sr13d, did not provide resistance to Ug99. These results, which were reported in *Plant* Journal, show that Sr13c can be used by breeders to develop new wheat varieties with robust stem rust resistance.

New stem rust resistance genes in a synthetic wheat line. Largo, a synthetic wheat line created by artificially reconstructing the ancestral hybridization of three wild species, was found to have resistance to stem rust. ARS researchers in Fargo, North Dakota, identified five stem rust resistance genes in Largo. Among these genes, three were previously identified genes—Sr9e, Sr13c, and Sr46—and two are potentially new Sr genes. These findings, which were reported in the *Genes/Genomes/Genetics Journal*, will help wheat breeders develop new stem rust-resistant wheat varieties.

Development of sugarcane aphid tolerant forage sorghum with diverse quality traits. Sorghum forage crops are a major commodity in the United States; its high yields, growth under drought conditions, and excellent forage quality, allows the crop to be grown in many areas of the country. Unfortunately, the majority of sorghum forages grown today are susceptible to the highly destructive sugarcane aphid. ARS researchers in Lubbock, Texas, developed a diverse set of forage sorghum inbreds and hybrids that are highly resistant to the sugarcane aphid. These breeding lines will be used for further sorghum improvement by ARS researchers, and will also be provided to sorghum stakeholders and sorghum breeding programs.

New Upland cotton germplasm with improved tolerance to Fusarium wilt. Fusarium oxysporum f. sp. Vasinfectum, race 4 (FOV4) causes Fusarium wilt disease in cotton. This disease is devastating to cotton production and to date, there is no source of resistance in Upland cotton. FOV4 was recently identified in West Texas and poses a threat to the cotton industry. ARS scientists in Lubbock, Texas, identified and released 17 Upland cotton germplasm lines with improved resistance to FOV4. This is the first set of Upland germplasm released to the public with FOV4 resistance.

Establishment of rice plants as an important tool for functional genomic studies to understand and manage plant disease resistance. The genetic complexity of the common bread wheat, which contains three different genomes, has been extremely challenging to researchers for carrying out studies to conclusively pinpoint the role of numerous insect/pest-responsive genes that are potentially involved in disease resistance. ARS researchers in West Lafayette, Indiana, demonstrated that this problem can be overcome by analyzing less complex model genomes, such as rice. Since rice resembles bread wheat in its responses to Hessian fly infestation at the physical and molecular level, the scientists found that rice plants are suitable for functional studies of genes that can help elucidate how wheat defends against Hessian fly and other destructive insect pests. This strategy, published in the *Journal of Plant Interactions*, can be employed by other plant researchers to characterize genes, study other plant-pest/pathogen interactions, and develop effective mitigation strategies that complement native resistance.

Greenhouse method to evaluate sunflower resistance to *Sclerotinia* basal stalk rot. Field trials for evaluating sunflower resistance to *Sclerotinia* basal stalk rot are time consuming and offer limited resolution for identifying resistance. ARS scientists in Fargo, North Dakota, and North Dakota State University and Iowa State University colleagues developed and validated a new method to evaluate basal stalk rot resistance. The new method is time- and space-efficient and enables testing multiple structured breeding populations for genetic mapping in a single year, compared to the need for multiyear, multi-location, inoculated field trials. Results from the new method were strongly correlated with field observations and the new method improved identification of highly resistant genotypes. The newly developed method has been applied to evaluate multiple mapping populations to improve the identification of genome regions that provide resistance to basal stalk rot.

Genomic selection incorporated into maize breeding. Genomic prediction, or genome wide selection, has been used for more than a decade in the U.S. seed industry to speed cultivar development. The Germplasm Enhancement of Maize (GEM) breeding process has been based on traditional breeding methods, but genomic marker assisted breeding has not been employed. Seed samples of 1,796 lines (representing 29 breeding populations) and associated yield trial evaluation data were submitted to a seed industry GEM Cooperator for genomic evaluation and prediction model building. Genomic prediction models for the breeding populations showed that yield was highly heritable and reliably predicted by the model. Heritability and prediction accuracy are lower than expected in elite modern United States germplasm, but are accurate enough to eliminate low yielding breeding lines prior to costly yield testing. Lines selected for yield trials will have higher average yield potential than the original population, which will result in higher yielding lines released by the GEM Program.

Release of high yielding soybean germplasm with highly diverse parentage. The limited genetic diversity in U.S. soybean cultivars is well documented and continues to limit advances in soybean breeding. Modern soybean cultivars from Japan are genetically very distinct from United States cultivars and are

an important source of diversity for applied breeding in the United States to create novel diversity. USDA-N7005, released in 2021, has a higher proportion of plant introduction germplasm—62.5 percent—from the U. S. National Plant Germplasm System genetic resources in its pedigree than any other southern U.S. germplasm released in the past 40 years. This germplasm release exhibits superior yield and agronomic traits, as well as resistance to root knot nematode and stem canker. This new release is a desirable breeding stock that public and commercial soybean breeders will find useful in developing new, diverse, and high yielding cultivars.

Sources of superior disease resistance identified for alfalfa. Alfalfa, the nation's third most valuable field crop, has an annual value of more than \$10 billion as a high-protein feed for dairy and beef cattle. It also protects soil by fixing biological nitrogen so that less fertilizer is required for subsequent crops. Spring blackstem and leaf spot disease causes yield and quality losses in alfalfa. Current commercial alfalfa cultivars lack effective genetic resistance to this disease, and chemical control is not economically feasible, so sources of disease resistance are needed for commercial cultivars. ARS researchers in Prosser, Washington, and St. Paul, Minnesota, developed improved methods to screen alfalfa for important diseases and identified new sources of resistance to spring blackstem and leafspot disease. These resistant plants have been incorporated into new breeding stocks that can be used to breed disease-resistant commercial alfalfa varieties.

Genetic mapping of resistance to *Sclerotinia* head rot and *Phomopsis* stem canker diseases. *Phomopsis* stem canker and *Sclerotinia* head rot are the two most serious diseases of sunflower and are a recurring problem in the Northern Plains due to an increasing trend of wet conditions in late summer. Historically, sunflower breeders breed for each of these diseases separately and hybrids typically do not possess resistance to both diseases at the same time. ARS scientists in Fargo, North Dakota, and University of Colorado researchers conducted genetic studies on a panel of domesticated sunflowers to identify regions of the genome associated with resistance to both diseases. Several common genomic regions were identified for resistance to both diseases, suggesting that several loci together may contribute to *Phomopsis* and *Sclerotinia* resistance in the same plants. This work provides genetic resources and genetic markers that will enable breeding for disease resistance to both diseases in the same plants, ultimately helping farmers reduce damage from these diseases, preserve high yields, and simplify crop disease management.

First USDA food-grade winter pea varieties released. Fall-sown (winter) peas have been sporadically grown in the United States for more than 20 years but for animal feed only, as no winter pea varieties were considered food grade, or acceptable for human consumption. ARS scientists in Pullman, Washington, developed and released three winter pea varieties: 'USDA Dint', 'USDA MiCa', and 'USDA Klondike.' USDA Dint and USDA MiCa are green peas and USDA Klondike is a yellow pea. These are the first food grade winter pea varieties released by USDA. These new winter pea varieties provide growers with an alternative fall-sown crop to winter wheat or winter barley and will confer additional benefits associated with using legumes in crop production systems, such as the natural production of nitrogen fertilizer and control of grassy weeds.

## **Component 2. Plant and Microbial Genetic Resource and Information Management**

Discovery of 33 toxin-producing, plant pathogenic fungi. During the past three decades, epidemics of the fungal disease Fusarium head blight (FHB) have caused economically devastating damage to wheat and barley in the United States and elsewhere around the world. FHB significantly reduces seed quality and yields and contaminates grain with toxins that pose a serious global threat to agricultural biosecurity, food safety, and human health. ARS researchers in Peoria, Illinois, characterized the genetic diversity of a global collection of 171 Fusarium strains either known or predicted to produce toxins. The 171 strains comprised 74 different species, including 33 that are new to science, and the species were distributed among 6 species groups that corresponded to the type of toxins they produce. The researchers found that species within only two of the six groups could cause FHB of wheat and contaminate grain with toxins. These data further suggest that the type of toxin produced contributes to the ability of these plant pathogens to cause disease. These findings will be of interest to plant disease specialists and quarantine officials who are focused on minimizing the threat these toxigenic molds pose to U.S. and world agriculture. Moreover, knowledge gained from this research should assist plant breeders in developing cultivars with broad-based resistance to FHB.

Microwave irradiation for eliminating bacterial leaf scorch from pecan graftwood. Pecan is an important crop for large-scale and small-scale producers in the southeast, southwest, and south-central United States, and in 2018 had a national farmgate value of \$425 million. Pecan bacterial leaf scorch disease caused by *Xylella fastidiosa* is widespread in U.S. pecan production areas and can be transmitted from pecan scions to rootstocks via grafting. ARS researchers in College Station, Texas, and university colleagues developed a thermal treatment that employs microwave irradiation and microwave absorbers to kill or deactivate *X. fastidiosa* in pecan scions. These techniques provide researchers and industry personnel with additional options for treating infected pecan graftwood to reduce the spread of the leaf scorch disease. Microwave irradiation is faster and requires less energy than current hotwater methods for treating *Xylella*-infected pecan graftwood. This innovation also furnishes a scientific foundation for developing novel treatments that incorporate microwave irradiation and microwave absorbers to control vascular pathogens of other crop species.

Superior germination protocols for evaluating the quality of seeds from wild plants. Wild plant species tend to produce seeds that germinate slowly or at different times. There are few protocols for accelerating or synchronizing germination to determine seed viability, so staining methods have substituted as a proxy approach for estimating seed quality. ARS scientists in Fort Collins, Colorado, developed and adapted protocols that can stimulate germination of seeds of wild species. The protocols combine temperature treatments, hormonal applications, and clay-based media or embryo extraction and growth in culture. These protocols can increase the accuracy and efficiency of seed quality assessments, especially of stored seeds, and enable wild-collected seeds to be germinated more readily for regenerating genebank accessions and producing plants for vegetation restoration programs.

<u>Identification of corn aroma compounds that alter fall armyworm behavior</u>. Fall armyworm is a major global threat in the production of corn and other crops. It is currently having a devastating impact in sub-Saharan Africa, where small-holder farmers often lose their entire crop to these voracious pests. The fast spread and nocturnal habits of these long-distance flyers mean that rapid detection of these moths is vitally important for their control. ARS scientists in Gainesville, Florida, identified several odors produced by corn that alter fall armyworm behavior. A number of these odors attract fall armyworm

and therefore could be used to trap and monitor fall armyworm populations. They could be part of an early warning detection system that would enable farmers to apply pesticides at the start of a fall armyworm infestation, potentially reducing damage to their crops. They also identified other compounds that can repel fall armyworm. These compounds are naturally produced by corn; consequently, long-term guided breeding strategies could be adopted to increase their levels, making corn plants less attractive to fall armyworm. These findings were reported in the *Journal of Chemical Ecology*.

High yield, high fiber quality germplasm lines containing 50 percent exotic parentage. New genetic diversity is desperately needed to broaden the genetic base and to improve the economic competitiveness of U.S. cotton. Upland cotton's narrow genetic diversity represents a major source of genetic vulnerability for the U.S. cotton industry. ARS researchers in Florence, South Carolina; College Station, Texas; and Maricopa, Arizona, in partnership with Cotton Incorporated and Clemson University, released five cotton lines with high yields and excellent fiber quality. They developed these lines from USDA National Plant Germplasm System (NPGS) genetic resources from Asia, Africa, and South America; 50 percent of the genetic makeup from these lines was derived from NPGS material that originated outside of the United States. The research showed that these new cotton lines have excellent fiber quality and excellent yield performance—significantly better than several commercial cultivars. These improvements provide public and private cotton breeders with several selections of improved germplasm lines that can serve as breeding parents to develop new commercial cultivars with increased genetic diversity, yield, and fiber quality.

Training the next generation of plant genetic resource managers. Plant genetic resource managers throughout the world are retiring in unprecedented numbers, threatening the successful transfer of institutional knowledge for safeguarding invaluable genetic materials in genebanks. To avoid losing this institutional knowledge and to communicate the latest technological advances in plant genetic resource management, ARS scientists in Fort Collins, Colorado, and Beltsville, Maryland, developed and released educational and training tools for plant genetic resource managers, users, and students. A public website, <a href="https://grin-u.org/">https://grin-u.org/</a>, was established to deliver training courses, eBooks, videos, and other educational resources to U.S. and international genebank personnel, universities, industry, other organizations, and individuals. These much-needed training resources will support and accelerate training the next generation of plant genetic resource managers.

Genetic fingerprinting of high-value coffee in Hawaii. Coffee significantly contributes to the U.S. economy; in 2015, the U.S. coffee industry estimated that coffee constituted around \$225.2 billion of the total U.S. economic output, approximately equivalent to 1.6 percent of the total gross U.S. domestic product. Fine-flavor Kona coffee, which is grown exclusively in Hawaii, is a high-value commodity that contributes substantially to Hawaii's agricultural economy. Nonetheless, Kona coffee is genetically quite uniform, leaving it potentially susceptible to virulent diseases, pests, and rapid environmental change. ARS researchers in Beltsville, Maryland, and Hilo, Hawaii, and Hawaii Agriculture Research Center collaborators analyzed the genetic content of coffee grown in Hawaii and coffee genetic samples safeguarded in the newly established ARS coffee genebank in Hilo, Hawaii. The analyses clarified the genetic relationships of coffee's genetic resources, the parentage of improved coffee varieties in Hawaii, and the genetic identity of Kona coffee. This information is valuable for guiding the expansion of the ARS coffee genebank collection and for supporting and advancing the Hawaii's coffee industry, which is an important part of the local economy.

<u>Vernonia - A novel trap crop for cotton agroecosystems</u>. The western tarnished plant bug *lygus* is a major pest of cotton in the United States and causes substantial yield loss if populations are not properly managed. Improvement of current integrated pest management (IPM) approaches could include the use of attractive plants as trap crops to lure pests away from the cash crop. ARS researchers in Maricopa, Arizona, identified Vernonia, an annual plant native to eastern Africa, as a potential trap crop for *lygus* and other pests such as the cotton flea hopper. Vernonia also provided a summer habitat for a wide range of pollinators not typically seen in cotton monoculture practices. The identification of Vernonia as a trap crop provides a novel IPM approach in cotton that may help growers reduce pest management costs and provide ecosystem services for pollinators. This work was published in *Journal of Insect Science*.

#### **Component 3. Crop Biological and Molecular Processes**

New reference genomes of pathogen that causes aflatoxin production in corn. Available genome sequences of *Aspergillus flavus*, one of the main pathogens that causes aflatoxin, have been invaluable for characterizing genes involved in aflatoxin production. However, an understanding of *A. flavus* diversity has been hindered by the lack of suitable and diverse references. ARS researchers in Tifton, Georgia, led a team of collaborators in constructing two reference genome sequences for two different *A. flavus* isolates. These isolates were chosen based on their variation in aflatoxin production and responses to reactive compounds associated with drought stress that increase aflatoxin production. These genome analyses revealed several key genes, including a novel gene that may be involved in oxidative stress tolerance and aflatoxin production. This information was used to sequence 264 genomes of different *Aspergillus* isolates from field soils in Georgia and corn plants in Mississippi. These data will contribute to additional comparative studies to identify novel aflatoxin regulators, shedding light on the origin and evolution of these new genes, and providing valuable tools for aflatoxin and crop research.

Maize silk expression atlas. Each year, growers across the globe produce approximately one million corn kernels per human on the planet. These corn kernels are produced through cross-pollination, which occurs when pollen from the male parent comes in contact with the silks from the female parent. Yet, despite their importance, the biological complexities of silk form and function are not well understood. ARS scientists in Ames, Iowa, and Iowa State University collaborators developed a comprehensive catalogue of how genes are turned on and off in corn silks. Published in *Plant Genome*, this "corn expression atlas" provides data for understanding how silks function in diverse environments. This research demonstrates, for the first time, the wide diversity of genes expressed during maize silk growth and function, including important roles in development, metabolism, physiology, and abiotic- and biotic-defense. These results are expected to be widely used in agricultural research focusing on stress responses and reproductive biology in plants.

<u>Novel disease resistance in barley</u>. Fungal pathogens are among the greatest threats to cereal grain production worldwide. ARS scientists in Ames, Iowa, applied genomic methods to identify a novel variant of SGT1, a protein vital for all life, in barley. SGT1 mutations are usually lethal because they can interfere with the fundamental role this protein plays in survival. However, the novel SGT1 variant found by ARS researchers contains a unique mutation that helps stabilize other disease resistance proteins and

increases overall robustness without triggering other potentially lethal changes. These findings demonstrate for the first time that this unique modification in the SGT1 protein can support disease resistance traits and can be used to facilitate greater disease resistance in crops.

A new type of wheat gene governing disease resistance. Septoria tritici blotch (STB) is a major fungal disease of wheat worldwide. ARS researchers in Fargo, North Dakota, and collaborators in France and The Netherlands discovered a gene from wheat that provides resistance to STB. The gene, Stb16, belongs to a class of genes that has not previously been associated with disease resistance in plants; it makes wheat resistant to many strains of the STB fungus and will enable plant breeders to develop new STB resistant varieties. This discovery was reported in *Nature Communications*.

The chromosome-level genome assembly of cranberry and its wild relative. Development of cranberry genetic resources started very recently, but genetic studies are now limited by the lack of a high-quality genome database, which is needed to facilitate understanding genetic mechanisms governing horticultural traits and further breeding efforts at the molecular level. ARS scientists in Madison, Wisconsin, reported the first chromosome-scale genome assembly of cranberry and a draft genetic code of its closest wild relative species. More than 92 percent of the entire estimated cranberry genome sequence was assembled into 12 chromosomes, which enabled gene model prediction and chromosome-level comparative analysis, divergence, and evolution with other plant species, including close relatives such as blueberry and kiwifruit. The chromosome-level cranberry genome sequence, and the draft genome sequence of its closest wild relative species, provide a much-needed resource for investigating the genetic architecture underlying trait variation. These genetic codes will support cranberry improvement efforts by facilitating the discovery of novel trait-gene associations applicable to molecular based breeding strategies.

Genomic mechanisms of climate adaptation in switchgrass. Understanding plant adaptation to long-term climate change and periodic environmental extremes has been limited to well-studied model systems. ARS researchers in Albany, California; Lincoln, Nebraska; Madison, Wisconsin; Griffin, Georgia; and Temple, Texas, collaborated with many other U.S. scientists to sequence the large and complex genome of the polyploid bioenergy crop switchgrass. Analysis of biomass and survival among 732 genotypes, which were grown across 10 common gardens that span 1,800 km of latitude, jointly revealed extensive evidence of climate adaptation. The scientists found that climate—gene—biomass associations were abundant and varied considerably among deeply diverged gene pools, and that gene flow accelerated climate adaptation during the postglacial colonization of northern habitats. The polyploid nature of switchgrass also enhanced adaptive potential because of the increased level of heritable genetic diversity.

Soybean gene restricting nitrogen fixation identified. Nitrogen is the most critical nutrient requirement for crop production. Legume crops such as soybeans can derive most of the nitrogen required for optimal growth and yield through symbiosis with nitrogen-fixing bacteria known as rhizobia. Despite knowing how rhizobia establish connections to form symbiotic root nodules, it was not clear why this is possible. ARS scientists and Huazhong Agricultural University (China) collaborators analyzed the DNA soybean genomes and identified a gene, GmNNL1, that limits the numbers of root nodules formed by rhizobia. When this gene is disabled, rhizobia can successfully colonize soybean roots, thus enhancing nitrogen fixation. These findings provide important insights into soybean—rhizobia compatibility and offer new options to design effective legume—rhizobia interactions for efficient symbiotic nitrogen

fixation. These findings will assist soybean scientists and breeders at government agencies, universities, and private institutes who want to improve soybean production with less fertilizer application.

Taking the tart out of cranberry fruit. Compared to most other fruit, cranberry fruit is high in organic acids, which causes cranberry fruit and fruit products to be very tart, thus requiring large amounts of added sugar to be more palatable. ARS scientists in Chatsworth, New Jersey, took advantage of natural variation in organic acid content in cranberry genetic resources to determine the genetic basis of acid accumulation. They identified chromosomal regions in cranberry associated with citric and malic acid production and successfully designed markers for this low acid trait. These genetic tools, published in *Tree Genetics and Genomics*, are now available for public and private breeders to develop new cranberry cultivars that provide products with less added sugar, which will be more desirable to consumers.

New type of wheat gene governing disease susceptibility. Septoria nodorum blotch (SNB) is a severe fungal disease of wheat worldwide. ARS researchers in Fargo, North Dakota, isolated a new gene in wheat, Snn3-D1, that makes wheat susceptible to SNB. This research, published in the Plant Journal, found that a portion of the Snn3-D1 gene involved in the recognition of the SNB pathogen in wheat plants resembles a component of a nematode gene involved in sperm movement. This is the first research to show common features between a nematode sperm-movement gene and a pathogen resistance gene in plants. This knowledge will allow researchers to devise appropriate strategies for the development of disease resistant crops.

Novel control of tomato fruit improving firmness and extending shelf life. A major limitation in delivering fresh, high-quality fruit to consumers is the natural propensity of ripening fruit to over-soften, which reduces consumer appeal and accelerates decay and product loss. Most techniques for ensuring firmness and shipping/shelf-life inhibit complete ripening and diminish consumer appeal. Researchers in Ithaca, New York, worked with Cornell and Zhejiang University collaborators and identified a tomato regulatory gene that primarily affects softening without affecting other aspects of ripening. Targeted inhibition of this gene, SILOB1, resulted in firmer fruit with longer shelf life and did not affect desirable ripening characteristics. These results identified a unique target for extending shelf life and minimizing softening without harming fruit quality, unlike other current options for controlling fruit softening.

Quantifying sensitivity of wheat powdery mildew to fungicides. Powdery mildew is a major disease of wheat and barley. The U.S. population of wheat powdery mildew had never been surveyed for susceptibility to quinone outside inhibitors (QoIs) or demethylation inhibitors (DMIs) fungicides used for its control. In other countries, the wheat powdery mildew population has lost sensitivity to both modes of action. ARS researchers in Raleigh, North Carolina, conducted a survey of powdery mildew isolates from 15 central and eastern states in the United States where wheat powdery mildew epidemics are common. More than 375 isolates were screened for sensitivity to two QoIs and two DMI fungicides. Known mutations that cause insensitivity to QoIs were not detected, but a range of QoI sensitivity was observed, suggesting its efficacy is diminishing. For DMIs, significant regional differences in sensitivity were detected, with lower sensitivity in the eastern United States. These results support the judicious use of fungicides by showing the need to rotate between the two fungicide chemistry classes and reduce unnecessary applications in the United States, management that will delay the loss of fungicide effectiveness against wheat powdery mildew.

Progress in reducing methane gas production in rice fields. Rice production is responsible for up to 11 percent of worldwide anthropogenic methane, a key greenhouse gas. ARS researchers in Stuttgart, Arkansas, and Beltsville, Maryland, showed that the structure of rice-soil microbial communities is genetically linked with high and low methane emitting rice genotypes. They also showed that high and low methane emitting genotypes had contrasting soil microbial profiles of methanogens (methane-producing bacteria) and methanotrophs (methane-oxidizing bacteria). They also demonstrated that the profile of particular methanogens and methanotrophs varies corresponding to the amounts of methane produced by rice cultivars. These findings suggest it may be possible to use the soil microbial profile as a potential phenotyping tool to select for rice breeding lines that generate fewer methane emissions, thus reducing the global agricultural production of greenhouse gases.

Identification of novel plant metabolic pathway controlling microbiome development under drought stress. Climate change drives increases in the global intensity and duration of drought. ARS scientists in Albany, California, and university collaborators demonstrated that drought leads to dramatic, highly conserved shifts in the crop root microbiomes that can enhance adaptation. They successfully implemented an "holo-omics" approach combined with genome-resolved metagenomics to identify a new role for iron metabolism in shaping the crop microbiome. This strategy provides new evidence that crop genetics related to nutrient status is related to microbiome management. The findings implicate iron metabolism in the root microbiome's response to drought and may inform efforts to improve plant drought tolerance to increase food security.

Brown midrib-12 plants have altered drought responses. In the United States, sorghum biomass is an important forage crop for livestock and is being developed as a bioenergy crop. Plant cell walls contain lignin, which is difficult to break down for cellulosic conversion to ethanol and for feed. However, brown midrib (bmr) sorghums have reduced levels of lignin. ARS researchers in Lincoln, Nebraska, and their collaborators found that the root response of brown midrib 12 (bmr12) plants was altered by drought. Subsequent gene expression analyses showed that bmr12 plants were primed to respond to drought even under well-watered conditions. These findings, which were published in *New Phytologist*, showed that changing plant cell walls to improve bioenergy conversion may improve plant responses to drought. This research provides a basis to further investigate the roles of cell walls in perceiving the environment, which is critical for developing forage and bioenergy crops in a changing climate.

<u>Utilizing the whole plant to understand disease resistance signaling</u>. Brown stem rot (BSR) reduces soybean yield by up to 38 percent. The causal agent of BSR is *Phialophora gregata*. BSR leaf symptoms are often misdiagnosed, making BSR resistance especially difficult to phenotype and utilize in breeding programs; in addition, *P. gregata* is a slow growing pathogen, so breeders typically must wait 5 weeks to access plants for resistance or susceptibility. ARS scientists in Ames, lowa, conducted whole genome expression analyses of infected and mock-infected BSR-resistant soybean plants and found infected and mock-infected leaves, stems, and roots follow the same defense pathways. They also showed that molecular approaches can detect resistance and susceptibility after only a few hours of infection. The genes and networks described here will be used to develop novel diagnostic tools to facilitate expedited breeding for BSR resistance. In addition, candidate disease resistance genes can introduce resistance to advanced soybean lines developed with traditional breeding or transgenic approaches.

Characterizing genetics of plant growth enhances genomic selection in wheat. Seasonal genetic variation in growth is a major source of grain yield variation in wheat. Although major genes underlying flowering time and plant height in wheat have been cloned, their importance in contributing to genetic variation for plant growth over time is not fully understood. ARS scientists in Raleigh, North Carolina, determined that almost all additive genetic variation in plant growth traits is associated with known major variants and novel moderate-effect quantitative trait loci (QTL). Most breeding populations in the southeastern United States segregate for known genes for these traits; consequently, genetic variation in plant height and heading date likely emerge from similar combinations of major and moderate effect QTL. This information, published in *BMC Genomics*, is being used to provide wheat breeders with more accurate and cost-effective genomic prediction models by targeted genotyping of key markers. This research will increase efficiency and reduce costs of genomics- assisted development of improved wheat cultivars.

Development of a biochemical model for cross-incompatibility systems in maize. Cross-incompatibility systems in maize determine what plants can successfully cross-pollinate each other. These systems have great potential for controlling genetic purity, including reducing adventitious presence of genetically-engineered traits in organic corn. Although these systems have been studied genetically for more than 100 years, researchers have only recently learned the molecular identity of the genes involved, and how they work is still a mystery. Working with researchers from the Indiana Crop Improvement Association and the Carnegie Institute, ARS researchers in Ames, Iowa, proposed a biochemical model for how the genes work to prevent pollination, based on the predicted functions of the newly-identified genes that control the processes. This model, published in *Plant Reproduction*, provides researchers with hypotheses to test, which will lead to a better understanding of how cross incompatibility systems work. It also provides a framework for engineering novel cross incompatibility systems with improved efficacy. Understanding cross incompatibility systems at the biochemical level will enable plant breeders to develop new varieties of maize with increased genetic purity.

Iron deficiency followed by phosphate deficiency induces novel gene expression changes in soybean. Plants grown in the field experience many abiotic stresses throughout the growing season, which can result in yield losses. To understand the genes and networks underlying stress tolerance, ARS scientists in Ames, lowa, measured gene expression in soybean that was subjected to iron deficiency stress and then subjected to phosphate deficiency stress. These sequential stresses more closely replicated field grown plant stress exposure. These analyses determined that sequential stress induces expression of a unique suite of genes not differentially expressed under repeated stress conditions. These novel genes are usually involved in highly specialized reproductive processes such as pollen development. However, after sequential stress exposure, these same genes switch to performing basic tissue processes, such as cell wall modifications. These findings improve the understanding of soybean response to complex nutrient deficiency stress exposure and will improve understanding the genes and networks underlying plant stress tolerance. This information can be leveraged by researchers to improve stress tolerance in soybean and other important crop species.

Genome sequence of the diploid cotton, Gossypium longicalyx. Gossypium longicalyx, a close relative of cultivated cotton, is an African species with valuable resistance to the reniform nematode, a major pest that limits cotton production in many areas. Gossypium longicalyx has been used in cotton breeding as an important source of reniform nematode resistance, but the process is very complex and often results in substantial linkage drag that pulls in undesirable genes. ARS researchers in College Station, Texas, working with national and international collaborators, sequenced and assembled the genome of G.

longicalyx. The assembled genome sequence and associated gene annotation, published in the journal G3-Genes, genomes, genetics, provide plant breeders with valuable tools to effectively develop cotton cultivars that are resistant to the reniform nematode. These resistant commercial cottons will increase productivity and profitability and will improve environmental quality by reducing or eliminating the use of pesticides for nematode control.

# Component 4.

### Information Resources and Tools for Crop Genetics, Genomics, and Genetic Improvement

<u>Using machine learning to predict corn yield from genetic, environment, management, and historical data</u>. Predicting how new cultivars will perform in new environments is a longstanding challenge in agriculture and has the potential to increase breeding effectiveness and producer profitability. ARS researchers in Columbia, Missouri, and Ithaca, New York, developed computational methods (known as machine learning, deep learning, or artificial intelligence) that increase prediction accuracy by seven percentage points compared to current state-of-the-art methods. These results, published in *Theoretical and Applied Genetics*, will potentially enable breeders to accelerate the development of breeding lines and cultivars for specific environments.

Pan-genomic resources provided by the Maize Genetics and Genomics Database (MaizeGDB). Crop research increasingly relies on knowledge of a pan-genome, which is a complete set of genes found across a species. Understanding pan-genomes is especially valuable in plants with high genomic diversity, such as maize, that can be exploited for crop improvement. ARS researchers in Ames, lowa, introduced a pan-genomic approach to hosting a genome sequence database, leveraging the numerous diverse maize genome sequences available and their associated datasets to efficiently connect plant genomes with traits of interest and their inheritance and control. During the past several years, MaizeGDB has transitioned from hosting a single reference genome sequence to hosting data and tools integrated across 44 diverse maize genome assemblies. Recent improvements at MaizeGDB include an improved method for gene annotation, a tool to visualize structural variation across genomes, and methods to connect functional annotations across genomes. This pan-genome approach provides a template for other crop databases. It is also a resource to facilitate improved crop performance by helping researchers understand the relationship between the genes in a plant and the traits observed in crop fields.

Tools for genome editing in alfalfa. Alfalfa is the third most widely grown crop in the United States, but breeding for increasing herbage yield, quality, and stand persistence has been slow. Using genome editing to modify the alfalfa genome can accelerate development of more productive and environmentally resilient cultivars, but it is complicated by the multiple copies of each gene in alfalfa. ARS scientists in St. Paul, Minnesota, developed novel genome editing reagents that more efficiently edited a regulatory gene in alfalfa. Mutations were identified for the first time through targeted sequencing of the mutated areas, significantly accelerating identification of mutant plants and reducing costs of identifying mutant plants by tens of thousands of dollars. DNA sequences were identified that will facilitate the tracking of mutations when cross pollinated to other alfalfa backgrounds. These tools will accelerate development of improved alfalfa cultivars for bioremediation, animal nutrition, and nutrient uptake.

Breeding Insight deploys field and genomic tools for five of six specialty crop and animal species. Specialty crops and animals are a large portion of U.S. gross agricultural revenue, but genetic improvement programs for these species have not had access to the data capture and genomic innovations that benefit major crop and animal breeding programs. The challenge with assisting these programs is in both constructing the genomic resources, or data, and in integrating and processing the billions of genomic and field data points needed to make informed breeding decisions. This year, Breeding Insight (BI) generated genomic resources for breeding of blueberry, alfalfa, sweet potato, grape, and North American Atlantic salmon. Additionally, BI developed databases and field data collection systems for each of these species. Putting this powerful information and these genomic tools into the hands of ARS specialty crop and animal breeders helps improve breeding decisions and meet public demand for more nutritious and flavorful foods.

Genotypic characterization of the U.S. peanut core collection. Cultivated peanut is an important oil, food, and feed crop worldwide. In work funded by the National Institute of Food and Agriculture, ARS scientist in Ames, Iowa, and a diverse team of scientists extracted and sequenced DNA from 812 select peanut lines in the U.S. peanut collection. The lines represented a range of phenotypic variation and were from a range of countries. Analyses identified 14,430 high-quality, informative markers across the collection. The 812 lines were divided into 5 distinct genotypic clusters that largely corresponded with botanical variety and market type, but not with country of origin. One genetic cluster encompasses accessions primarily from Bolivia, Peru, and Ecuador, which is consistent with these having been the earliest lines cultivated by ancient farmers. Comparing these lines with their predicted parents suggested sub-genome exchanges are an important source of diversity. These diverse regions are likely novel sources of resistance and phenotypic variation. Markers associated with these regions can be applied by peanut breeders and growers to develop improved peanut varieties.

Acquisition of Kemin Hu stones as a new collection for the U.S. National Arboretum. Viewing stones are rocks that have been selected and displayed for the purpose of aesthetic appreciation. The custom of collecting viewing stones originated in China with evidence dating back to the Han dynasty (206 BCE – 220 CE) and spread to Japan and Korea before reaching the rest of the world. Stones may be abstract in form to represent a microcosm of the whole universe or resemble the wonder and beauty of nature. Kemin Hu, a noted collector of Chinese viewing stones, transferred much of her collection to the U.S. National Arboretum. U.S. National Arboretum staff are working with the National Bonsai Foundation to produce a catalog of the collection, which consists of more than 100 stones valued at more than \$570,000. The stones will be displayed in the National Bonsai & Penjing Museum.