

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

National Program 301 • Plant Genetic Resources, Genomics, and Genetic Improvement

FY 2017 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; 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, 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.

During fiscal year 2017, this National Program was composed of three 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 – Crop Genetic and Genomic Resources and Information Management*
- *Component 3 – Crop Biological and Molecular Processes*

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 2017, but rather are selected based on impact and level of contribution to meeting the National Program goals.

Component 1 – Crop Genetic Improvement

Durable sunflower downy mildew and rust resistance. Downy mildew and rust are two devastating diseases that seriously reduce yields for sunflower producers, and commercial confection sunflower

breeders rely on ARS for inbreds with disease resistance. But very few suitable confection (eatable) inbred sunflower lines with high levels of resistance to downy mildew and rust resistance are available for commercial confection sunflower breeders. Therefore, ARS scientists in Fargo, North Dakota, developed and released three germplasm lines resistant to both downy mildew and rust. The germplasm carries one downy mildew and one rust resistance gene, with different genes in each germplasm providing resistance to all known races of North American rust and downy mildew, representing the first confection germplasm with combined resistance to both downy mildew and rust. Molecular markers related to both disease resistance genes were developed and provided to the sunflower industry, enabling breeders to develop additional hybrids with resistance to multiple pathogens, thus assuring sustainable sunflower production in the presence of these two devastating diseases.

Sugarcane aphid resistant sorghum. Sugarcane aphid has become a major pest in sorghum in the last few years. ARS scientists in Lubbock, Texas, developed two new pollinator (restorer or R) lines designated LBK1 and LBK2 (tested as R.11259 and R.11143) that showed significant tolerance to sugarcane aphid. These R lines can help breed sorghum hybrids resistant to sugarcane aphid. These two lines have been transferred to four seed companies and have been adopted in their breeding programs.

Strawberries perform better under low tunnels. Strawberries are economically valuable to farmers and are popular with consumers who expect availability all year long; however, in much of the United States, traditional strawberries produce fruit only three to four weeks each year. To produce strawberry fruit for several months, farmers need to use a repeat-fruiting strawberry variety that fruits nearly all year long, and protect it from mid-summer outdoor conditions. Therefore, ARS scientists in Beltsville, Maryland, compared the performance of repeat-fruiting strawberries under two different production systems (raised beds with and without low tunnels) to determine the impact of day length, brightness, soil moisture, humidity, and temperature on strawberry yield. They found the higher temperatures under low tunnels, especially in early spring and late fall, resulted in a much longer harvest season. Strawberry yield increased as light increased, and also with warmer temperatures up to about 28 degrees-Celsius, above which yields dropped due to excessive heat. Further evaluations showed that yields were more strongly associated with soil temperatures than air temperatures. This new discovery will be useful to strawberry growers and scientists studying ways to help farmers increase the length of the strawberry season to match consumer demand. The new discoveries have been published in scientific journals and presented at grower and professional meetings. Strawberry growers are rapidly adopting the low tunnel production system.

Two USDA potato varieties chosen by McDonald's for the production of French fries. The retail market for potatoes used for French fries in the United States represents approximately \$1.5 billion before the added value of retail sales. The quick service restaurant sector prefers lighter colored French fries with a wider range of storage temperatures that can resist the conversion of starch to sugars, which promotes an unacceptably dark French fry. But breeding and identification of new potato varieties with these enhanced attributes involves careful testing and data collection across multiple locations and years. Therefore, ARS scientists in Aberdeen, Idaho, collaborated with the University of Idaho, Washington State University, and Oregon State University to develop suitable varieties for the French fry industry. The research team released 'Blazer Russet' in 2005 and 'Clearwater Russet' in 2008, and partnered with growers, processing companies, and the restaurant industry to conduct extensive testing for more than 10 years. McDonald's recently accepted these two varieties for use in their French fry business.

Mcdonald's now uses only seven varieties for French fries, and four of the seven originated from the ARS-Aberdeen breeding program. In addition to the benefits of enhanced culinary characteristics, greater fry recovery, less processing waste, and higher marketable yields, both varieties represent improved sustainability in production with more efficient nitrogen use and greater tolerance to temperature and water stress, and with less tuber defects than industry standard varieties. These offerings will have high impact in providing significant economic benefits for potato growers and deliver a high quality product to consumers.

Release of soybean germplasm line with superior tolerance to high temperature stress. High heat (greater than 90 °F) damages soybean seed by reducing seed germination and seed vigor, as well as increasing seed wrinkling, seed coat impermeability, and green seed discoloration. Unacceptable seed quality and economic loss are major challenges for soybean producers in environments with consistently high temperatures, such as the Mississippi Delta region. But there has been little attention in commercial breeding programs to address the issue. Therefore, ARS researchers in Stoneville, Mississippi; Columbia, Missouri; Jackson, Tennessee; and Raleigh, North Carolina, discovered a heat tolerance gene, and developed and released a maturity group IV soybean germplasm line to seed companies and public breeders. This line maintains excellent seed germination and high seed quality under elevated temperatures. 'DS25-1' is the first U.S. soybean germplasm release to address the heat tolerance problem in soybean, and it will enable breeding for improved seed quality in high temperature stress environments. Commercial and public soybean breeders are using DS25-1 in their breeding programs to develop heat tolerant soybean cultivars for producers.

New pinto bean cultivar has slow darkening trait and improved market quality. Late season rains can delay harvesting of pinto beans and cause the beans to darken prematurely, and growers in the United States receive a reduced price for darkened beans that amounts to annual losses in excess of \$30 million. Current varieties lack the genetic potential to restrict darkening. Therefore, ARS researchers in Prosser, Washington, in cooperation with scientists at North Dakota State University, developed and released the new pinto bean cultivar 'Scout.' 'Scout' has the slow darkening seed trait that protects pinto beans from premature darkening, and it also has better canning quality than the industry pinto bean standard 'Othello.' 'Scout' provides growers in the western United States and intermountain plains with a new bean cultivar that has excellent value and market quality.

Identification of a new virus associated with blueberry fruit drop disease. Blueberry fruit drop disease was detected in blueberry plants in the Pacific Northwest more than a decade ago. Symptoms include plants that drop all of their fruit before harvest, when the berries are unripe. The cause of the disease was unknown, making eradication difficult, so ARS researchers set out to determine the cause of blueberry fruit drop disease by examining infected plants. A new virus, called Blueberry fruit drop-associated virus (BFDaV), was cloned and sequenced from small circular DNA isolated from plants exhibiting fruit drop symptoms. The virus was detected in 35 of 35 symptomatic plants, and 31/31 asymptomatic plants tested negative for the virus, showing a strong correlation between the virus and the disease. In a national blueberry virus survey carried out in 2015 and 2016, from the major blueberry producing states that included about 4,200 samples, this virus was only detected in northwest Washington and British Columbia, and in an 'Aron' blueberry plant from Finland housed at an ARS unit in Corvallis, Oregon. Due to its very limited distribution and slow rate of spread in the field, efforts are underway to eradicate this virus from blueberry fields in North America.

Genetic characterization of beneficial nitrogen-fixing rhizobacteria. A crucial factor contributing to the historical success of peas, lentils, and chickpeas is their ability to form symbiotic relationships with beneficial rhizobacteria that convert atmospheric Nitrogen to ammonia (NH₃), which can be used as fertilizer by grain legumes and subsequent rotational cereal crops, and plant breeding has been proposed as a method to increase biological nitrogen fixation (BNF). But little is known about how BNF is affected by different plant lines and strains of nitrogen fixing rhizobacteria. Therefore, ARS researchers in Pullman, Washington, completed a genetic characterization of 156 isolates of rhizobacteria collected from field grown peas and chickpeas. The entire collection of characterized isolates was deposited into the National Rhizobium Germplasm Resource Collection. This work has resulted in the release of a characterized reference set of rhizobia strains for improving capacity for BNF in peas and chickpeas. The availability of this resource provides new information about the BNF capacity of different plant lines when paired with specific strains of nitrogen fixing rhizobacteria.

Sugar beet germplasm with resistance to multiple diseases released. Diseases are a limiting factor for sugar beet production worldwide. Rhizomania is a serious disease found in all sugar beet growing areas in the United States. Because of the cost of fumigation to control rhizomania and its negative impact on the environment, the use of rhizomania-resistant cultivars is employed to manage this disease. There are two main genes (Rz1 and Rz2) in commercial germplasm used to develop resistant cultivars to rhizomania. ARS scientists in Fort Collins, Colorado; East Lansing, Michigan; and Kimberly, Idaho, used genetic markers to successfully develop two sugar beet populations, one with the Rz2 gene (FC1740), and the other with both the Rz1 and Rz2 genes (FC1741). The two populations have been released to commercial seed companies for the production of not only hybrid cultivars with resistance to rhizomania, but also to beet curly top virus and fusarium yellow, two other devastating diseases of sugar beet in the United States.

Development and assembly of a complete draft genome of hop. Identification of molecular markers for use in selection regimes is highly dependent upon the use of a complete draft genome covering all chromosomal regions. Previous quantitative trait locus (QTL) studies identifying molecular markers in hop utilized a draft assembly covering only 63% of the genome—leaving open the possibility that the best molecular markers would be overlooked. Therefore, ARS scientists in Corvallis, Oregon, along with collaborators at Oregon State University and Pacific Biosciences, sequenced and assembled a new draft hop genome that covers approximately 98 percent of the genome. This research discovered that up to 3 percent of the hop genome consists of structural variants of DNA sequences and also identified highly repetitive DNA consisting of transposable elements and other repeat structures. Previous draft genomes were made up of relatively short stretches of DNA sequence data with average assembled size of such sequences being 40,000 base pairs while the new draft genome has an average size of assembled sequences equaling 750,000 base pairs. This new draft genome will enable the precise identification of molecular markers and genes linked to traits of economic importance in hop as well as enable other molecular tools such as unknown variety identification.

Anthracnose fruit rot in tomato and pepper elucidated. Anthracnose fruit rot is one of the most serious diseases affecting the production of tomato and pepper in the United States. Although anthracnose is generally considered to be a disease of ripe fruit, increased incidence of disease on immature pepper fruit has been observed. ARS scientists in Beltsville, Maryland, in collaboration with Rutgers University,

collected fungal samples from infected fruit from the Mid-Atlantic and Northeastern United States to characterize the genetic diversity and virulence of these samples. Extensive diversity among fungal samples was identified. They also identified which samples caused immature fruit to rot, rather than ripe fruit. Based on the fungal diversity in a production region, growers can now determine whether to implement disease management strategies at immature or mature stages of fruit development. This information on pathogen population diversity also enables breeders to develop new cultivars resistant to more aggressive strains of the anthracnose pathogen.

Infrared thermography reveals how freezing occurs in wheat under natural conditions. Depending on timing and growth stage of wheat, complete loss of a crop is possible when freeze events occur in the spring. In the last two years in some regions of the United States, a 40 to 60 percent loss of yield was attributed to unexpected spring freezes. The heat given off by water when it freezes can be visualized with an infrared camera. Using this technology, ARS scientists in Raleigh, North Carolina, determined that under laboratory conditions, many wheat tissues remain unfrozen even when temperatures are below freezing and some parts of the plant are frozen. When moving the cameras to the field, we found that plants in the field always froze from the bottom to the top, even though the tops of the leaves were 6 or 8 degrees colder than the bottom. In addition, older leaves always froze before younger leaves, as was also observed in the lab. Additionally, we found that even though many times “leaf-tip burn” is observed after a winter and spring freeze, the tips of leaves never freeze independent of the whole leaf. This suggests that even though an entire leaf freezes, the tips of leaves are less freezing tolerant and die while the rest of the leaf survives. This provides a basis for studying gene expression within the plant to determine differences in freezing tolerance mechanisms within the plant. The field analysis confirmed that laboratory freeze tests accurately duplicate freezing within plants under natural freezing conditions.

First rapid genetic diagnostic test developed for early detection of vascular wilt of celery. Celery is one of the most valuable crops per acre in the United States, with a 2015 farmgate value of more than \$450 million from 30,000 acres of production. But celery production is threatened by the highly destructive but poorly understood fungal disease termed vascular wilt. ARS scientists in Peoria, Illinois, in cooperation with university colleagues, applied leading-edge DNA genetic analyses to determine the fungal pathogen’s genetic diversity. The genetic analyses indicated that the several fungal races that infect celery evolved independently. This information will help guide the direction of ongoing research into this disease, and also provide information needed to develop disease control strategies that are effective against these genetically diverse pathogens. In addition, the DNA data were developed into the only effective molecular diagnostic test for detecting these distinct races. That information can help prevent the introduction and spread of these pathogens into disease-free celery production areas within the United States.

Cranberry fruit rot resistance molecular markers developed. Sustainability of the cranberry industry is threatened by widespread and increasing losses due to fruit rot in the field, as well as increasing restrictions on using fungicides for disease control. A long-term solution is to develop rot-resistant varieties. ARS scientists in Chatsworth, New Jersey, and in Madison, Wisconsin, together with university collaborators, developed a composite genetic map of cranberry with 6,000 markers, and utilized the map to identify markers linked to 19 genetic loci for rot resistance. Genomic regions that control yield and berry weight were discovered as well. Cranberry breeders are already using these markers in their

breeding programs to develop improved cranberry cultivars with improved fruit rot resistance, higher yield and superior fruit quality.

Completion of the first genetic map for mango. Mango ranks as the fifth most-consumed fruit in the world, after citrus, banana, grapes, and apple. Millions of acres are grown worldwide, with about 2,300 acres in the United States, although U.S. acreage is growing, and U.S. producers have had a substantial influence on the supply of new cultivars for international trade. But producing a new cultivar of a tree crop like mango requires 15 years or more of breeding with traditional approaches. Detailed knowledge of the location on the mango genome of genes that control key traits such as fruit color, shelf life, disease resistance, etc., could substantially improve the efficiency of selecting those traits via associations of controlling genes with genetic markers. ARS researchers in Miami, Florida, and cooperators in Australia, Brazil, Indonesia, Senegal, and Thailand have created the first genetic map for mango. By knowing where the genes that regulate the superior traits are on the genetic map, mango breeders can select superior trees at the seedling stage with genetic markers and remove seedlings that will not develop the desired trait. Considering that most of the cost of mango breeding involves maintaining and evaluating the trees over years, a genetic map gives U.S. and international breeders a powerful tool to reduce their costs and improve their chances of more rapidly breeding new, improved mango cultivars.

Drought: Automated root imaging system improvement. As a result of the dramatic impacts of drought on U.S. agricultural productivity, there is increased demand for high throughput tools for measuring older and larger root systems, to improve our understanding of the genetics that control rooting traits that underlie crop productivity. ARS researchers in Ithaca, New York, developed an improved 3D root imaging system for use on a range of agricultural crops. The system incorporates improved plant root growth media, new operating and imaging software, reduced imaging time, increased system capacity and throughput, and provides for the safe storage of critical experimental data.

Discovery and characterization of a hypoallergenic soybean. Soybean seeds are a major source of edible oil and protein, and are among the eight most significant allergenic foods for humans. Several seed storage proteins in soybean have all been shown to be allergenic, and they are present in all currently grown soybeans. Therefore, in an effort to identify soybean lines that lack the major allergens, ARS researchers in Columbia, Missouri, screened the NPGS soybean germplasm collection and identified a line that is missing one of the allergenic proteins. Genomic resequencing allowed identification of the causative molecular genetic reason for the absence of this allergen. Additionally, a new computational method was developed to predict the impact of this gene on soybean seed composition. This new information was used to develop an experimental soybean line with seeds that lack three of the major soybean allergens. These new lines will be useful to soybean breeders for the development of high-yielding soybean germplasm with lower allergenic effects.

Genomic region controlling nitrogen use efficiency identified in Great Plains hard winter wheat. Wheat varieties that efficiently capture and convert available soil nitrogen into grain protein are key to sustainably meeting the rising global demand for grain protein. Both historically important and contemporary varieties and breeding lines were included in the set of 299 winter wheat lines that was tested by ARS scientists in Manhattan, Kansas, and University collaborators. Differences in plant height and flowering date explained much of the variation in nitrogen use efficiency. A region of the wheat genome on the long arm of chromosome 2D also was important for nitrogen use efficiency. The most

favorable gene(s) in this region are not widely used in current varieties, which indicates that wheat nitrogen use efficiency could be improved by selection using markers in this region of the wheat genome.

PCR assays developed that can detect over 800 loci in barley in one reaction. Next generation marker systems rely on random sequencing, which results in markers that may or may not be linked to important targets of selection by breeders. ARS researchers in Pullman, Washington, screened over 2,000 primer pairs derived from previously mapped single nucleotide polymorphic (SNP) loci to identify highly informative SNP markers for genomic selection in western germplasm. They supplemented this set with markers derived from known genes that are targets of selection in breeding programs. A targeted amplicon sequencing panel was developed for the regional barley programs with over 500 oligonucleotides that saturate the barley genome that can be utilized in genomic selection. This panel allows next generation sequencing methods to be used in breeding programs with known targets of selection that are important for plant breeding.

Key enzyme in lignin synthesis characterized in sorghum. Sorghum biomass (stalks and leaves) serves as an important forage crop for livestock. In addition, sorghum is being developed as a bioenergy crop for advanced or second generation biofuels production. Advanced biofuels are derived from the breakdown of the cellulose and hemicellulose components of biomass into sugars, and their subsequent conversion into biofuel molecules. A third biomass component, lignin, impedes breakdown of biomass in either livestock digestive systems or bioenergy conversion processes. Cinnamoyl-CoA reductase (CCR) gene encodes an enzyme involved in the synthesis of lignin. ARS scientists in Lincoln, Nebraska, together with scientists from Washington State University examined how this enzyme makes precursors to lignin in sorghum. Two classes of CCR enzymes were discovered, which have different roles in lignin synthesis. The second class of CCR enzymes is involved in making a specific type of lignin associated with plant defenses against pathogens. Collectively, this research gives a new perspective on the dual functions of this enzyme in lignin synthesis, and may lead to ways to protect plants from pathogens or insects. In addition, this research provides new ways to alter biomass composition of sorghum and other grasses for improved bioenergy conversion.

Carrot gene flow deciphered. Studies of gene flow between crops and their wild relatives have implications for both farming and breeding management practices as well as understanding the risk of transgene escape. Wild carrot occurs widely across the United States, but gene flow between wild and cultivated carrots has not been studied. ARS scientists in Madison, Wisconsin, with collaborators in Tennessee utilized molecular genetic markers to evaluate carrot gene flow in two regions of the United States and discovered substantial gene flow indicating that both carrot pollen and seed transfer genetic information among wild and cultivated populations in the same geographic region. This research provides seed companies with knowledge of risks that wild carrots impose on carrot seed production, and insights into carrot pollination biology.

Sugar beet resistance genes identified, cloned and functionally defined. Plant diseases and pest problems are responsible for decreases in crop yields. Scientists in Beltsville, Maryland, utilized an enrichment technique to discover and characterize sugar beet root genes involved in pest and disease resistance responses. One of the several genes that were selected for further analysis was cloned and reconstructed for expression in genetically modified plants to determine its role in insect and disease resistance. Findings revealed a new structure of this gene that is uniquely associated with sugar beet and

may be responsible for broad resistance to fungal pathogens and insect pests. Plant biologists will use this information to design new strategies for developing improved plant varieties with enhanced disease and pest resistance that will benefit farmers by increasing yields and the quality and nutritional value of cultivated crops for human consumption.

Component 2 – Crop Genetic and Genomic Resources and Information Management

Resistance identified for new types of wheat stem rust. Wheat, a staple of life for billions worldwide, had a farm gate value of ca. \$10 billion in the United States in 2015. For centuries, wheat production has been threatened by a highly virulent microbe, wheat stem rust, which rapidly changes genetically into new dangerous types. New and highly virulent races of the wheat stem rust pathogen, known as Ug99, have arisen in Africa and are a severe threat to U.S. and world wheat production. To protect the U.S. wheat crop from Ug99, new research tools are needed to help wheat breeders identify new sources of genetic protection. ARS researchers in Aberdeen, Idaho, and St. Paul, Minnesota, discovered genetic markers that speed the selection for the resistance gene Sr28—effective against Ug99—from wheat breeding stocks. Also, a potentially new type of genetic resistance that acts at the adult wheat plant stage was identified and its location mapped on the wheat genome. These results provide research tools that substantially aid U.S. wheat breeders with developing new wheat varieties resistant to stem rusts.

Big Data Toolbox: Determining optimal breeding strategy for crop performance and yield. While computer-aided simulation may be useful in designing optimal breeding strategies, the complexity of simulation is a barrier for breeders to take the first step. ARS researchers in Ithaca, New York, developed a simple and flexible computational toolkit called the Breeding Scheme Language (BSL). The BSL tool allows users to more easily simulate breeding options and estimates of benefit from selection under specific genetic criteria and options. This tool will be useful for breeders to evaluate breeding schemes and to choose an optimal breeding strategy among a number of possible ones, as well as provide a training platform for plant breeders.

More efficient technique for detecting “seed aging.” The ARS National Plant Germplasm System (NPGS) conserves more than 500,000 individual germplasm samples in the form of seeds. Monitoring the viability of all of those seed samples during storage is an essential NPGS operation, but it is labor-intensive, consumes valuable germplasm, and does not predict when seeds would begin to die. ARS scientists in Fort Collins, Colorado, have introduced a new method to detect “seed aging” that measures the integrity of a key genetic molecule, RNA, within seed cells. This new assay has the potential for automation, generates highly quantitative data about seed aging, closely corresponds to germination potential of seeds, and consumes about 1/10 the number of the valuable seeds required for germination tests. Implementing this technology will ensure that early effects of seed deterioration will be detected in NPGS seed collections while reducing the labor costs and number of seeds required for such tests.

Thousands of new genetic markers for genetically improving pea. Pea production in the United States generates more than \$600 million/year in farm gate value, but higher-yielding varieties are needed for this crop to remain commercially competitive. Incorporating new genetic diversity into the pea crop holds the key for unlocking additional yield potential for all types of pea market classes. But, breeders need additional genetic markers—signposts that can guide them to new genetic variants for higher yield.

ARS researchers in Pullman, Washington, and international and U.S. university cooperators identified more than 66,000 high quality genetic markers from more than 400 pea genetic samples in the NPGS. These markers, accessible worldwide through ARS' Germplasm Resource Information Network (GRIN)-Global database, plus the NPGS' pea germplasm samples, are now available for researchers and breeders to identify new genes in pea for increasing yield and farmer profitability.

Big Data Toolbox: The Triticeae Toolbox Plant Breeder's Software. Important grains such as wheat, rye, and barley belong to the family triticeae. ARS researchers in Ithaca, New York, developed software for triticeae breeders termed The Triticeae Toolbox (T3). T3 enables plant breeders and researchers to combine, visualize, and interrogate the wealth of data collected by numerous researchers across the nation and around the world to assist in breeding of cereal crops. T3 assists plant breeders in generating trial reports, selection indices, trial comparisons and visualizing a number of aspects of distinct and diverse data. T3 makes the slow and difficult task of crop breeding and improvement more efficient for those undertaking this important task needed to meet the food security needs of a growing world population. T3 software is available under the General Public License and is freely downloadable.

New genetic markers for accelerating breeding black raspberries resistant to pests. The value of black raspberry production in California, Oregon, and Washington combined is about \$7.2 million for the fresh market and \$9.5 million for the processed product. But in those states the current standard commercial cultivar is highly susceptible to the large raspberry aphid, which is a vector for the raspberry mosaic virus complex, which causes such a rapid decline in plant health that fields must be re-planted with new bushes after only three to four growing seasons. ARS scientists in Corvallis, Oregon, identified a genetic marker in the black raspberry genetic material, closely associated with resistance to the aphids and viruses, which enables breeders to rapidly distinguish resistant from susceptible plants at the early seedling stage, accelerating development of new resistant cultivars for the black raspberry industry.

Genes conferring resistance to rust, anthracnose, and angular leaf spot diseases. Rust, anthracnose, and angular leaf spot diseases are devastating in the Americas and Africa, the largest common bean production regions of the world. And the black-seeded Ouro Negro dry bean cultivar is unique in containing resistance to all three diseases. But to capitalize on the resistance in breeding programs, an understanding of the resistance mechanisms is needed. Therefore, ARS scientists in Beltsville, Maryland, in collaboration with researchers at the Universidade Estadual de Maringá, Maringá-PR, Brazil, discovered that the resistance in Ouro Negro is conferred by three different single, and dominant genes. Using DNA technologies, ARS researchers showed that these genes are located very close to each other on the bean chromosome, and are inherited together. The scientists discovered DNA markers that identify these genes which are now being used by breeders to verify that all three genes get transferred when they breed new varieties of commercial beans to be grown in different parts of the world.

Genome deciphered for bacterium that fixes nitrogen in soybean. Bradyrhizobium bacteria has the ability to form nodules on the roots of legume plants, where nitrogen fixation can occur, and crop plants like soybean, the second largest field crop in the United States, can produce its own nitrogen through its association with the bacteria. But very little is known about the genetics of the rhizobium organism, which restricts research to identify the genes responsible for the synergy between the soybean plant and the bacterium. Therefore, participating in an international collaboration, ARS scientists in Beltsville, Maryland, sequenced the genome of a specific species of rhizobium, *Bradyrhizobium elkanii*. The

availability of the complete genome sequence will be valuable in uncovering functions of all of the genes responsible for soybean-rhizobium interactions, and nitrogen fixation. The sequence will be useful to companies, universities, and government laboratories that have the mission to understand the association of soybeans with these specific bacteria, and improve soybean production by increasing nitrogen fixation.

Component 3 – Crop Biological and Molecular Processes

Drought: Traditional row crops versus perennial biomass crops. The national drought of 2012 provides important data for comparing the benefits and risks, in terms of carbon and water cycling, from traditional row crops and perennial biomass grass crops production. Data was collected and analyzed from 2008, through the major Midwestern drought of 2012, and through the 2013 recovery, by ARS scientists in Urbana, Illinois, and university collaborators. While maize and soybean showed a significant reduction in carbon uptake and water loss due to limited soil moisture supply, the perennial grasses maintained higher productivity and water use, due to their deeper established root systems, throughout the drought. Despite higher productivity during the drought, however, the perennial grasses showed lower water use efficiency, due to greater access to soil moisture coupled with extremely dry atmospheric conditions. Moreover, the perennial biomass crops lagged in productivity in 2013, due to a lack of soil moisture recharge, although there was no lag effect on annual crop productivity. Therefore, while perennial crops are more resilient than annual row crops during extreme drought conditions, their higher water use during a drought can negatively impact productivity in following years.

High carbon dioxide levels affect sweet corn's response to insect pests. Variations in atmospheric conditions will likely impact American agriculture, thus threatening future supplies and food safety. ARS researchers in Gainesville, Florida, therefore sought to determine if high carbon dioxide levels affect how sweet corn defends itself against insect pests. They discovered that the amount of airborne chemical signals (odors) produced by caterpillar infested sweet corn was reduced when plants were grown under high carbon dioxide conditions. These odors help protect sweet corn from caterpillars as they act as locator signals for parasitic wasps that lay their eggs in the caterpillars, thus killing them. These findings, published in the *Plant Cell and Environment* journal, suggest that growers (particularly organic growers) that use such wasps as a form of biocontrol to reduce crop damage from caterpillars may need to modify their pest management strategies if atmospheric carbon dioxide levels rise.

Discovery of a group of genes that enhances fiber quality in cotton. Improving fiber quality is a top breeding goal in most cotton breeding programs, and applying DNA markers to improve fiber quality is not yet widely used. But identification of genes or genomic locations affecting an agronomic trait is a prerequisite to the use of DNA markers to improve a specific trait in plant breeding. Therefore, using a unique random-mated population that was derived from crosses involving 11 cotton cultivars, ARS scientists in New Orleans, Louisiana, identified a major group of genes on chromosome 7 of the cotton genome, that work in concert with each other to improve fiber quality. This group of genes has large effects on fiber strength, uniformity, and short fiber content, and moderate effect on length. The scientists developed DNA markers to identify the cluster, which were released to the public, along with the genetic populations containing the genes. Selection based on these DNA markers can simultaneously improve fiber strength, length, and uniformity in Upland cotton. Germplasm with these genes, and

associated DNA markers identified in this research are being used in cotton breeding programs to improve fiber quality while maintaining yield via marker assisted selection strategy.

Breeding: 'Honeysweet' GE plum is released. Sharka is a devastating disease of stone fruit caused by plum pox virus (PPV) that resulted in the near collapse of the plum industry in Eastern Europe and caused significant losses in most plum producing regions around the world. ARS scientists in Kearneysville, West Virginia, in collaboration with international partners, demonstrated that 'Honeysweet' trees planted in PPV stricken regions have remained largely virus and symptom free after over two decades of field trials. 'Honeysweet' was officially released as a publicly available cultivar in the United States. The first trees are now available for purchase from U.S. nurseries, and the group is currently working with international partners to make 'Honeysweet' available in the European Union.