

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

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

FY 2023 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;
- Apply biotechnological and genetic engineering methods to a broad variety of crop species, and develop new methods to address their potential unintended effects on production systems;
- Genetically improve a broad spectrum of major, specialty, and new crops;
- Produce higher yielding diverse crop plants that use water and other inputs more efficiently and contain genetic protection against crop production barriers, such as diseases, pests, and environmental extremes;
- Increase our knowledge of the structure and function of crop genomes and of plant biological and molecular processes; and
- 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 collaborates with diverse public and private sector partners to 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 2023, NP 301 scientists participated in research collaborations with scientists from the following 59 countries:

Argentina	France	Netherlands	South Africa
Australia	Germany	New Zealand	South Korea
Bangladesh	Ghana	Nicaragua	Spain
Belgium	Greece	Nigeria	Taiwan
Brazil	Haiti	Norway	Tanzania
Canada	Honduras	Pakistan	Thailand
Chile	Hungary	Panama	Turkey
China	India	Peru	Uganda
Colombia	Indonesia	Poland	United Arab Emirates
Costa Rica	Israel	Portugal	United Kingdom
Czech Republic	Italy	Qatar	Uruguay
Denmark	Ivory Coast	Romania	Uzbekistan
Dominican Republic	Japan	Saudi Arabia	Vietnam
Egypt	Kenya	Scotland	Zambia
Ethiopia	Mexico	Senegal	

The quality and impact of NP 301 research was evidenced during FY 2023 by the following:

- 517 refereed journal articles published,
- 284 cultivars and breeding lines released,
- 3 new invention disclosures (patents only) submitted,
- 3 new patent applications filed, and
- 1 new patent issued.

During fiscal year 2023, 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, and

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

Research in Component 1 led 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 was 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. This component also 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 a few selected NP 301 accomplishments, presented according to the relevance to the respective NP 301 Research Components. The FY 2023 accomplishments presented here were selected based on impact and level of contribution to meeting the National Program goals.

Component 1. Crop Genetic Improvement

Reducing agricultural greenhouse gas production. More than 80 percent of the natural and synthetic nitrogen incorporated into our food systems is lost before it reaches the consumer. This inefficiency accounts for 97 percent of U.S. agricultural greenhouse gas emissions (nitrous oxide, methane) and more than 60 percent of water pollution by nitrates. The CERCA (Circular Economy for Reimagining Corn Agriculture) project, launched this year by ARS, focuses on corn, the single largest player in the U.S. agricultural nitrogen system. The goal of CERCA is to develop corn genetic resources that, in concert with agronomic practices, can shift the corn growing season earlier to capture natural soil nitrogen more efficiently, reduce corn's intrinsic need for nitrogen, and recycle nitrogen back to the soil at the end of season (currently the case with perennial plants). The CERCA project is led by ARS scientists from across the country and university collaborators (27 total labs, led by Cornell) whose integrated research program encompasses crop modeling, agronomy, genetics, and physiology to accomplish these goals.

McDonald's accepts 'Teton Russet' potato as Gold Standard variety for their French fries. 'Teton Russet', a new potato variety developed by ARS scientists in Aberdeen, Idaho, and university cooperators, was accepted by McDonald's for French fry production. This variety has higher vitamin C and protein and lower asparagine contents than the industry standard 'Russet Burbank'. Lower asparagine content contributes to reducing carcinogenic acrylamide levels in fries and other processed potato products. McDonald's currently accepts only eight potato varieties for its French fry processing worldwide, five of which are from ARS breeding program. The ARS-university potato breeding team was also awarded a Technology Transfer award by the ARS Pacific West Area for collaborative research that led to the variety's development. The team included ARS, Oregon State University, and Washington State University scientists and the Pacific Northwest Potato Variety Management Institute, led by Washington State, Idaho, and Oregon potato commissions.

A major QTL associated with peanut smut resistance. Peanut smut is not currently found in the United States but has been devastating peanut production in Argentina. It is a serious emerging disease that threatens peanut production worldwide, including the United States. The value of the U.S. peanut crop is greater than \$1 billion annually, and finding genetic resistance to smut preemptively will protect the U.S. peanut industry by enabling rapid development of smut-resistant cultivars in case the pathogen arrives in the United States. ARS researchers in Stillwater, Oklahoma, and Tifton, Georgia, with colleagues in Argentina, Alabama, and Georgia, identified a major quantitative trait locus (QTL) that is associated with strong resistance to peanut smut, and tracked it to chromosome 12 of peanut. Accurate DNA markers for this QTL were developed, and multiple public peanut breeding programs within the United States and Argentina are effectively applying those markers to select peanut lines with resistance to that disease.

Improved club wheat variety ‘Cameo’ available in Fall 2023. Club wheat, a major export commodity, is grown commercially only in the U.S. Pacific Northwest. Farmers need new club wheat cultivars with improved disease resistance and end use quality to remain commercially competitive. ARS scientists in Pullman, Washington, and Washington State University collaborators developed the new ‘Cameo’ club wheat cultivar through classical and molecular breeding techniques. Compared to other club wheat cultivars grown in eastern Washington and northern Idaho, Cameo has better agronomic performance, better stripe rust resistance, greater tolerance to soilborne mosaic virus and acid soils, and similar excellent club wheat quality. Cameo was available as registered seed to seed companies and farmers in the region in the fall of 2023.

Release of new USDA-ARS hop cultivar ‘Vista’. New high-yielding hop cultivars with improved traits are needed to advance the U.S. hop industry. ARS scientists in Corvallis, Oregon, and Oregon State University collaborators released ‘USDA Vista’, a high yielding, disease-tolerant aroma line. In 2022 this cultivar was planted on approximately 50 acres across the U.S. Pacific Northwest with additional acres planted during 2023. The release of this new cultivar provides brewers with a new hop cultivar with a unique flavor profile that excels in India pale ale (IPA) and pale ale styles but also has niche use in fruity sour beers. U.S. producers benefit from the production of a high-yielding new cultivar that is seedless and picks cleanly. Additionally, the cultivar is harvested later than other cultivars and allows a grower to add production acreage without eliminating other hop lines currently in their inventory.

The MAGIC peanut: a new tool for peanut research and breeding. Populations derived from the crosses of two parents have been the standard research tool used in mapping peanut genes. But this approach hindered developing genetic markers for agronomic traits because of the structure of the peanut genomes. Applying recent advances in genome sequencing and computational bioinformatics, ARS researchers in Tifton, Georgia, developed a multi-parent advanced generation inter-cross (MAGIC) population composed of more than 2,700 genetically-distinct lines derived from 8 diverse parents. Based on analyses of 310 of those lines, the MAGIC Peanut population contains a balanced and even representation of genetic material from all 8 parents. This MAGIC Peanut will be made available to the peanut research and breeding community to facilitate the genetic mapping of key traits such as disease resistance, high oleic acid, and shelling percentage, which will enable breeding high yielding peanuts with better disease resistance and quality.

Chickpeas for alternative protein content and better human nutrition. In general, overconsumption of saturated fatty acids is associated with health risks, including Type II diabetes and coronary artery disease (CAD), whereas consumption of unsaturated fatty acids is associated with health benefits. Chickpea is a rich source of protein and dietary minerals, but little was known about the concentrations of different fatty acids in chickpea or how these traits were inherited. That lack of knowledge impeded progress for developing chickpeas with reduced concentrations of palmitic acid, an undesirable saturated fatty acid in human foods. ARS researchers in Pullman, Washington, and Clemson University collaborators evaluated concentrations of oleic acid, linoleic acid, alpha linolenic acid, and palmitic acid in diverse chickpea lines and commercial varieties and identified lines with up to 46 percent and 72 percent of the recommended daily allowances of the unsaturated fatty acids linolenic acid and alpha-linoleic acid, respectively. They identified genetic markers associated with concentrations of palmitic acid, along with several genes that may be involved in regulating concentrations of fatty acids in chickpea. Other collaborators are incorporating lines identified with low palmitic acid concentrations

into breeding programs to develop new chickpea varieties with more nutritious fatty acid profiles that can contribute to improved health.

‘USDA Lumina’ strawberry cultivar released. Strawberries that fruit early in the strawberry season are highly desirable to capture higher prices and attract customers to U-pick farms early each year. Several early-season cultivars are available, but each lacks one or more key traits. ARS researchers in Beltsville, Maryland, released ‘USDA Lumina’, an early-season strawberry that combines all these key traits: winter-hardiness; high yield; resistance to anthracnose fruit rot and low botrytis fruit rot; and large, sweet, beautiful fruits that do not split open in the field or turn dark in refrigerated storage. USDA Lumina plants were sent to three U.S. nurseries and one Canadian nursery for propagation and a U.S. plant patent application is in process. USDA Lumina is expected to have the greatest value to growers in the U.S. Mid-Atlantic regions and other regions with hot growing conditions where other cultivars fail to perform.

‘USDA Fortress’, an improved winter feed barley. The acreage devoted to feed barley was equal to that of wheat in Oklahoma until the 1970s, but in the past 5 years, interest has increased in growing barley as an alternative to wheat. The last feed barley cultivar released in Oklahoma in 1990 was resistant to greenbug (GB) feeding but did not have resistance to the new invasive pest Russian wheat aphid (RWA). ARS researchers in Stillwater, Oklahoma, developed and released ‘USDA Fortress’, a new winter feed barley and the first U.S. barley with resistance to GB, RWA, and the new invasive aphid Hedgehog grain aphid. It received USDA plant variety protection and is licensed to Oklahoma Genetics, Inc. for seed production.

Stacking rust and downy mildew resistance genes from wild relatives of sunflower. Rust and downy mildew (DM), serious fungal diseases that lower crop yield and quality in confection sunflowers worldwide, have become more important in North America in recent years as the pathogens evolved to overcome host-plant resistance. Combining multiple genes (stacking) is a common practice in crop breeding to ensure longer lasting resistance. ARS scientists in Fargo, North Dakota, and North Dakota State University colleagues developed two triple-stacked confection sunflower germplasm lines with two rust and one DM gene conferring resistance to the most common and serious races of rust and DM. Genetic markers for the three genes were also provided to facilitate stacking these genes. These multiple disease-resistant lines provide the sunflower industry with long-lasting sources of disease resistance for the continued improvement of the crop.

Pima and Upland cotton germplasm with resistance to Fusarium wilt. For almost 2 decades, the soilborne fungal pathogen *Fusarium oxysporum* f. sp. *vasinfectum*, race 4 (FOV4) has impacted cotton production in California’s San Joaquin Valley, causing plant wilt and death and millions of dollars in economic losses. Recently, this pathogen was identified in New Mexico and near the Texas High Plains, the largest Upland cotton producing region in the United States, and U.S. cotton growers urgently need resistant commercial cotton varieties. ARS scientists in Lubbock, Texas, developed three Pima-like cotton germplasm lines with improved resistance to FOV4 by crossing cotton lines from the United States and Uzbekistan. They also worked with university cooperators to develop and release 17 Upland cotton germplasm lines with improved resistance to FOV4. These lines were derived from 6 diverse cross-combinations of 10 parental lines with different genetic backgrounds. This is the first set of Upland cotton germplasm with FOV4 resistance released to the public. Improved FOV4 resistance in commercial

cotton will reduce the vulnerability of the cotton industry to this fungal pathogen and help avoid the production disruptions that affected cotton fields in California, Texas, and New Mexico.

USDA-N5001, a high yielding maturity group V soybean germplasm with high seed yield and protein content. Soybean stakeholders are extremely concerned about recent U.S. soybean cultivars with low seed meal protein content that falls below the minimum market standard of 47.5 percent. To remain competitive in the international market, U.S. soybean growers and processors require high-yielding soybeans with seed meal containing greater than 48 percent protein. Nonetheless, increasing protein content without reducing seed yield is challenging, because soybean protein content is negatively correlated with yield. ARS researchers in Raleigh, North Carolina, recently released the germplasm line USDA-N5001, which has seed yields that match or surpass currently grown commercial cultivars and 2-3 percent increased protein content in seed meal. This line is valuable to public and private soybean breeders for developing high-yielding soybean cultivars with meal protein levels exceeding 48 percent.

‘USDA-Tiara’ purple bran rice: delicious and nutritious. Consumers want flavorful food that is also nutritious. Most rice is consumed in the milled form where the outer bran layer is removed, but the bran contains most of the protein, vitamins, fiber, and antioxidants contained in the grain. ARS researchers in Stuttgart, Arkansas, conducted research for 16 years to develop ‘USDA-Tiara’, a long-grain rice variety that has purple bran enriched with anthocyanins, the antioxidant found in blueberries. It is aromatic with a nutty, popcorn-like flavor and gives small independent growers the opportunity to maintain their distinct market brands and expand industry offerings with a commercially appealing rice variety, unlike large rice mills that co-mingle rice varieties. ‘USDA-Tiara’, now commercially grown in the southern United States, can deliver greater value to rural communities and enhanced nutrition for consumers.

‘USDA-Spiers’ rabbiteye blueberry released. ‘USDA-Spiers’, a new, non-patented early-season rabbiteye blueberry cultivar developed by the ARS researchers in Poplarville, Mississippi, is derived from rabbiteye blueberry cultivars and selections and produces an abundance of medium to large, attractive light blue and very firm berries with resistance to rain-induced splitting. Mature ‘USDA-Spiers’ bushes are moderately spreading with narrow crowns, and compared to most rabbiteye blueberry cultivars, grow more slowly and require little pruning to manage excessive vegetative growth. ‘USDA-Spiers’ is an ideal choice for blueberry production in the USDA plant hardiness zones 8a thru 9a.

Novel cherry tomato lines developed. Flavor and nutritive value are important determinants of consumer acceptance of tomato varieties. ARS scientists in Beltsville, Maryland, developed novel cherry tomato lines with compact plant sizes that are productive, do not require staking and are adapted to ground or raised bed culture. Two of these lines carry the recessive gene for pink colored fruit and accumulate high concentrations of anthocyanin in fruit peel, resulting in purple-pigmented ripe fruit. Fruits are flavorful and provide nutritive benefits of both lycopene and anthocyanin. The third line produces red cocktail-size cherry tomatoes with superior flavor and resistance to fruit crack. These lines will be released as public cultivars and as a germplasm source of novel traits for commercial breeding programs.

Genome sequence of an important cotton species. *Gossypium herbaceum*, one of the four types of cultivated cotton, is grown and is well-adapted to challenging environmental conditions in Africa. Information about the *G. herbaceum* genome is essential for identifying the genetic control of these desirable adaptations and incorporating these genes into cotton breeding for other regions adversely

affected by extreme weather. ARS researchers in College Station, Texas, and national and international collaborators sequenced, assembled, and annotated the *G. herbaceum* genome. This accomplishment provides the building blocks to further define the genetic control and development of cotton fiber, as well as to identify genes for tolerance to environmental stresses. The genome sequence also serves as a blueprint for further research on the evolution of cotton and as a valuable tool for genetically improving the U.S. cotton crop, which had an annual farmgate value of \$6.4 billion in 2022.

Increased trait diversity of Germplasm Enhancement of Maize (GEM) lines through application of genomics-based predictions. The Germplasm Enhancement of Maize (GEM) Project is a large international public-private partnership for improving sustainability of corn production by expanding genetic diversity and introducing novel traits into elite maize lines for commercial breeding. Since 1993, GEM has delivered 346 elite lines with novel traits from exotic germplasm such as disease resistance, drought/heat stress tolerance, grain with high amylose content, and superior silage yield and nutritional quality. Demands are increasing for GEM lines that address environmental sustainability and emerging pests and diseases, so ARS researchers in Iowa and North Carolina and industry collaborators developed sophisticated genomic tools to meet demands for increasing trait diversity. More than 1,000 breeding lines and released GEM lines were provided to 20 GEM stakeholders for the 2023 planting season for disease documentation trials, environmental response observations, line development operations, and genetic analyses. An additional 5,400 packets of yield plot seed were distributed to seven cooperators for yield trial testing and observation. Genomics-based tools have enabled the GEM project to deliver more germplasm resources and share more information to stakeholders for incorporating new genetic diversity and beneficial traits in their breeding programs. These efforts also potentially reduce genetic vulnerabilities by broadening the U. S. maize germplasm base.

Machine vision techniques to quantify tuber shape and classify hollow heart defect in potato breeding populations. Potato tuber size, shape, and defect susceptibility are some of the most important characteristics that influence cultivar acceptance within the potato processing industry. Tuber size and shape determine compatibility with potato processing machines (peelers, cutters, fryers, etc.), while size, shape, and defect susceptibility influence the portion of usable raw product after cutting and trimming. ARS scientists in Prosser, Washington, and Aberdeen, Idaho, developed a latent trait approach (tuber biomass profile) for quantifying potato shape features that is independent of tuber size and aspect ratio (length-to-width ratio). They also developed a deep learning model to classify tubers affected by an internal tuber defect called hollow heart. These accomplishments enable inexpensive and reproducible quantification of these features from hundreds-to thousands clones and tuber samples generated in each potato breeding and evaluation cycle. This quantification is needed for potato breeding programs to apply statistical genetics models and marker-assisted selection for these traits. The results will significantly improve identifying and selecting potato cultivars that meet the needs of breeders and the potato processing industry.

Broad-spectrum stem rust resistance gene discovered. Stem rust has re-emerged as one of the most important diseases of wheat. The pathogen continues to evolve new, aggressive races that threaten U.S. and global wheat production, and wheat breeders urgently need new genes to develop cultivars with robust resistance. ARS researchers in Manhattan, Kansas, identified a new broad-spectrum resistance gene, Sr64, that confers resistance to all known races of stem rust fungus from wheat, including one of the most potent strains, Ug99. Sr64 is tightly linked to the resistance gene Wsm1, which is effective against wheat streak mosaic virus (WSMV) and Triticum mosaic virus (TriMV). The two genes are on the

same piece of genetic material derived from a wheat wild relative, so they can be inherited together in breeding crosses. DNA genetic markers were developed so that breeders can readily select for the combined resistance to WSMV, TriMV, and stem rust in their breeding populations.

New sources of leaf rust disease resistance in emmer wheat. Wheat leaf rust is one of the most devastating fungal diseases of wheat and new sources of disease resistance are continuously needed to sustain global wheat production. ARS researchers in Albany, California, and Fargo, North Dakota, and collaborators at Cornell University and North Dakota State University conducted a genome-wide survey of leaf rust resistance genes in 180 cultivated emmer wheat germplasm accessions. They identified 90 chromosomal regions that are associated with leaf rust resistance and determined that 24 and 66 regions possibly contain known and novel leaf rust resistance genes, respectively. These results provide new genetic resources for determining if these genes should be incorporated into resistance breeding programs for modern wheat varieties.

Combining coffee leaf rust resistance and coffee nematode tolerance through grafting. Root-knot nematodes, a devastating pest in Hawaii coffee production, reduce yield and plant vigor. With the recent discovery of coffee leaf rust (CLR) disease in Hawaii, growers are now challenged on two fronts. Producers wanted to grow new CLR-resistant coffee cultivars but the susceptibility of these cultivars to the root-knot nematode was unknown. ARS researchers in Hilo, Hawaii, confirmed that CLR-resistant coffee cultivars 'Obata 'and 'Tupi-HI 'are susceptible to root-knot nematodes, but could be grafted onto nematode-tolerant coffee rootstocks. This research will enable Hawaii coffee growers to produce plants tolerant to both CLR and nematodes.

Defining soybean seed composition targets for alternative proteins. Plant sources of suitable high-protein raw materials and foods must become more climate resilient and accessible to meet the rapidly changing nutritional needs of the global human population in the face of climate change. ARS scientists in Columbia, Missouri, partnered with other ARS scientists and food scientists and engineers in academia and industry to develop new soybean germplasm and perform a baseline study that combined sensory analysis with chemical characterization of seven novel ARS seed composition germplasm lines. They separated germplasm into six seed composition categories based on oil, carbohydrate, oxidizing enzyme, and aromatic characteristics and found significant differences for sensory attributes and chemical composition. Based on that knowledge, they developed new soybean germplasm with suitable seed composition that can be sustainably produced to meet current and future food demands. This knowledge also will be important for tracking changes that affect flavor and functionality after seeds are processed for oil and protein products.

Discovery of genes and associated DNA markers for breeding crown rust resistance in oats. Crown rust (CR) causes significant oat yield losses worldwide and therefore is a key target for oat improvement under the ARS Oat Grand Challenge Initiative. ARS researchers in Fargo, North Dakota, and St. Paul, Minnesota, evaluated a large multi-parent oat population for CR at the adult growth stage. Six genes were discovered that provide durable CR resistance and were incorporated together into oat cultivars. High-throughput DNA genetic markers were developed to track these genes and the source germplasm was publicly released. These findings and new tools will enable more rapid development of improved oat varieties with robust resistance to CR.

Genetic mapping of pollinator preference in sunflower. Nectar is a floral reward that encourages bees and other insects to pollinate crop plants. Previous research with sunflowers and many other crop species showed bees prefer varieties with more nectar. ARS scientists in Fargo, North Dakota, and colleagues at the University of Colorado and North Dakota State University discovered the chromosomal location of the sunflower genes that determine nectar volume. They found four key genetic markers that enabled breeding sunflowers with increased nectar volume and also found that genes near some of the genetic markers resembled other genes known to influence nectar production in other plant species. Identifying genetic markers for nectar volume can enable sunflower breeders to increase nectar rewards in sunflower hybrids grown worldwide, with benefits to farmers and valuable insect pollinators.

Assessing southern highbush blueberry germplasm collection for fruit quality attributes. Breeding new blueberry cultivars with enhanced fruit quality requires simple, accurate, and cost-effective assays to select individuals with these characteristics. ARS researchers in Poplarville, Mississippi, used high throughput phenotyping tools to characterize the southern highbush blueberry germplasm collection for berry diameter, berry weight, firmness, pH, total polyphenol, total acids, glucose, fructose, total glucose, and total sugars content. Those traits were highly variable across the germplasm collection, with glucose and fructose the predominant sugars in tested genotypes. Furthermore, between 27 to 39 percent of the tested genotypes displayed a firmness value of 160 g/mm or above, which is considered a superior value. This information is critical for identifying superior genotypes for future crosses and advanced evaluation. In addition, the firmness tester and the discrete analyzer used in this research could potentially improve the efficiency and precision of phenotyping.

Field trials demonstrate resistance to Fusarium wilt in celery. Fusarium wilt disease is caused by the soilborne pathogen *Fusarium oxysporum* f.sp. *apii*, with races 2 and 4 the primary pathogens affecting celery production in California. Genetic resistance through breeding is the most effective control for this disease. ARS researchers in Salinas, California, and University of California collaborators coordinated field trials in soils infested with race 2 and race 4 to identify improved disease resistance in new celery cultivars from private-sector breeding companies. The data can support release of resistant varieties from private breeding companies. The researchers identified resistant cultivars and conducted field day events at the trial locations that were attended by growers, shippers, breeders, and public-sector and private company researchers. The event generated considerable interest in promising new varieties from the private sector.

New Hessian fly-resistant durum pasta wheat lines for effective insect management. The Hessian fly, an economically important pest of U.S. wheat, annually causes severe yield losses. In 2023 wheat stakeholders identified Hessian fly resistance as one of the critical needs for resilient wheat production. Planting wheat cultivars with Hessian fly resistance genes is still the most effective and economical strategy to manage this insect pest, but elevated temperature weakens host plant defense and immunity, which causes normally insect-resistant plants to become susceptible. ARS researchers in West Lafayette, Indiana, found 14 durum pasta wheat lines resistant to Hessian fly biotypes 'L' and 'vH13' and three pasta durum wheat accessions maintained complete resistance to the highly virulent Hessian fly biotype L at the increased temperature of 30°C (normal growth temperature is 18°C). These new pasta wheat cultivars are valuable resources for breeding elite wheat lines for durable resistance to Hessian fly and for efficient crop management across different wheat growing regions.

Cloning a rust resistance gene in sunflower. Rust, one of the most important diseases of sunflower worldwide, is best managed through resistance genes (R genes). Understanding the genomic location and function of R genes is important to successful breeding for rust resistance and management. The rust R gene R11 in the sunflower line HA-R9 confers resistance to most serious races of rust and was previously believed to be located on a small region in sunflower chromosome 13. ARS scientists in Fargo, North Dakota, identified the R11 gene as a single nucleotide sequence of 3,996 base pairs and caused specific changes (mutations) to the gene which resulted in susceptibility to rust. The specific chemical pathways that provide resistance to rust were identified, enabling more effective use of this and other rust-resistance genes in sunflower breeding.

New maize lines with reduced aflatoxin and good hybrid yield ability. Aflatoxin contamination of maize grain creates risks for animal and human health and causes economic losses for growers, particularly in the southeast United States. Developing maize hybrids with genetic resistance to aflatoxin contamination by the fungus *Aspergillus flavus* will help to minimize these risks. ARS researchers developed new elite lines that are aflatoxin resistant and increase grain yields when produced as hybrids (called General Combining Ability, GCA). ARS scientists in Tifton, Georgia, identified 18 aflatoxin-resistant lines that were evaluated in hybrid combinations for GCA and yields. In separate experiments, aflatoxin content was also quantified by inoculating the maize with the fungus. Two new aflatoxin resistant lines, GT1209 and GT1309, were identified with consistently positive GCA for yield, demonstrating the progress with improving both traits in maize hybrids adapted to the southern United States.

Breeding coupled with management essential for soybean seed protein improvement. Progress with breeding soybean varieties with high protein content has been promising. Nevertheless, growers could benefit from management practices that more rapidly deliver in high-protein soybean meal desired by end users. Consequently, information is needed about how management strategies in the southeast United States affect soybean protein content. ARS researchers in Raleigh, North Carolina, conducted field experiments to examine tillage and plant density effects on soybean protein production from multiple high yielding soybean genotypes. Although tillage practices did not impact seed protein, higher planting density led to higher seed protein concentration in two of the three southeast U.S. environments tested. Additionally, there was no yield penalty to higher seed protein concentration in the soybeans grown in higher planting densities. These results suggest that southeast U.S. growers could adopt agronomic strategies to maintain stable protein concentration of high protein varieties or maximize seed protein content.

An improved genotyping platform to assist plant breeders. Single nucleotide polymorphisms (SNPs), mutations abundantly interspersed throughout plant DNA, can be exploited for plant breeding because they often closely associate with genes that confer desirable traits. Consequently, plant breeders expend considerable effort to accurately identify SNPs with greater efficiency and at a lower cost. ARS scientists in Fargo, North Dakota, simplified SNP detection in sugarbeet by developing new rapid detection protocols that involve affordable, readily available lab equipment rather than the more elaborate and expensive laboratory instruments previously used. This improved SNP detection method has effectively advanced sugarbeet breeding and is also applicable to the genetic improvement of other crops.

Soybean: a new crop model to study iron deficiency chlorosis (IDC) in plants. IDC reduces crop quality and yield. Studies from model plant species have demonstrated that shoots control or influence iron uptake in roots, but these analyses were conducted days and weeks after the onset of iron stress. ARS scientists in Ames, Iowa, grafted nearly identical soybean lines Clark and IsoClark (iron stress tolerant and susceptible, respectively), to the Clark rootstock to demonstrate that the rootstock can drive iron stress tolerance in IsoClark leaves. In contrast, the IsoClark rootstock cannot confer iron stress tolerance in Clark leaves. Analyses of grafted plants 30 and 120 minutes after iron stress identified 518 and 846 differentially expressed genes in leaves and roots, respectively. Grafts with a Clark rootstock induced genes involved in iron uptake and utilization at 30 minutes in the root and by 120 minutes in the leaves, regardless of the leaf genotype. These results suggest that an unknown mobile signal initiated in roots regulates iron stress responses in soybean leaves. More information about the complicated physiological differences between crop and model plant species will help develop crops with improved IDC tolerance, reducing economic losses and increasing yield.

Discovery of a novel barley greenbug resistance gene. Greenbug, a serious threat to cereal production worldwide, causes yield losses that are predicted to increase because of global warming. New greenbug resistance genes are urgently needed to combat the continuous emergence of novel greenbug biotypes. ARS scientists in Stillwater, Oklahoma, and Manhattan, Kansas, identified a novel greenbug resistance gene, Rsg3, in the Chinese landrace PI 565676 on the long arm of chromosome 3H. Two DNA genetic markers flanking Rsg3 were developed to facilitate rapid breeding of Rsg3 into locally adapted barley cultivars, which will be valuable for sustainable barley production.

Inheritance for partial resistance to *Verticillium dahliae* race 2 in two resistant lettuce sources. *Verticillium* is a serious pathogen affecting numerous economically important vegetable crops and few chemical solutions are available to manage it in lettuce. New races of this pathogen are being identified on multiple crops throughout California. Resistant varieties are the best solution to control diseases caused by *Verticillium*, and determining how sources of resistance in vegetables are inherited can help identify the best strategy for incorporating resistant germplasm into breeding programs. ARS researchers in Salinas, California, determined that the most efficient breeding strategy for *Verticillium* race 2 partial resistance into lettuce is to make selections at late generations from a large population. Lettuce breeders can adopt this strategy to develop improved, resistant germplasm.

Improving flood tolerance increases soybean resilience during crucial growth. Flooding is becoming an increasing concern for U.S. soybean production due to an increase of intense rainfall events, which are associated in part with climate change. In the southeast United States, most soybean fields have experienced spring floods at planting, which, along with drought, have always been one of the most severe challenges to U.S. soybean production. One, if not the only, sustainable approach to improve performance under flood stress is to develop flood-tolerant soybean lines. ARS researchers in Raleigh, North Carolina, developed and evaluated breeding lines for the response to flood stress at three critical growth stages for production—germination, early vegetative growth, and early reproductive growth. The breeding lines with the highest level of flood tolerance maintained a germination rate of higher than 80 percent after 8 hours of flooding, whereas the germination rates of the susceptible check varieties were significantly lower and ranged from 58 to 63 percent. During early vegetative and reproductive growth the flood-tolerant lines averaged a 30 percent reduction in foliar damage and a 10 percent increase in biomass. In 2023 ARS scientists in Raleigh released USDA-N6006, the first high-yielding soybean germplasm to combine elevated seed oil and flood tolerance with adaptations to U.S. southeast

production. It should be valuable to soybean breeders and growers as a source of flood tolerance and high oil.

Elite hard red spring wheat (HRSW) lines with novel resistance to fusarium head blight (FHB). FHB is a major threat to wheat and barley production in the United States and worldwide. Through the US Wheat and Barley Scab Initiative, ARS is developing integrated solutions to protect those crops in the field, which includes developing improved varieties with genetic resistance. ARS scientists in Lincoln, Nebraska, and North Dakota State University collaborators developed and released a new wheat line, WGC002, that contains *Fhb7*, a novel wild grass-derived FHB resistance gene. In addition, resistance gene-specific DNA genetic markers were developed to enhance the utility of *Fhb7* in wheat breeding. ARS scientists in Lincoln, Nebraska used these resources for marker-assisted backcrossing breeding, deployed three HRSW lines with the *Fhb7* gene and confirmed their superior FHB resistance in both the greenhouse and field. These lines can provide excellent options for HRSW growers in the upper Great Plains to improve wheat productivity and reduce the economic losses caused by FHB.

Development and release of new peanut cultivar, TifNV-HG. Peanut farmers need cultivars that eliminate the need of fungicide treatments in production fields, thereby increasing farmer profits and protecting the environment. ARS scientists in Tifton, Georgia and their collaborators developed peanut breeding populations and applied marker-assisted selection to identify nematode resistance and the high oleic to linoleic (O/L) fatty acid seed oil trait. They developed the peanut cultivar TifNV-HG, highly resistant to both the peanut root-knot nematode and tomato spotted wilt virus (TSWV) and containing seed oil with a high ratio of oleic to linoleic acid. When tested in fields without nematode pressure TifNV-HG delivered yields that were at least equivalent to other currently grown peanut varieties. When tested in fields with nematode pressure, TifNV-HG delivered yields at least equivalent to other nematode resistant cultivars and significantly higher than susceptible cultivars. TifNV-HG yielded a higher quality grade of peanuts compared to the previously grown variety, TifNV-High O/L. Growers will reap high economic benefits from this new variety and peanut breeders are using it to develop new resistant cultivars.

Novel gene confers a wide spectrum of resistance to wheat powdery mildew. Powdery mildew, caused by the fungus *Blumeria graminis* f. sp. *tritici* (Bgt), infests wheat worldwide. Novel genes for powdery mildew resistance that do not disrupt other important traits are urgently to develop wheat cultivars. ARS scientists in Stillwater, Oklahoma, and Manhattan, Kansas, identified a new powdery mildew resistance gene in German cultivar PI 351817 that confers resistance to all U.S. representative Bgt isolates. User-friendly, high throughput genetic markers were developed for incorporating this new gene into locally- adapted wheat lines. The gene and its DNA genetic markers can be widely employed to enhance wheat powdery mildew resistance in the U. S. wheat crop.

Microbiome and metabolome genetic markers linked to improved postharvest sugar beet storage. Sugar beet roots stored under postharvest conditions before processing can suffer millions of dollars in sugar quality losses every year. ARS researchers in Kimberly, Idaho, conducted postharvest storage studies with disease resistant and susceptible sugar beet germplasm to evaluate them for disease resistance, root weight loss, sugar content, and associated microbiomes and metabolomes. Microbiome and metabolite-related markers were identified that are genotype-specific and affect postharvest storage quality of sugar beet roots. These markers are now applied to identify sugar beet germplasm with lower sucrose losses during storage.

CRISPR/Cas9 gene editing to generate mutant plants associated with metal ion uptake and accumulation. Crops grown in soils contaminated with heavy metals can take up those metals and introduce those contaminants into the food chain; conversely, plants that take up heavy metals can also be used to decontaminate soils. More information is needed about the genetic traits associated with heavy metal accumulation and tolerance in plants, to identify those plant that effectively remove heavy metals from soil. Several promising candidate genes involved in metal uptake were recently identified by genome wide association studies. To validate these genes, ARS scientists in St. Paul, Minnesota, generated five mutated *Medicago truncatula* (a model plant system) plants by CRISPR/Cas9 gene editing. The mutants were screened for targeted mutations and the heritable transmission of the mutations was demonstrated along with removal of the foreign genes by genetic segregation. The plants were analyzed under different concentrations of metal ions and metal accumulation was quantified by x-ray fluorescence microscopy. Mutants that can accumulate toxic metal ions were identified which hold promise for removing toxic metals from contaminated farmland soils. Farmers and environmental managers can grow these plants or other species with these mutations to reduce the negative impact of these contaminants on the environment and consumer.

Host-plant resistance to disease identified in watermelon. Cucurbit downy mildew (CDM) is a serious threat to the production of cucumber, cantaloupes, and watermelon in the U. S. and in other cucurbit production areas worldwide. New sources of host-plant resistance and genetic markers for those traits are particularly needed to control the spread of the CDM pathogen in watermelon. ARS researchers in Charleston, South Carolina, surveyed germplasm from the ARS National Plant Germplasm System genebank in Griffin, Georgia, and identified several samples that are resistant to CDM, and also identified gene loci associated with CDM-resistance in watermelon. The CDM-resistant germplasm samples and genetic markers for gene loci associated with resistance can accelerate progress for breeding enhanced CDM-resistance in watermelon cultivars.

Improved method to screen for potato physiological defects. Successful new potato cultivars require resistance to physiological disorders such as blackheart, which results in tuber discoloration. But the only ways to screen for such disorders are slow, costly field trials that generate highly variable results depending on poorly understood, uncontrollable environmental conditions. ARS scientists in Prosser, Washington, developed a rapid 4-day assay for blackheart resistance that can be conducted under laboratory conditions that mimic elevated temperatures that can induce blackheart. This method provides a new capacity for determining the physiological basis of blackheart and will improve the ability of potato breeding programs to evaluate breeding lines for resistance and reduce the occurrence of blackheart, one of the leading causes of consumer complaints for fresh market potatoes.

Novel genes that increase crop yield. Yield, among the most important traits in crop breeding, is essential to sustainably meeting the food security needs of a growing world population. Genetic and molecular analyses of crop nutrient content and response to growth regulators often include yield assessments beyond specific analysis of targeted traits to ensure that any proposed trait enhancements do not adversely impact yield. ARS researchers in Ithaca, New York, and collaborators at Boyce Thompson Institute and the Federal University of Vicosa, Brazil, demonstrated that two genes involved in fruit development—pigment accumulation (carotenoids) and fruit ripening response (ethylene)—significantly increase yield in tomato. In both cases, specific amino acid modifications are associated with the positive yield increase, suggesting that altered function and not loss-of-function in these genes

is responsible for this important change in yield. Although tomato is an important crop worldwide, both these genes occur across diverse plant and crop species, so yield increases likely can be attained in crops other than tomato though modifying these genes.

Component 2. Plant and Microbial Genetic Resource and Information Management

Climate change impacts on the USDA/ARS National Plant Germplasm System. Rapid global warming (climate change) is affecting plant germplasm in genebanks and in nature throughout the United States. ARS researchers in Fort Collins, Colorado; Pullman, Washington; Beltsville, Maryland, and university and botanical garden cooperators published the first systematic assessment of the current and possible future impacts of climate change on USDA/ARS National Plant Germplasm System (NPGS) conservation operations. Specific examples describe how higher temperature and drought currently affect genebank operations. In addition, a web-based application was developed to provide NPGS genebank managers with predictions for future temperatures and rainfall at their specific locations. Strategies for adapting NPGS genebank operations to climate change were developed. The importance of plant germplasm for enabling human society to adapt to climate change was also explained in an e-book. These publications disseminate recommendations and tools that represent critical first steps for safeguarding plant germplasm in the NPGS and other genebanks to ensure its long-term access for research and breeding.

ARS has the world's largest hemp genetic resource collection. To expand and underpin domestic and international production of hemp as a grain, fiber, and secondary metabolite crop, plant breeders and researchers need access to highly genetically and geographically diverse hemp genetic resources (HGR). But until 2 years ago, few such genetic resources were publicly available. At the direction of Congress, the USDA-ARS National Plant Germplasm System (NPGS) genebank in Geneva, New York, established a HGR collection that is now the largest in the world, and the only one with substantial associated-trait data such as cannabinoid profile, plant architectural traits, and other critical agronomic characters. Newly acquired diverse HGR were evaluated at five unique growing environments resulting in 11,000+ data points which were generated in accordance with the USDA/ARS Hemp Phenotyping Handbook. These data are now available at the NPGS Germplasm Resource Information Network (GRIN)-Global. The ARS Cotton Quality Laboratory in New Orleans, Louisiana, the ARS National Center for Agricultural Utilization Research in Peoria, Illinois, and university cooperators also generated additional fiber, secondary metabolite, and seed fatty acid/protein data. Providing access to highly characterized HGR can substantially intensify and accelerate hemp breeding and research for developing high-value and locally adapted hemp cultivars and other HGR.

Genetic resources for breeding drought-tolerant lentils. The annual U.S. market value for lentils, an important pulse crop containing high-quality protein and other important nutrients, was \$180 million in 2023. But rapid global warming challenges U.S. lentil production, and traits for adaptation to extreme temperatures are needed. ARS researchers in Pullman, Washington, and university collaborators evaluated several hundred lentil samples in the U.S. National Plant Germplasm System genebank with high-throughput reflectance methods and multispectral radiometers and identified several samples with drought tolerant attributes. Breeders can use these samples to obtain traits to breed resilient, drought-tolerant lentils for sustainable agricultural production to benefit both lentil growers and consumers.

Genetic markers for key reproductive trait in mangos. Most fruit trees are highly variable genetically and are propagated clonally by cuttings and grafting, which maintains the gene combinations for controlling horticulturally important traits. That is the case for mango, one of the most important tree fruits with a \$60 billion+ global industry value. For some mango trees, a complicated reproductive system involves “polyembryony”, which usually results in embryos that are genetically identical to the mother plant and therefore can enable clonal propagation of mango cultivars through seeds, thus bypassing grafting cuttings. But introducing the polyembryony trait into important cultivars through breeding requires several years, because breeding lines must set fruits and then polyembryonic traits must be selected. Developing genetic markers associated with polyembryony will enable identifying polyembryonic seedlings very early in the breeding cycle. ARS scientists in Miami, Florida, applied genome sequencing and genome-trait association methods to identify molecular markers for the polyembryony trait in mangoes. This can help accelerate selecting and incorporating the polyembryony trait in mango, and potentially also provide important insights for manipulating the polyembryony trait in other crops.

GRIN Taxonomy, the internationally recognized, trusted resource for plant taxonomy of agricultural plants. Using correct scientific plant names is critical for researchers, farmers, industry, and regulators. But those names can frequently change to reflect scientific advances, international rule changes governing correct names, and the discovery of scientific names that were assigned in the past. Stakeholders rely on GRIN Taxonomy, managed by ARS in Beltsville, Maryland, to provide reliable, accurate information for names of economically important plants. During the reporting period, 27,460 new plant names were added to GRIN Taxonomy to fulfill requests from germplasm curators and numerous stakeholders. Crop wild relative, economic usage, and noxious and rarity data were extensively restructured, facilitating the complete overhaul of all the GRIN Taxonomy webpages, several of which had been unavailable for years. Additionally, data on wild relatives for 390 major and minor crops are now available that classify more than 6,000 primary, secondary, tertiary, and graft-stock genetic relatives for these crops, along with more than 4,100 agronomically important traits such as tolerance to drought, heat, and disease. These data can be searched in multiple ways through the GRIN-Global public website. By providing accurate data on economically important plants, GRIN Taxonomy assists breeders, regulators, researchers, farmers, and industry in communicating effectively as they identify and manage their plant resources.

Elucidating the genetic structure of mountain ash, a potential crop plant. Mountain ash (*Sorbus*) and its close relatives are valuable for ornamental landscape plants and for fruit that has food, medicinal, and beverage uses. But crop improvement of *Sorbus* species is hampered by their genetic complexity and the lack of quantitative evaluations for *Sorbus* genetic resources conserved in genebanks. ARS researchers at the National Clonal Germplasm Repository in Corvallis, Oregon, and the Woody Landscape Germplasm Repository at the U.S. National Arboretum in Washington, D.C., characterized and clarified the genetic relationships among samples of *Sorbus* species conserved in those genebanks. The resulting information provides a historical context to the complicated genetic structure of *Sorbus* species and their relatives and a framework for more effective *Sorbus* genetic resource conservation and incorporation of those materials into future *Sorbus* breeding programs.

Guayule genetic resources developed during WWII in Manzanar War Relocation Center added to USDA/ARS National Plant Germplasm System. The USDA/ARS National Plant Germplasm System (NPGS) collection in Parlier, California, manages the only publicly available source for genetic resources of

guayule, an alternative source of natural rubber currently under development for production in arid parts of the United States. Breeders continuously seek new sources of genetic diversity to further improve this crop. But unfortunately, most of the genetic resources that had been accumulated in the United States by the Emergency Rubber Project during WWII had been lost before the NPGS was established, and new sources of guayule genetic resources are scarce. Recently, seed was donated to the NPGS Parlier genebank from the family of former internees at Manzanar, where Japanese-American agronomists had also worked on genetically improving this important alternative rubber crop. This scientifically and historically valuable plant material will be maintained by the NPGS at Parlier, and seed will be available for distribution through the GRIN-Global website in 2024.

Component 3. Crop Biological and Molecular Processes

Impacts of sorghum starch on human gut microbiome. Sorghum containing mutations in the Granule Bound Starch Synthase gene (also termed ‘waxy’) produces grain with altered starch composition. This altered starch composition affects the physical properties of the starch and increases its digestibility. ARS and University of Nebraska researchers in Lincoln, Nebraska, used *in vitro* experiments and mouse models to examine how normal and waxy sorghum grain affect the human colon microbiome. The normal sorghum starch was more resistant to digestion, which increased bacteria populations beneficial to human health, compared to waxy grain, which has potentially undesirable effects on the human gut microbiome. But mixing starch from normal sorghum with waxy grain starch restored the growth of these beneficial bacteria. Examining the effects of grain starch traits on the human microbiome opens new avenues for improving sorghum to benefit human health.

Predicting optimal cross combinations to accelerate soybean genetic improvement. Parental selection and crossing to combine traits from desirable parents are the first essential steps in plant breeding. Usually, these crossing decisions are based on information about pedigrees, parental genotypes, or specific desirable traits. Soybean breeding population development can take 5 or more years, so poor choices for cross combinations can impede breeding progress for years. ARS researchers in Beltsville, Maryland, and University of Georgia collaborators developed genetic marker-based selection models, evaluated their predictive accuracy for soybean seed yield, and identified the best predictive models under different experimental conditions. The predictive models can be applied to soybean as well as other crop breeding programs at the earliest stage of the breeding cycle to accelerate genetic gain and reduce the number of breeding cycles needed to produce a cultivar.

High ozone concentrations increase crop sensitivity to drought. Ozone is a damaging air pollutant that often accompanies heat waves and droughts, which are detrimental to plant productivity. More regional-scale information is needed about whether plant responses to drought and heat vary at different ozone concentrations. ARS researchers in Urbana, Illinois, combined remotely sensed and ground-level monitoring data to investigate interactions between ozone, drought, temperature, and plant productivity across the Northern Hemisphere. For most North American vegetation types and climatic zones, high ozone concentrations increased plant susceptibility to atmospheric drought. Plants were more vulnerable to low humidity under high ozone concentrations, but these concentrations did not increase vulnerability to high temperature stress. As background ozone concentrations continue to increase globally, understanding their impacts on plant productivity is important for modeling ecosystem carbon and water balances. This research demonstrates that ozone is a new factor that

should be considered more directly in global models of plant productivity and reveals the critical role of ozone in altering plant responses to low humidity.

Optimizing crop-microbiome interactions that promote crop resilience to drought. Drought significantly reduces crop yield and health each year, and drought severity and duration are anticipated to increase nation-wide in the coming decades. In addition to ongoing efforts to improve drought tolerance through breeding and crop engineering, alternative methods are needed to mitigate drought stress in crops. ARS researchers in Albany, California, identified specific root-associated microbes, the plant genes that promote colonization by these microbes, and applied that knowledge to develop synthetic microbial communities that can alter root and shoot traits in sorghum and other grain crops and that enhance crop productivity during drought. This discovery paves the way for developing alternative, potentially more efficient technologies to maintain crop productivity under abiotic stress.

A novel protein quantitative trait locus (QTL) from a soybean wild relative. Soybean contains very low genetic diversity compared to many other crops primarily because it is a self-pollinating species. This lack of genetic diversity has been exacerbated by the domestication process, the introduction of only a few original soybean types to the United States. To ensure long-term genetic gain potential for seed yield and nutritional potential, ARS researchers in Beltsville, Maryland, and University of Missouri collaborators developed and performed genetic analyses on populations of a wild plant species most closely related to soybean. They identified several new sites in the genome of that wild species that were correlated with changes in seed protein and oil content. One site was identified that increased seed protein levels but had no significant impact on seed oil content. In contrast, many other genetic sources were found that elevate seed protein but significantly reduce seed oil and/or seed yield. Additional research located other sites on that genome with potential genes for further analyses and breeding selection tools for this potential new protein gene. This research could generate soybean germplasm/cultivars that are richer in protein and identify genes for this trait that can be more easily cloned.

New insights into wheat resistance to powdery mildew. The wheat powdery mildew resistance gene Pm1a provides resistance to *Blumeria graminis* f. sp. tritici (Bgt), the fungus that causes wheat powdery mildew. Pm1a has conferred greater-than-expected durable disease resistance in the United States. ARS researchers in Raleigh, North Carolina, found that a single previously identified gene on the Bgt chromosome known to interact with Pm1a did not fully explain global patterns of virulence and its lack of virulence associated with Pm1a. A genome-wide association study revealed a second Bgt gene that also interacts with Pm1a. This highly unusual “two-gene” discovery likely at least partially explains the greater durability of Pm1a in the United States, because separate mutations in each Bgt gene would be necessary for the fungus to infect wheat that carries Pm1a. The discovery is of strong interest for research on host-pathogen interactions and suggests that wheat breeders seeking durable resistance should use Pm1a in geographic regions where virulence is not yet elevated.

Predictive models for productivity gains and climate resilience in maize. Continued increases in maize productivity will be needed to support increasing demands for grains. Changes in climate will require maize hybrids with distinct characteristics that maintain and increase grain yield. Researchers in Ames, Iowa, applied a modeling approach to predict how maize hybrids might need to be bred in response to changes in climate. The changes in hybrid characteristics required to sustain increases in productivity in response to climate change greatly resembled the changes needed to sustain yield increases for maize

grown in the current climate. This significant result demonstrates that similar breeding approaches and maize germplasm can sustain productivity increases in the face of a changing climate. These findings will inform breeders regarding how to adapt maize breeding programs to continue realizing future genetic gain for yield.

Component 4.

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

Artificial intelligence enhances analyzing plant protein sequence variations. For decades determining three-dimensional (3-D) plant protein structures was time-consuming and costly, which impeded research in plant structural biology and functional genomics by limiting protein comparisons and understanding their roles and utility for trait improvement. ARS scientists in Ames, Iowa, developed a suite of powerful tools that employs machine learning for rapid 3-D protein structure prediction and analysis, which dramatically streamlines protein comparisons within maize and across diverse species, from crops to humans and yeast. This technological achievement paves the way for deeper explorations of maize's genetic diversity, accelerating research and enhancing our understanding of maize gene functions, ultimately driving advancements for improving crucial maize traits.

Breeding Insight powers ARS breeding. Specialty crop and livestock are central to human nutrition, wellbeing, and cultural preservation. Together their production is responsible for more than \$150 billion in cash receipts annually. ARS and university partner teams who breed these species develop outstanding biological and practical know-how but often lack specialized expertise in DNA-based genomics-powered breeding or advanced information technologies. ARS and Cornell University's collaborative Breeding Insight project centralizes that expertise and adds flexibility to apply advanced genomic and information/automation technologies for the genetic improvement of diverse species across the country. The project expanded its support to genetic improvement of 19 agricultural species in 2023, encompassing 32 breeding teams located in 18 states. Genomic markers that accelerate breeding were developed for an additional 5 species. Information and machine learning technologies powered the integration of historical data and automated the collection of new field data for 18 species, resulting in a 190 percent increase in database records available as compared to 2022. In sugarcane, blueberry, citrus, and sweet potato, for example, ARS breeders are collecting data faster and with fewer errors and, for first time, leveraging all aggregated historical datasets to improve precision in selection. Breeding Insight enables U.S. breeders to accelerate the delivery of more nutritious, climate-resilient crops and livestock.

A database for carrots and related crops. Crop improvement programs generate large datasets with information about crop traits and genetic variability. These datasets often reside in databases organized by crops or by families of related crops that are the subject of research and breeding. The recent sequencing of the carrot genome rapidly generated large datasets, so ARS researchers in Madison, Wisconsin, developed a database called CarrotOmics to collect and organize agricultural and genetic data for carrot and related crops. The images and genomic data collected comprise a database of great interest to plant researchers, crop breeders in public and private sectors, crop production and processing industries, and consumers. The carrot breeding stocks and genetic information in the CarrotOmics database generated by ARS research serve as a valuable foundation for ongoing research and breeding for this crop.