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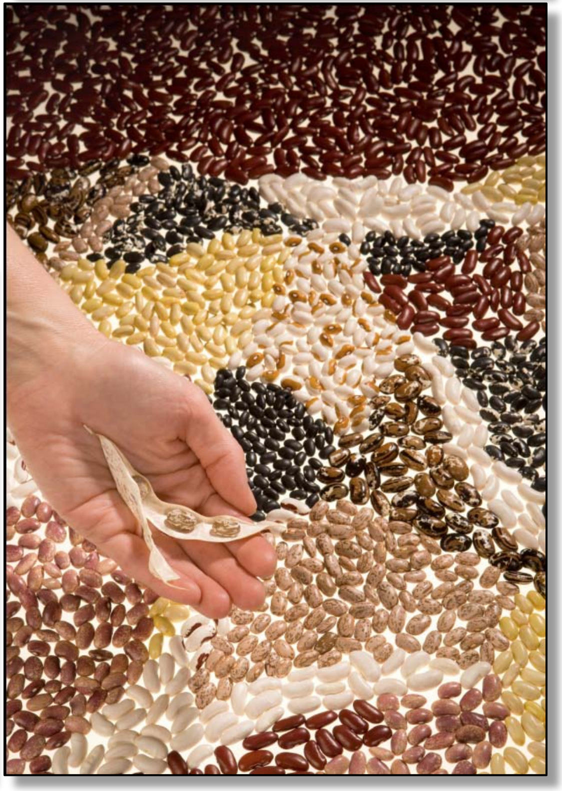
National Program 301:
Plant Genetic Resources, Genomics, and Genetic Improvement

ACCOMPLISHMENT REPORT 2006-2011



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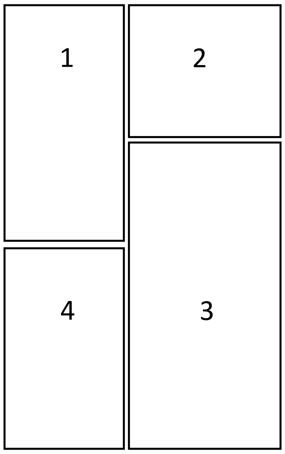

1. ARS Geneticist Sherry Flint-Garcia, Columbia, Missouri, examines and shells corn seed from the “Nested Association Mapping” (NAM) experimental population. *Photo by Peggy Greb/ARS.*

2. Rather than the 3 to 10 years normally required for a seedling plum to produce fruit, FasTrack plum lines carrying the early-flowering gene developed by ARS scientists in Kearneysville, West Virginia, which produces fruit in less than a year after being planted from seed. *Photo by Chinnathambi Srinivasan/ARS.*

3. Beans are one of the staple crops that feed people all over the world. This photo shows the diversity of dry beans, including pinto, great northern, black, and kidney. The Agricultural Research Service works to develop beans with resistance to various diseases. *Photo by Peggy Greb/ARS.*

4. Carrots freshly harvested from a farm garden near Stanwood, Washington. ARS researchers have developed many colorful varieties of carrots with improved nutritional traits. *Photo by Doug Wilson/ARS.*

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

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BACKGROUND AND GENERAL INFORMATION

This report is a distillation of some of the most significant research accomplishments of the past 5 years achieved by scientists working in the USDA Agricultural Research Service's (ARS) National Program 301, Plant Genetic Resources, Genomics, and Genetic Improvement**.** The largest of the 18 ARS National Programs, NP 301 encompasses 177 research projects focused collectively on a vision of furnishing genetic, genomic, and bioinformatic tools, information, genetic resources, and crop varieties to enhance American agricultural productivity and ensure a high quality, safe supply of food, fiber, feed, ornamentals, and industrial products. To attain that vision, this National Program pursues a mission of breeding improved germplasm and superior crop varieties; developing and applying new genetic and analytical bioinformatic tools; and safeguarding and utilizing plant genetic resources and associated genetic and genomic databases.

The rationale for NP 301 is based on the recognition that the U.S. systems of crop production face formidable challenges. Some of the most exacting challenges involve successfully adapting these systems to the accelerating rates of change in factors affecting agricultural productivity, including more frequent climatic extremes, emerging pests and diseases, rapid water and soil depletion, and more severe shortages and higher costs of other inputs. The challenges can only be met by technologies that optimally harness the inherent genetic potential of plant germplasm. Such technologies will enable those production systems to maximize profits, as well as add to the security of supply, price stability, and market competitiveness. In addition, they will reduce crop losses from genetic vulnerabilities and deliver nutritious food to consumers.

The research encompassed by NP 301 is crucial to developing safe, secure, and efficient crop production systems. It addresses these vital goals by incorporating and integrating a broad spectrum of agronomic and horticultural disciplines from genetics, genetic resource management, and plant breeding to genomic technology, information management, and novel bioinformatic analytical approaches. As this report documents, during 2006-2011, NP 301 has made a significant positive impact on crop agriculture and agricultural research, both in terms of the sheer number and quality of accomplishments and the breadth of issues addressed. The technologies generated by NP 301 include more rapid and efficient methods for identifying useful properties of genes and genomes, and for manipulating genetic and genomic material and information. They encompass more effective breeding strategies, based on more comprehensive knowledge of crop genomic structures, and on bioinformatic methods for integrating information regarding variation in genomic structure with knowledge of phenotypic variability in key crop traits. More cost-effective and accurate molecular markers have improved the efficiency of gene identification and mapping. More rapid gene analyses and mapping methods and strategies have determined the function of particular genomic segments. Genebanks and genome and germplasm databases have safeguarded and delivered huge volumes of germplasm and new genomic data. Breeders developed superior varieties that enabled producers to maximize yields of high-quality products with minimum inputs; reduce water and soil limitations or contamination; and lower production costs.

The ever increasing demands for NP 301's research and service products demonstrate that this National Program's contributions and impact on U. S. agriculture have steadily increased over the years. During the last 5 years, ARS scientists distributed a record number of crop germplasm accessions, while devising more innovative genomic, genetic, and crop improvement approaches. They released valuable enhanced germplasm and superior cultivars for many crops. The agricultural research community accessed ever more frequently the growing amounts of data maintained in the ARS-developed crop germplasm and genome databases.

PLANNING AND COORDINATION FOR NP 301'S 5-YEAR CYCLE

Prior to the start of the current 5-year research cycle, ARS scientists and administrators held a workshop in October 2005 with customers, stakeholders, and research partners to identify major crop agricultural issues and research priorities. At the workshop, three major research components for NP 301 were formulated as the basis for research programmatic planning.

Subsequent to the 2005 Customer/Stakeholder Workshop, a writing team composed of ARS scientists and National Program Leaders drafted the current NP 301 Action Plan. Incorporating input from customer/stakeholder interaction, their knowledge of the science subject matter, and input from other ARS scientists and cooperators, the writing team identified the priority needs that ARS could address with its research. These individual research needs were aggregated into problem statements under each of the three research components. The Action Plan was finalized in 2006 and guided development of new individual NP 301 research projects that began the new 5-year research cycle in 2008.

All of the individual plans for NP 301 research projects include statements of the agricultural problem being addressed; the anticipated products or information to be generated by the project; how the planned research contributed to mitigating or solving the larger National Program problem statements; and timelines and milestones for measuring progress toward achieving project objectives. All project plans for NP 301 were evaluated prospectively for scientific quality by external peer review panels. Project plans were then revised in response to review panel recommendations and implemented.

Coordination and planning for NP 301 are the tasks of the National Program Leaders who constitute the NP 301 leadership team. The National Program Leaders also coordinate NP 301 activities with other ARS National Programs and with other agencies and departments. Some of the interagency research coordination associated with NP 301 is conducted through the Interagency Working Group on Plant Genomes, which includes leaders from ARS, the USDA

National Institute for Food and Agriculture (NIFA), USDA Forest Service, National Science Foundation (NSF), Department of Energy (DOE), National Institutes of Health (NIH), Office of Management and Budget (OMB), and the Office of Science and Technology Policy (OSTP). NP 301 National Program Leaders also confer and coordinate NP 301's research with colleagues from other USDA agencies, including the Economic Research Service, the Animal and Plant Health Inspection Service (APHIS), and the Foreign Agricultural Service (FAS), as well as with the U. S. Agency for International Development (USAID), and the U. S. Department of State.

Customer and stakeholder interaction and research coordination have continued to play important roles in helping National Program Leaders guide NP 301 research to maintain its relevance to U.S. agriculture. As shown in Appendix 4, NP 301 National Program Leaders attended or organized numerous research planning, coordination, and stakeholder workshops during 2006-2010. These workshops addressed crop/commodity-specific challenges, emerging high-priority issues, and new scientific developments in the fields of crop genetic resource management, genetics, genomics, genome databases, germplasm enhancement, and breeding.

Input for programmatic change can also come from other sources, as well. In 2009, the external Retrospective Review Panel reviewing the accomplishments of the 45 projects in National Program 302, Plant Biological and Molecular Processes, suggested that the research in that National Program should be integrated into NP 301. They noted the broad-scale methodological and conceptual convergence for the research conducted by the two national programs. After some discussion, National Program Leaders and ARS administrators decided to merge the two national programs as of October 2010. The NP 301 Action Plan that was developed in 2006 was revised in 2010 to add a fourth component to encompass the NP 302 research components and objectives. The current NP 301 Action Plan can be viewed online at: (www.ars.usda.gov/SP2UserFiles/Program/301/NP301ActionPlanRevised-April2010.pdf).

STRUCTURE OF NP 301

With the merger with NP 302 in October 2010, the NP 301 Action Plan now includes four Research Components:

Component 1: Plant and Microbial Genetic Resource Management.
This component encompasses ARS' efforts to conserve and distribute a wide diversity of plant genetic resources and selected microbes important for crop agriculture or industrial use. ARS researchers and genebank curators increase the value and utility of these conserved materials and associated information by characterizing their genetic contents and evaluating their agricultural merit.

Component 2: Crop Informatics, Genomics and Genetic Analysis.
In this component, researchers maintain and deliver huge volumes of crop genome sequence and genomic map data in state-of-the-art genome databases. New bioinformatic analytical tools are devised and applied. Crop genome structures are elucidated, key agricultural traits are mapped in crop genomes, and their underlying genetic bases are determined.

Component 3: Genetic Improvement of Crops.
For this component, ARS scientists develop innovative approaches for breeding plants more efficiently and effectively. They enhance unimproved crop germplasm and breed superior crop varieties for release to producers, or incorporation into public or private sector breeding programs for further improvement.

Component 4: Plant Biological and Molecular Processes.
Research in this component emphasizes understanding the function of agriculturally important crop genes and devising the means for manipulating their expression. The biological processes underlying crop productivity and quality are elucidated. New means of assessing risks associated with plant genetic engineering are developed.

For organizational and administrative purposes, specific NP 301 research projects are assigned membership in only one of these research components. However, these categories do not act as barriers or "stovepipes" that impede research across the National Program. Rather, many of those individual research projects contribute to the goals of multiple NP 301 research components or to several national programs (see Figure 1).

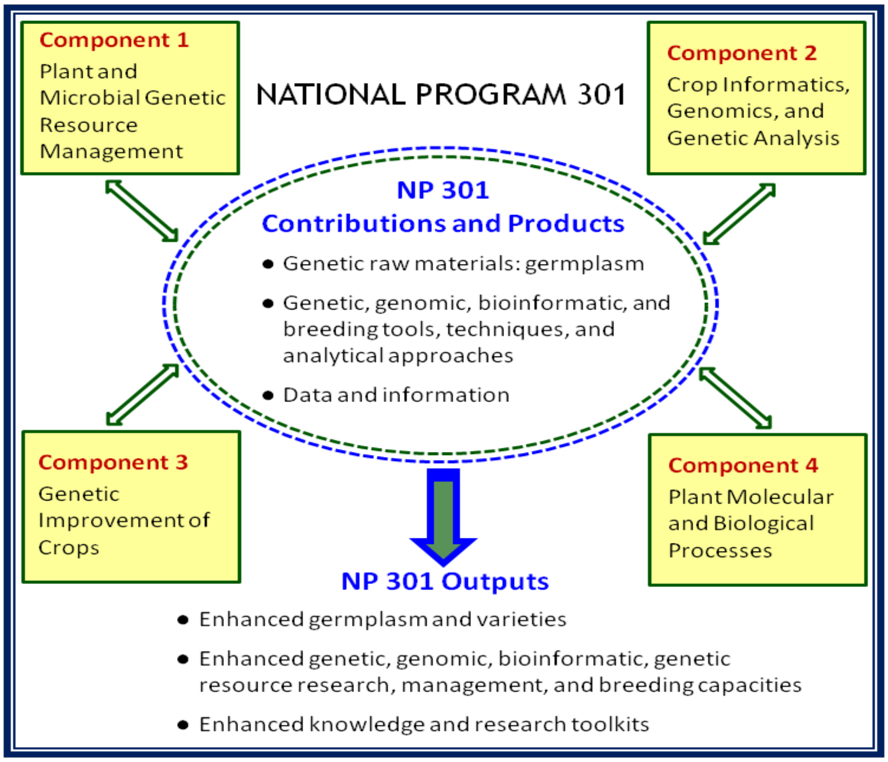

Figure 1. The research components of NP 301 are interactive and collaborative and often blend resources to contribute towards accomplishments within specific National Program goals.

For example, an ARS researcher might obtain seed from one of ARS' 21 crop genebanks (Component 1) in order to develop a new drought-tolerant or disease-resistant variety (Component 3). Once developed, the new variety might then enter directly into agricultural production, thus contributing to a key NP 301 product. Additionally, new information about the genes or the trait of the variety that confers the drought tolerance or disease resistance results from that research, and is added to the crop genome or germplasm database (Components 1 and 2). The seeds of the newly developed variety are incorporated into the genebank inventory for conservation and use by other breeders (Component 1). Researchers in genetic/genomic projects

(Components 2 and 4) could utilize that new information in determining the genetic basis for particular traits, which in turn could accelerate breeding future varieties.

As one example (see Figure 2) of how this functions operationally, NP 301 researchers enlisted the methods, tools, and genetic resources of NP 301 as a whole, across all research components, to devise an innovative approach to tree breeding. In many instances when products emerge from these processes, collaborations are established with industry or university partners to facilitate transfer of this technology to the end user and/or customer (See Component 3).

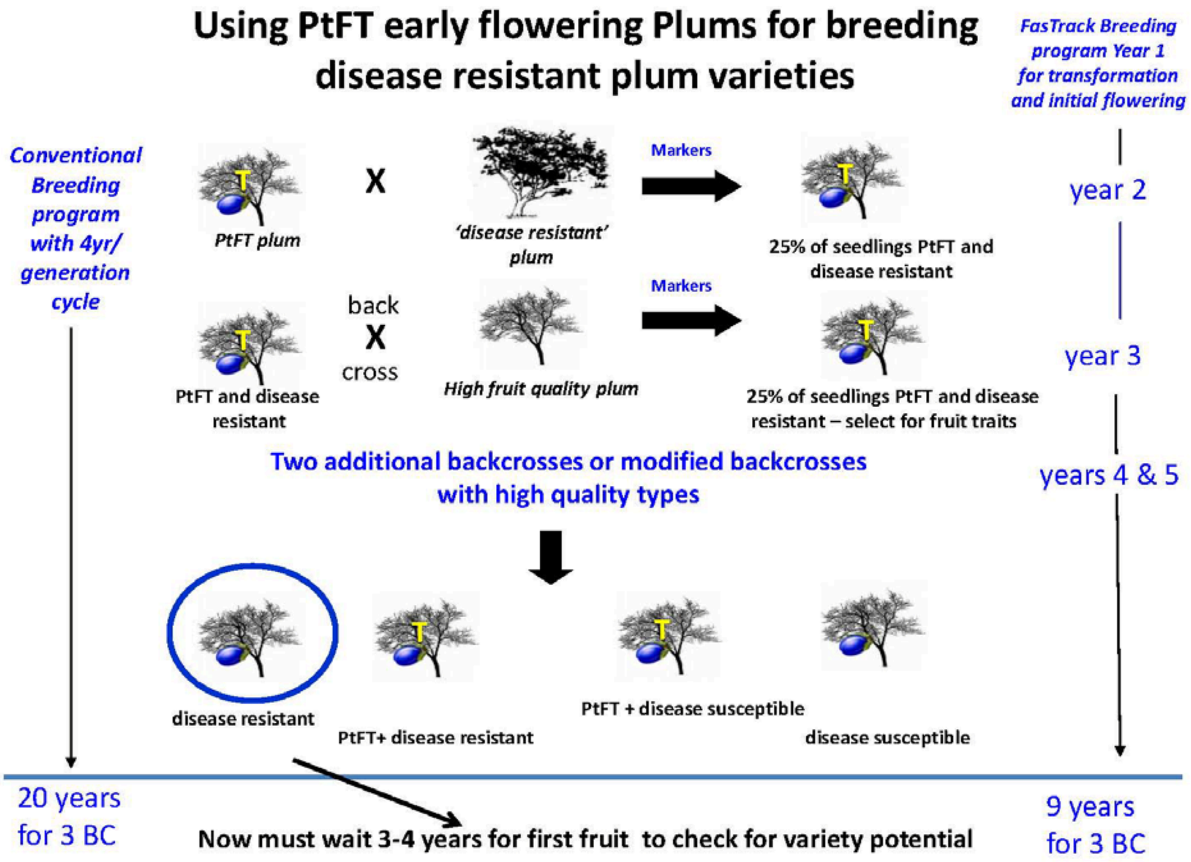

Figure 2. The FasTrack fruit tree breeding approach developed by NP 301 researchers in Kearneysville, West Virginia, can dramatically reduce the period needed to generate new fruit tree varieties. The "finished variety" incorporates no transgenes. This single innovative method incorporates elements from each of NP 301's four Research Components: germplasm, genetic analyses, breeding, and genetic engineering. PtFT= a gene from a Populus (poplar) species that governs flowering. See accomplishment under Research Component 3.

Similarly, other NP 301 researchers incorporated NP 301's full breadth of genetic resources, genomic tools, and data analytical procedures to construct the most powerful approach currently known for dissecting the genetic bases of quantitative traits for any species, Nested Association Mapping (see Figure 3 next page).

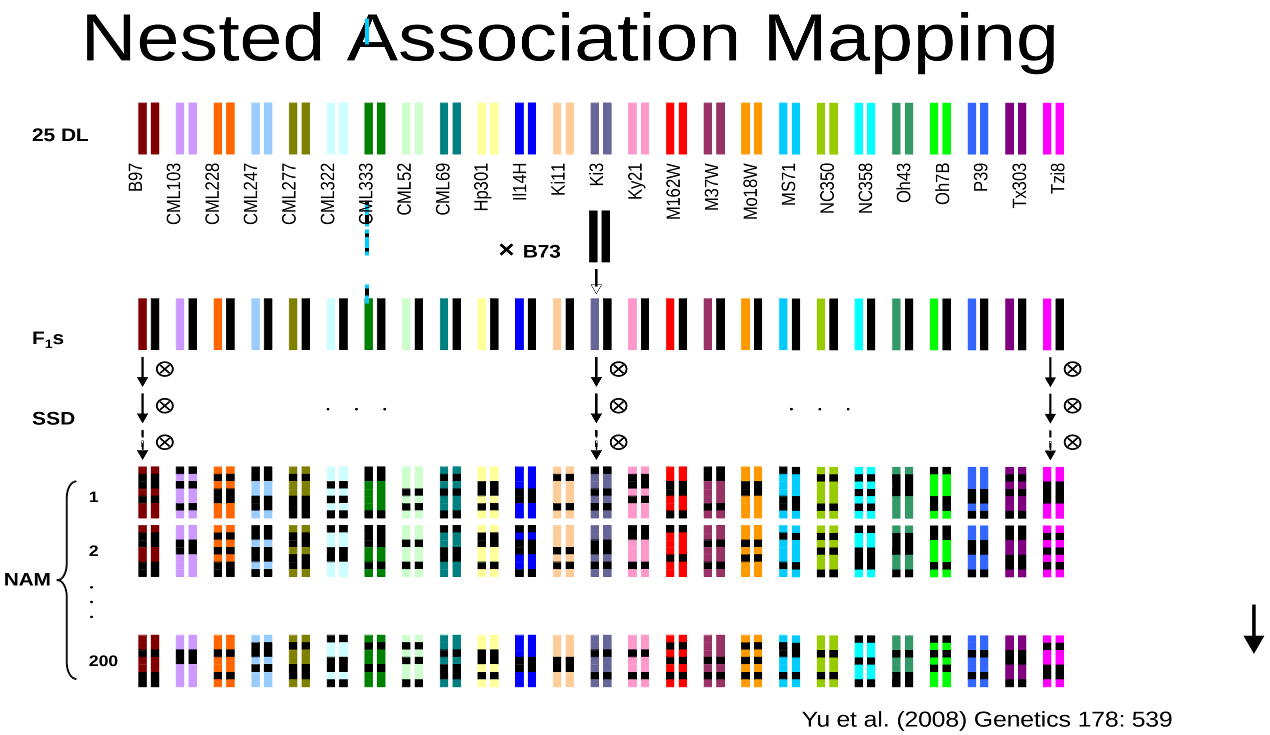

Figure 3. The Nested Association Mapping approach for analyzing the underlying genetic bases of quantitatively-inherited traits was developed by NP 301 researchers in Ithaca, New York; Columbia, Missouri; and Raleigh, North Carolina, and their collaborators. This method generates and analyzes massive volumes of genotypic and trait phenotype data. It also generates a set of recombinant inbred lines (in this case, maize) that facilitates a wide variety of future genetic analyses. See research accomplishment under Research Component 3. (Yu et al. 2008 Genetics 178: 539).

The accomplishments of NP 301 were often attained in close cooperation with public and private sector collaborators. As compared to other ARS National Programs, NP 301 research projects are unusual in their close cooperation and integration with university research efforts. In fact, many NP 301 researchers are co-located on land-grant university campuses. The ability of ARS to partner with land-grant universities has enabled university strengths and capacities to complement NP 301 efforts. This strong ARS-university cooperation has developed into joint research projects supported not only by ARS, but also by National Plant Genome Initiative awards from the NSF, NIFA, and the DOE which enabled NP 301 to expand research objectives and their contributions to the overall National Program goals. As an added benefit of this relationship with the universities, NP 301 researchers have helped to train some of the next generation of agricultural researchers. Since 2006, NP 301 scientists have trained and mentored 2,000 post-doctoral fellows, and graduate and undergraduate students who contributed significantly to NP 301 research accomplishments (see Appendix 4).

During the past 5 years, the progress and achievements of NP 301 were also strengthened significantly by collaborative and coordinated research supported by non-governmental organizations, private industry, and international partners. This support was often in the form of in-kind contributions, trust agreements, or Cooperative Research and Development Agreements (CRADAs). Once again, NP 301 was unusual in the high degree to which its research was integrated into commodity and other targeted research communities. NP 301 National Program Leaders and scientists often participated in strategic planning and coordination efforts with these commodity research communities so as to integrate public-sector and private-sector partners into cooperative research. These partnerships enabled NP 301 to effectively leverage its resources with external resources to conduct and transfer research that addresses critical agricultural problems.

NP 301 RESEARCH CONTRIBUTES TO THE OVERALL ARS CROP PRODUCTION AND PROTECTION RESEARCH EFFORT

As described previously, the technologies generated by NP 301 encompass upstream, fundamental genetic research (particularly under Component 2), breeding and genomics research that directly enhances crop production and protection (particularly under Component 3), and delivery of key genetic raw material, genetic tools, and information to the global research community (particularly Components 1 and 2). The dynamic interactions of NP 301 with other Crop Production and Protection national programs are shown in Figure 4.

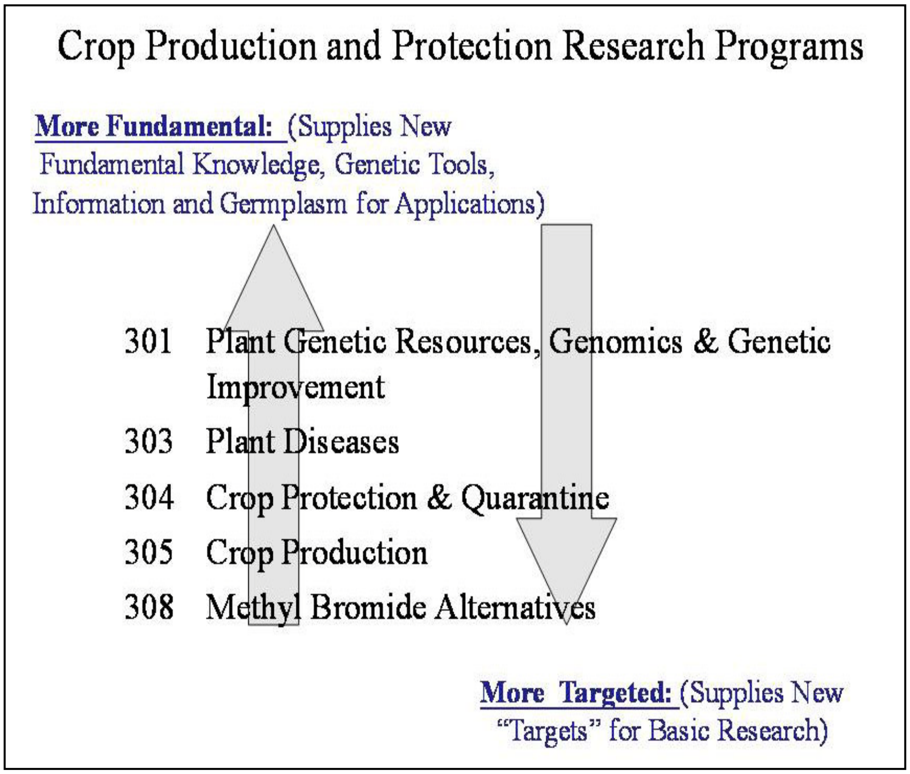

Figure 4. NP 301 research is integrated with the other national programs within the Crop Production and Protection section.

The arrows in the figure depict the different types of research products that the individual National Programs contribute to the overall Crop Production and Protection enterprise. National Program 301 supplies knowledge, research tools, and improved genetic resources which other National Programs can apply to solving agricultural problems. For example, NP 301 researchers can breed improved germplasm or varieties resistant or tolerant to diseases (targets for NP 303), pests (targets for NP 304), or environmental stresses (targets for NP 305). Those genetic materials can complement and/or mutually-reinforce applied biocontrol measures (NP 303, NP 304, and NP 308) and more efficient production methods (NP 305 and NP 308) to collectively increase crop production efficiency and producer profitability.

NP 301 also contributes to other ARS national program areas not depicted in this diagram, particularly, NP 306 (Quality and Utilization of Agricultural Products), NP 307 (Bioenergy and Energy Alternatives), and NP 215 (Rangeland, Pasture, and Forage Systems). The genetic knowledge, research tools, and improved genetic resources furnished by NP 301 can help these national programs meet their targets, e.g., higher quality produce (NP 306); improved feedstock crops that are more efficiently transformed into biofuels (NP 307); and higher yielding and/or more persistent forage, pasture, and rangeland crops (NP 215).

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

In this report, NP 301 accomplishments and their impact are organized and presented according to research components and their constituent problem statements, as described in the Action Plan. Under the problem statements, accomplishments are reported with particular reference to specific products, groups of products, or other themes.

For the most part, the content of this accomplishment report is derived from the 2006-2010 NP 301 annual reports and the reports of its constituent research projects. This report stresses the actual or projected impacts of those accomplishments and, where relevant, cites key publications or Web URLs documenting those accomplishments. NP 301 currently encompasses 177 research projects (see the listing of projects in Appendix 1). Publications authored by NP 301 scientists are compiled in Appendix 2, and cultivars and germplasm populations released by NP 301 scientists are listed in Appendix 3. Publications that document specific accomplishments are also cited with the narrative text.

The body of research which now constitutes Component 4, Plant Biological and Molecular Processes, was assessed during the 2009 external retrospective review of NP 302. Consequently, research achievements for Component 4 are not subject to review a second time here. Instead, selected examples of progress for Component 4 since the 2009 review are listed in this report to help place in context their interrelationships with Components 1, 2, and 3. The 2009 NP 302 Accomplishment Report, which provides more extensive details of research achievements for the years 2004-2009, is available online at:
www.ars.usda.gov/research/programs/programs.htm?np_code=302&docid=18813

Finally, it is important to note how NP 301 achievements and accomplishments were documented. In the same way that only selected accomplishments are reported, the details of those accomplishments are described selectively to illustrate the overall variety of products and knowledge generated by this National Program. In some cases, the results from an individual study focused on only one crop are described. In other instances, similar research or service achievements are aggregated and summarized across the breadth of the National Program.

This report was prepared for an external (to USDA) retrospective review of NP 301 to assess how well this National Program attained its projected goals, as outlined in its current Action

Plan. Accordingly, the purpose of the retrospective review is not to judge the performance of individual NP 301 research projects, but to gauge the overall impact of the National Program. Consequently, the report does not attempt to catalogue all individual accomplishments of NP 301's constituent research projects. Individual scientists or projects are not identified by name in the narrative text. Instead, their achievements are described in the context of contributions towards accomplishing NP 301's stated commitments to U. S. agriculture.

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COMPONENT 1: Plant and Microbial Genetic Resource Management

During the last 60 years, the ARS has maintained a long-term commitment to conserving and managing a broad spectrum of plant and plant-associated microbial genetic resources and information associated with that germplasm. It has also vigorously encouraged use of these genetic resources in research, breeding, education, and production. During the last decade, these genetic resource management efforts have been conducted under the framework of ARS' National Program 301 Research Component 1: Plant and Microbial Genetic Resource Management. Only two of the individual NP 301 projects (at Peoria and Beltsville) that are associated with this research component manage microbial germplasm; all others manage plant genetic resources, and collectively constitute the U. S. National Plant Germplasm System (NPGS). The locations for the NPGS genebanks are shown in the map below (Figure 1). Many of the NPGS projects contribute to Component 1 and to Component 3, Problem Statement 3B, as presented in this report. ARS' 5-year goals and specific commitments for this research component are presented under the subsequent Problem Statements.

ARS NATIONAL PLANT GERMPLASM SYSTEM (NPGS) GENEBANKS

Figure 1: Across the United States, ARS maintains 21 genebanks that manage the U. S. national plant germplasm collections. The crops safeguarded at the genebanks are listed on the following page.

ARS NATIONAL PLANT GERMPLASM SYSTEM (NPGS) GENEBANKS

Stuttgart, AR – Rice genetic stocks.

Palmer, AK – Arctic and sub-Arctic germplasm

Davis, CA – Almond, fig, grape, kiwi, mulberry, olive, persimmon, pistachio, pomegranate, stone fruit, walnut, and tomato genetic stocks.

Parlier, CA – Arid land regeneration site; lesquerella, meadowfoam, jojoba, guayule, and yucca.

Riverside, CA – Citrus and related genera, and dates.

Fort Collins, CO – National Center for Genetic Resources Preservation; Base seed collection, base collection of selected clonally-propagated crops.

Washington, DC – Woody landscape genera.

Miami, FL – Annona, avocado, mango, Passiflora, sugarcane, Tripsacum, and tropical and subtropical ornamental and medicinal plants.

Griffin, GA – Regional Plant Introduction Station; Cowpea, watermelon, peanut, capsicum pepper, sorghum, warm season forage and turf grasses, tropical and subtropical legumes, annual clovers, mung bean, sesame, okra, eggplant, tropical squash, and sweet potato.

Hilo, HI – Acerola cherry, atemoya, breadfruit, carambola, guava, litchi, lychee, macadamia, papaya, Passiflora, peach palm, pili nut, pineapple, rambutan, and tea.

Aberdeen, ID – Barley, barley genetic stocks, oat, rice, rye, triticale, and wheat and its wild relatives.

Ames, IA – Regional Plant Introduction Station; Maize, melon, cucumber, squash, carrots, spinach, amaranths, sweet clovers, temperate zone millets, oilseed brassicas, sunflower, cuphea, ornamentals, mints, umbels, flax, and soybean genetic stocks.

Urbana, IL – Maize genetic stocks and soybean and its wild annual and perennial relatives.

Geneva, NY – Regional Plant Introduction Station; Apple, vegetable brassicas, onion, buckwheat, radish, celery, sour cherry, hardy grapes, highland squash, and tomato.

Beltsville, MD – National Germplasm Resources Laboratory; GRIN database, plant exchange.

Columbus, OH – Herbaceous ornamentals.

Corvallis, OR – Blackberry, blueberry, cranberry, currant, filbert, gooseberry, hop, mint, pear, raspberry, and strawberry.

Mayagüez, PR – Tropical quarantine site; bamboo, banana, plantain, sapotaceous tree fruit, tropical ornamentals, sorghum, and cacao.

College Station, TX – Cotton and cotton genetic stocks and pecan.

Pullman, WA – Regional Plant Introduction Station; Alfalfa, bean, chickpea, vicias, lupines, beets, lettuce, cool season grasses, lentil, garlic, pea and pea genetic stocks, clovers and other forage legumes, and safflower.

Sturgeon Bay (Madison), WI – Potato.

PROBLEM STATEMENT 1A: *Efficiently and Effectively Manage Plant and Microbial Genetic Resources*

This Problem Statement focuses on meeting the key challenges of fulfilling the ever-increasing demand for crop and microbial genetic resources and associated information. Crop breeding and genomics, critical tools for enhancing crop productivity, depend on ready access to genetic resources for enhancing crop productivity, thereby furnishing adequate and affordable food, feed, fiber, and fuel. To meet those key challenges, the U.S. National Plant Germplasm System (NPGS) must expand its collections, and its genetic resource regeneration, maintenance, documentation, and distribution programs. Crop-specific or microbe-specific managerial priorities and strategic plans must be developed to enhance existing collections in close consultation with Crop Germplasm Committees and other consultative groups. Gaps in the NPGS collections must be filled by acquiring imperiled genetic resources, especially for wild relatives of crops – an especially rich trove of genetic variability for crop improvement.

Once acquired, NPGS collections must be secured both at the primary and back-up genebank sites, and maintained under conditions that promote optimal longevity, health (pathogen-negative), and trueness-to-type. Research is especially needed to develop methods for long-term preservation of clonally-propagated crops and for reliable germination and propagation. Accessions must be regenerated reliably to provide sufficient quantities of viable propagules. They must be distributed rapidly and reliably to a global clientele. The NPGS Germplasm Resources Information Network (GRIN) requires upgrading to more efficiently and effectively deliver the wide variety of information required for successful genetic resource curation and use. The NPGS must also augment and upgrade genebank facilities (cold rooms, greenhouses, etc.) and other infrastructure to successfully safeguard genetic resources, and enhance genebank capacities for managing the current collection and thousands of new genetic seed stocks generated by genomics research.

The NP 301 Action Plan identified nine anticipated products which were expected to result from research addressing the needs expressed in Problem Statement 1A. The anticipated products now serve to help measure the national program’s progress during the last 5 years in meeting the needs of crop researchers and producers. A sampling of accomplishments that illustrate those products for this problem statement follow the list of Anticipated Products.

ANTICIPATED PRODUCTS IN ACTION PLAN:

1. Strategic genetic resource management plans and priorities, aligned with ARS agency goals, are developed by genebank curators, in close consultation with Crop Germplasm Committees and new system-wide consultative group(s).
2. Genebank collections of agriculturally important organisms, especially microbes, specialty crops, and genetic/genomic stocks, are expanded strategically.
3. Timely infrastructure maintenance and facility upgrades ensure the secure, long-term, *ex situ* genetic resource maintenance.
4. Expanded international germplasm exchanges and linkages fill critical gaps in collection coverage.
5. New regeneration protocols and techniques increase collection quality, ensure genetic integrity, and enhance genetic resource management efficiency.

6. More effective methods and strategies for long-term maintenance protect genetic resource vigor, viability, and health, especially from seed-borne diseases.
7. A broad spectrum of genetic diversity in the form of viable and well-documented germplasm is conserved.
8. Efficient and timely distribution increases the frequency and use of germplasm in research and in crop genetic improvement.
9. High-quality, comprehensive characterization, evaluation, and genebank curatorial data are readily accessible, either from a transformed, upgraded, well-maintained GRIN, from databases housed at GRIN as the primary site, or at sites linked to GRIN.

PROBLEM STATEMENT 1A: ACCOMPLISHMENT OVERVIEW

During the last 5 years, the total plant germplasm holdings in the NPGS have expanded about 14 percent, as a result of plant explorations, exchanges, and acquisition of discontinued collections or research programs. Its genetic resource maintenance efforts also expanded substantially, especially for clonally-propagated crops, as detailed in the text below. The NPGS capacity for delivering germplasm-associated information to clientele was enhanced substantially during the last 5 years. In conjunction with Bioversity and the Global Crop Diversity Trust, the NPGS revamped GRIN into a new version, dubbed GRIN-Global (see www.grin-global.org/index.php/Main_Page) that will become the international standard plant genebank information management system. As of this writing, GRIN-Global is being implemented at selected genebanks internationally. The NPGS success in delivering data associated with its genebank accessions is documented by the greatly increased use of information from GRIN. The number of unique computers that accessed and consulted data from the NPGS GRIN Web site annually increased from about 800,000 during 2006 to nearly 2 million in 2010 (see Figure 2A).

The global demand for NPGS germplasm also grew dramatically. In 2006, about 150,000 different accessions were distributed to requestors worldwide, but during the last 2 years (2009-2010) more than 200,000 accessions were distributed each year, or about one-third of the entire collection each year (see Figure 2B). The increase in demand was especially pronounced for accessions of horticultural crops; bioenergy crops; previously proprietary elite commercial varieties whose intellectual property rights protection had expired; and genetic stock generated as part of large-scale genomics research projects. These distributions helped to strengthen research and breeding programs worldwide.

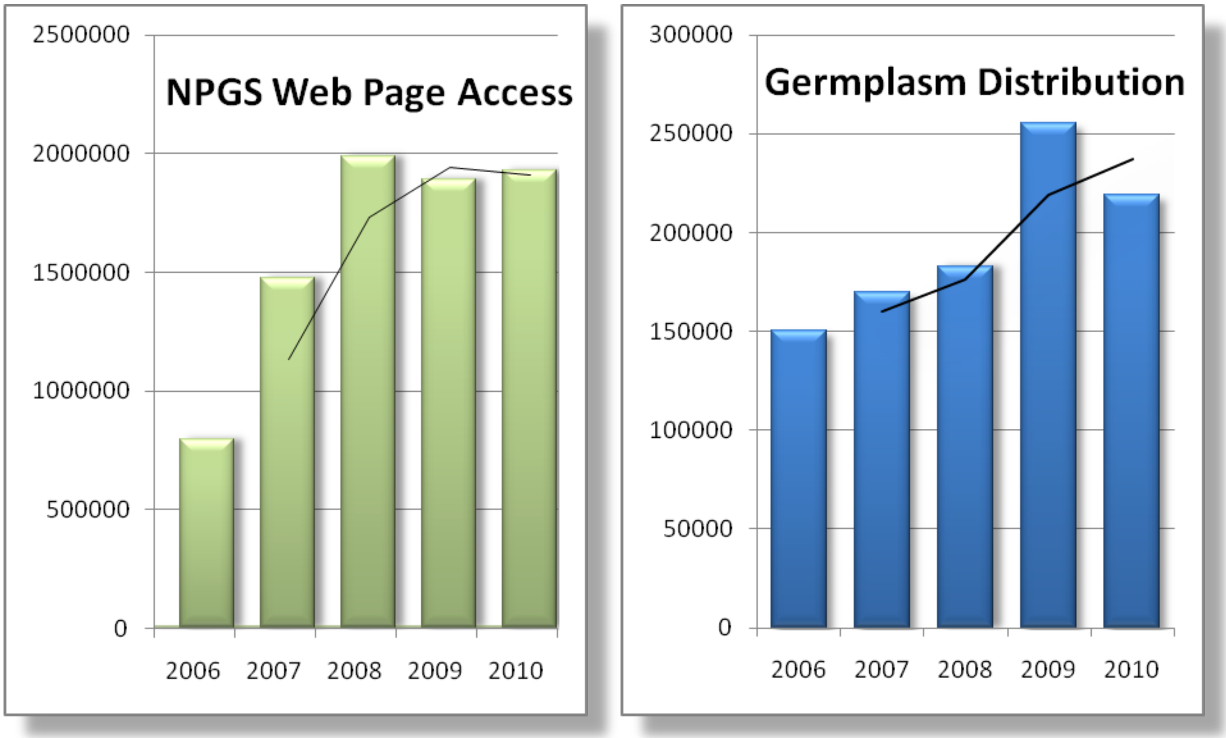


Figure 2: A. During the last 5 years, the number of individual computers accessing GRIN online more than doubled. **B.** The total number of germplasm accessions distributed by the NPGS has steadily increased since 2006.

Problem Statement 1A: Selected Accomplishments

Anticipated Product 1: *Strategic genetic resource management plans and priorities, aligned with ARS' agency goals, are developed by genebank curators, in close consultation with Crop Germplasm Committees and new system-wide consultative group(s).*

During the last 5 years, ARS curators and researchers contributed substantially to strategic crop genetic resource management planning and priority setting at local, national, and international levels, organizing regularly scheduled or special ad hoc meetings and co-authoring plans and strategies for more efficient and effective managerial programs. Furthermore, two new Crop Germplasm Committees and the National Plant Germplasm Coordinating Committee were formed to enhance the capacity for strategic planning and priority setting.

ARS curators help formulate global conservation strategies. Under the aegis of the Global Crop Diversity Trust, ARS curators and other genebank personnel participated in formulating global conservation strategies, which identified the priority genebank collections for bananas and plantain, barley, cacao, citrus, maize, rice, sorghum, and strawberry, and developed regional crop conservation strategies for Central Asia and the Caucasus. The information gained during these consultations is also valuable for formulating NPGS collection development strategies. For one example, see www.croptrust.org/documents/cropstrategies/strawberry.pdf

Hummer, K.E. 2009. Global conservation of strawberries: a strategy is formed. Acta Horticulturae 842:577-580

National Plant Germplasm Coordinating Committee established. In 2006, staff of the NPGS, NIFA, and administrators of several State Agricultural Experiment Stations (SAES), formed the new National Plant Germplasm Coordinating Committee (NPGCC) (see the Web site at: http://escop.ncsu.edu/ViewCommittees.cfm?comid=41) as a mechanism to assess, develop, and recommend strategies for improved coordination and communication of NPGS activities and to evaluate the current funding models for the NPGS and report findings to the SAES directors, ARS, and NIFA. During the last 5 years, the NPGCC communicated to SAES directors the high priority for adequately supporting the ARS genebanks; acted to restore full funding to the ARS potato genebank; and catalyzed support for increasing the base funding for the Geneva, New York, genebank.

Two new NPGS Crop Germplasm Committees established. To meet the increased demand and interest for germplasm and information, the NPGS formed two new Crop Germplasm Committees (CGCs). The Specialty Nut CGC, formed in 2009 (www.ars-grin.gov/cgi-bin/npgs/html/mbrship.pl?SPECIALTY-NUT), has already drafted crop vulnerability statements for economically-important nut crops such as hazelnut (Corylus), pistachio (Pistacia), and chestnut (Castanea), and formulated and received funding for two competitive germplasm evaluation grants. The Medicinal and Essential Oils CGC, established in late 2010, is currently developing a list of priority crop genera for increased NPGS conservation efforts in the future. They formulated and received funding for a plant exploration trip to be conducted in the United States during 2011. These two new CGCs provide a formal mechanism for securing expert technical input for genetic resource management plans and priorities. They also catalyze a greater awareness of current genetic resource management efforts for these crops.

Plant Germplasm Operations Committee extends coordination of NPGS-wide operations. Each year, curators and site managers from across the NPGS convene to discuss strategies for addressing NPGS-wide issues and for planning responses to common challenges. The presentations from the 2010 Plant Germplasm Operations Committee **(**PGOC) meeting (www.ars-grin.gov/npgs/sitemenu/pgoc/pgoc2011/presentations_page.html) document the breadth and depth of the topics addressed, and the PGOC's impact with developing effective NPGS-wide managerial practices and solutions. Various PGOC subcommittees worked actively between the annual meetings on new and recurrent challenges. During the last 5 years, staff from national genebanks in Canada, Mexico, Brazil, and South Korea attended the annual meetings, providing an international perspective on common problems and potential solutions.

Documenting crop wild relatives occurring in the United States. More than 700 primary, secondary, and tertiary gene pool relatives for 20 of the most economically important crops were identified in the GRIN database. A Web interface (www.ars-grin.gov/~sbmljw/cgi-bin/taxcrop.pl) was constructed to query and retrieve information to assist scientists in the United States and other countries with developing strategies for filling gaps in genebank collections, and for setting priorities for in situ conservation of these crop wild relatives.

Anticipated Product 2: ***Genebank collections of agriculturally important organisms, especially microbes, specialty crops, and genetic/genomic stocks, are expanded strategically.***

Since 2006, ARS' total plant germplasm holdings within NPGS have expanded about 14 percent, from 474,000 accessions of 11,800 species to more than 540,000 accessions of 13,500 species in 2010. The expansion was a result of plant explorations, exchanges, and acquisition of discontinued collections or research programs. This expansion safeguarded and made readily available thousands of additional plant genetic resources.

For example, the NPGS played a critical role in safeguarding collections donated by the home institutions of discontinued research programs. The Griffin, Georgia, genebank acquired the late Professor Norman Taylor's (University of Kentucky) highly diverse, globally important collection of more than 1,000 accessions from about 200 species of *Trifolium* (clover), and secured thousands of samples of subtropical and subtropical tropical legumes from a discontinued breeding program and the University of Florida.

Furthermore, the NPGS collections expanded significantly its holdings of voucher/reference samples for crop varieties which are registered by the Crop Science Society of America, and voucher/reference samples for varieties for which Plant Variety Protection Certificates have expired.

Ellis, D.D., Campbell, K., Grotenhuis, J.A., Jenderek, M.M., Pedersen, J.F. 2010. Crop registration: The pathway to public access of plant genetic materials to build crops for the future. Crop Sci. 50:1151-1160

Bretting, P. K. 2007. The U. S. National Plant Germplasm System in an era of shifting international norms for germplasm exchange. Acta Hort. 760:55-60

Highlights of some specific germplasm acquisitions follow; the collections of current or potential bioenergy crops were particularly enhanced. Note that international germplasm exchanges (especially plant explorations) are covered in more detail under Anticipated Product 4.

Key genetic tools for grain improvement research are safeguarded. The genomes of the major grains wheat and barley are so large and complicated that analyzing their genetic functions and structures require special genetic tools. ARS genebank curators in Pullman, Washington, greatly expanded the national collection of *Brachypodium* (purple false brome), a small, rapidly flowering grass, with a relatively tiny genome that has been completely sequenced. The 200 new accessions included the specific line whose genome had been sequenced. By safeguarding and distributing these different lines, NPGS genebanks furnished crucial genetic tools for catalyzing efforts to map and manipulate key traits for genetically improving major crops. These accessions complement and facilitate the use of the large NPGS collections in Aberdeen, Idaho, of wheat, barley, and other small grains germplasm (more than 125,000 accessions), which are widely requested for research and crop improvement.

Bockelman, H.E., Valkoun, J. 2010. Barley germplasm conservation and resources" Chapter 7 in "Barley: Improvement, production, and uses" Blackwell Publishing ed. S.E. Ullrich. Barley: Improvement Production and Uses. 144-159."

Expansion of the switchgrass and other warm-season grass germplasm collections. The ARS warm-season grass collection for species native to the United States did not contain adequate genetic diversity to meet the needs of requesters. The switchgrass (*Panicum*) collection was expanded with 94 accessions collected by ARS researchers at Griffin, Georgia, from areas in the United States previously underrepresented. The newly collected switchgrass accessions were tested for germination, regenerated, characterized genotypically using simple sequence repeat (SSR) markers, and the ploidy levels were determined. These efforts provided well-characterized, readily available, and viable material to scientists for bioenergy feedstock development. Also, germplasm for several other native warm season grass species (e.g., *Andropogon, Sorghastrum, Schizachyrium,* and *Bouteloua*) was made available through seed increases. Collection site data were obtained from historical records for more than 1,000 *Andropogon* accessions and made available online. These efforts collectively have increased the availability, broadened the diversity, and enhanced associated information for the native warm season grasses important for forage and bioenergy applications.

Targeted acquisitions of wild plant species from the western United States. In addition to the new genetic resources acquired by plant explorations, NPGS collections expanded through strategic acquisitions of materials from other collections. Thousands of new accessions of plants native to the United States, many from the western states, including many crop wild relatives, were transferred from the Department of the Interior's Seeds of Success program to the NPGS. These collections provide the raw materials for identifying superior new genes, traits, and products for crop producers, consumers, and industry. These native plants also are potentially valuable for land restoration and stabilization.

Johnson, R.C. 2008. Genebanks pay big dividends to agriculture, the environment, and human welfare. Public Library of Science Biology. www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.0060148

Genetic resources for mining maize diversity. Maize is the most diverse crop in the world, but much of that useful genetic variation is found in maize not adapted to U.S. agriculture. ARS researchers at Ithaca, New York, genetically characterized the largest set of mapping lines for complex trait dissection in any species; more than 5,000 diverse maize inbred lines. Seed for those lines were deposited at the ARS Maize Stock Center in Urbana, Illinois, where they have been distributed to dozens of scientific groups who are determining the genetic bases for numerous agronomically important maize traits.

Sachs, M.M. 2009. Cereal germplasm resources. Plant Physiology. 149(1):148-151.

McMullen, M.D., Kresovich, S., Sanchez-Villeda, H., Bradbury, P., Li, H., Sun, Q., Flint Garcia, S.A., Thornsberry, J., Acharya, C., Bottoms, C., Brown, P., Browne, C.J., Eller, M.S., Guill, K.E., Harjes, C., Kroon, D., Lepak, N.K., Mitchell, S., Peterson, B.E., Pressoir, G., Romero, S.M., Oropeza Rosas, M., Salvo, S.A., Yates, H., Hanson, M., Jones, E., Smith, S., Glaubitz, J., Goodman, M., Ware, D., Holland, J.B., Buckler IV, E.S. 2009. Genetic Properties of the Maize Nested Association Mapping Population. Science. 325:737-740.

Statistical maps and models for ecogeographical adaption in mountain brome. Mountain brome (*Bromus carinatus*) is a fast growing native perennial grass with high potential for revegetating highland forest lands. ARS researchers at Pullman, Washington, working cooperatively with Forest Service geneticists collected seed and data of this species from 145 locations. The association of plant traits with environmental variables at individual collection sites was analyzed by regression models, and several adaptation zone maps for this species were developed. These maps and information provide management tools to identify, based on their adaption, the optimal germplasm sources for revegetating those mountainous regions.

> Johnson, R.C., V. J. Erickson, N. L. Mandel, J. B. St Clair, K. W. Vance-Borland. 2010. Mapping genetic variation and seed zones for Bromus carinatus in the Blue Mountains of Eastern Oregon, U.S.A. Botany 88: 725–736.

Acquisition of endangered wild mustard species. The Zapata bladderpod (*Lesquerella thamnophila*) is an endangered species and recovery efforts are currently underway to upgrade its status to threatened. Bladderpod species are specialty oilseed crops with unique fatty esters that can serve as high temperature lubricants and heavy duty detergents. The ARS genebank at Parlier, California, acquired samples from the seven known wild populations and increased the seed to assist with re-establishing the species in nature, thereby safeguarding genetic material of this endangered plant.

Discovery of the third site in Mexico for a rare wild relative of maize. Some types of teosinte, the ancestor to maize, are generally cross-fertile with maize, enabling teosinte to serve as a potential source of genes for maize improvement. ARS scientists from Fort Collins, Colorado, and their collaborators in Mexico have found another field location for a rare wild perennial teosinte (*Zea perennis*) in Mexico. Previously, only two sites for this perennial species were known in Mexico. This find is significant as it represents a potentially unique population of this key evolutionary relative of maize.

> Taba, S. W. Wen, V. Chavez Tovar, R. Rivas, Y. T. Kato Angel, and D. Ellis. 2011 Identification and characterization of a population in situ of perennial teosinte found in Ziracuarétiro, Michoacán, Mexico. Abstract P71, 53rd Annual Maize Genetics Conference, 17-20 March 2011, St. Charles, IL U. S. A.

Anticipated Product 3: *Timely infrastructure maintenance and facility upgrades ensure the secure, long-term, ex situ genetic resource maintenance.*

Well-maintained, updated, and efficiently operated genebank facilities are vital for ensuring the long-term security of invaluable plant genetic resources. During 2006-2011, the overall conditions, security, and capacity of ARS genebank facilities were improved substantially through investments from special funding allotments and from recurrent operational resources. These facilities are considered integral to national and global food security. Consequently, during the last 5 years, the U. S. Department of Homeland Security provided extensive support to enhance physical security at genebank locations, including building alarms, security fencing, electric gates, and surveillance cameras.

A new high-security, low temperature vault for safeguarding duplicate samples of NPGS accessions was completed at Fort Collins, Colorado. Major building repairs and equipment upgrades were also accomplished at this site. Low-temperature/low humidity seed storage rooms were added, expanded, or upgraded at several NPGS sites, including College Station, Texas; Geneva, New York; Griffin, Georgia; Pullman, Washington; Sturgeon Bay, Wisconsin; and Urbana, Illinois. Seed and germplasm processing, seed testing, and digital imaging facilities were improved at College Station, Texas; Geneva, New York; and Parlier, California. At Peoria, Illinois, a new secure collection room for the ARS microbial culture collection was constructed and occupied providing secure, key-card limited access and 24-hour video monitoring of the collection's refrigerators and freezers.

Modern, well-equipped, and energy-efficient greenhouses (glass and/or plastic-clad) and growth chambers deliver the key capacity to conserve and propagate accessions that require special care, protection against virulent diseases and pests, and/or are not adapted to the climate at the genebank site. Greenhouses were added, expanded, renovated, and/or re-equipped at Ames, Iowa; Columbus, Ohio; Corvallis, Oregon; Geneva, New York; Riverside, California; Sturgeon Bay, Wisconsin; and Washington, D.C. Several of the genebanks that specialize in clonally propagated crops (College Station, Texas; Corvallis, Oregon; Davis, California; Miami, Florida; and Riverside, California) added, expanded, or renovated screen houses, shade houses, or field orchards that safeguard germplasm plantings.

The "passport" and descriptive information associated with the NPGS's accessions are invaluable for properly conserving these materials and encouraging their use. The GRIN information system computers and operational staff re-located to new, upgraded facilities at Beltsville, Maryland, during the last 5 years. Furthermore, a new application computer server was purchased to safeguard and rapidly deliver the millions of data points on the GRIN database.

Anticipated Product 4: *Expanded international germplasm exchanges and linkages fill critical gaps in collection coverage.*

Since 2006, the NPGS international germplasm exchange program continued to make progress, despite increased difficulties with securing permission to acquire materials internationally. Thanks to NP 301's activities and substantial support from other U. S. government agencies, the NPGS international benefit-sharing program was expanded, not only facilitating access to difficult to obtain materials, but also generating substantial improvements for the plant genetic resources infrastructure and managerial capacity for developing nations. These efforts helped protect thousands of germplasm accessions held by genebanks in developing nations.

Expanded plant explorations and exchanges. Between 2006 and 2010, ARS supported 74 plant explorations and three plant exchanges to acquire additional germplasm for long-term preservation in genebanks. Thirty-two of the explorations took place within the United States and another 42 occurred in 21 other countries. The targeted taxa included a wide range of crop species and their wild relatives – *Actinidia, Allium, Arachis, Beta, Carya, Chenopodium, Corylus, Daucus, Ficus, Fragraria, Glycine, Helianthus, Humulus, Juglans, Lactuca, Malus, Mentha, Medicago, Morus, Olea, Panicum, Parthenium, Phaseolus, Pistacia, Pisum, Prunus, Punica, Pyrus, Ribes, Rubus, Solanum, Trifolium, Triticum, Vaccinium, Vitis*, and many types of ornamentals and forage and range grasses. Many of these explorations focused on crop centers of origin or centers of diversity for crop wild relatives, such as wild sunflowers in the United

States; wild onions, garlic, and carrots in Tunisia; wild peanuts in Paraguay; temperate fruits and nuts in Japan; and wild beets in Morocco. The hundreds of accessions collected were incorporated into the NPGS where they are safeguarded and made available to researchers worldwide.

Bamberg, J.B., Del Rio, A.H., Fernandez, C.J., Salas, A., Vega, S.E., Zorilla, C., Roca, W., Tay, D. 2010. Comparison of "remote" versus "easy" in situ collection locations for USA wild Solanum (potato) germplasm. American Journal of Potato Research. 87:277-284. Available: www.springerlink.com/content/247308885401 24jt/fulltext.html.

Iketani, H., Hummer, K.E., Postman, J.D., Imanishi, H., Mase, N. 2010. Collaborative exploration between NIAS Genebank and USDA ARS for the collection of genetic resources of fruit and nut species in Hokkaido and the Northern Tohoku Region. J. of Jap. Bot. 26:13-26.

Expanded international benefit sharing. ARS scientists collaborated during 2006-2011 with the USDA Foreign Agricultural Service and the ARS Office of International Research Programs in an expanded portfolio of benefit-sharing projects associated with genetic resource exchange. These projects included:

- Developing a tissue-culture facility; installing field irrigation in a newly developed area; purchasing a medium-term, seed storage unit; and supporting seed increases and characterization of eggplant germplasm in Kerala, India;
- Collecting, evaluating, preserving, and distributing germplasm of landraces and wild relatives of traditional fruits and vegetables in Bangladesh;
- Supporting the collection and evaluation of crop wild relatives; training staff in advanced research techniques and maintenance of genebank facilities and equipment; and purchasing research equipment and supplies in Sri Lanka;
- Providing funding to two institutes to develop medium-term storage facilities in Armenia;
- Supporting complementary (ex situ and on farm) conservation and increased utilization of agrobiodiversity in native farming communities in Ecuador;
- Supporting major structural renovation and remodeling for the seed conservation laboratory, cold-storage facility, molecular biology laboratory, in-vitro conservation laboratory and propagation greenhouse, offices, and library; updated the germplasm-documentation system; inventoried and documented existing collections; and re-organized and backed up in long-term storage crop collections in Peru; and
- Upgraded facilities, trained Guyanese scientists at U.S. laboratories; provided field equipment; established field genebanks; and conducted workshops on national access and benefit sharing legislation in Guyana.

Collectively, the preceding efforts bolstered the genetic resource conservation and management capacities of several developing nations, as well as providing facilitated access to plant genetic resources for the global crop research and breeding communities.

Williams, D.E. 2006. Total makeover for crop diversity conservation in Peru. p. 11-12. In: R. Raymond (ed.), Geneflow ’06. Bioversity International, Rome.

NORGEN enhances germplasm conservation linkages. NORGEN, the plant genetic resources network for PROCINORTE (Cooperative Program in Agricultural Research and

Technology for the Northern Region), was created by the Inter-American Institute for Cooperation on Agriculture to stimulate formal cooperation among Canada, Mexico, and the United States in sharing information, development of cooperative projects, capacity building, training, and integration of activities with other genetic resources networks within the Americas and around the world. During the last 5 years, NORGEN conducted a joint genetics and breeding project on dry beans (*Phaseolus)*, and trained Canadian and Mexican genebank personnel in the new GRIN-Global germplasm information system (see the Web site: www.procinorte.net/Pages/Genetic%20Resources%20Task%20Force.aspx). NORGEN enhanced cooperation by identifying common problems that impede closer international linkages and exploiting opportunities for coordinated actions among the national genetic resources programs in the three countries to promote the conservation and sustainable use of plant genetic resources.

NPGS-EMBRAPA partnership facilitates germplasm exchange. ARS and its Brazilian counterpart, EMBRAPA, established LABEX (Laboratory Exchange Program) as a framework for fostering joint Brazilian-U. S. agricultural research programs, especially through long-term personnel exchanges. Expanding the volume of plant genetic resources exchanged between the NPGS and EMBRAPA was also a primary objective for LABEX. During the last 5 years, the LABEX program catalyzed the exchange of more than 500 samples of a rice core collection from EMBRAPA, and more than 1,100 accessions of multiple species from the NPGS. Thus, LABEX represents a successful new type of mutual capacity building mechanism which might serve as a model for additional bilateral or multilateral international germplasm exchanges.

The accomplishments for the following three Anticipated Products for Problem Statement 1A are so closely integrated that they are presented together to avoid duplication. The accomplishments for these Anticipated Products are grouped into the categories of seed-propagated crops; clonally propagated crops; long-term storage/safety duplication of NPGS and microbial germplasm; and microbes. These groupings reflect the similar managerial methods and approaches for the each of the classes of germplasm.

Anticipated Product 5: ***New regeneration protocols and techniques increase collection quality, ensure genetic integrity, and enhance genetic resource management efficiency.***

Anticipated Product 6: ***More effective methods and strategies for long-term maintenance protect genetic resource vigor, viability, and health, especially from seed-borne diseases.***

Anticipated Product 7: ***A broad spectrum of genetic diversity in the form of viable and well-documented germplasm is conserved.***

Seed-propagated crops:

Conserving 540,000 accessions in ARS' genebank system according to the best curatorial practices represents a daunting logistical and economic challenge. During the last 5 years, more than two-thirds of the fiscal, personnel, and facility resources at NPGS genebanks were expended for genetic resource maintenance and regeneration. More than 90 percent of NPGS'

540,000 accessions are seed-propagated, so as a system NPGS has emphasized managing seeds. The supply and quality of more than 140,000 of those seed accessions (approximately 26 percent of total) were rejuvenated via field or greenhouse increases. The germinability and viability of more than 305,000 of those accessions (about 56 percent of total) were tested. This extensive NPGS-wide program helped to ensure the availability and viability of a globally important germplasm collection.

More efficient and effective methods for regenerating seed-propagated genetic resources. Regenerating seed-propagated accessions often involves cultivation in individual plots, controlled pollination, harvesting of plots separately, and cleaning seeds in small batches. These operations are costly in terms of personnel, land, and equipment. Collectively, they constitute most of the overall cost for managing seed-propagated germplasm accessions. Primarily because of increasingly strong demand from the research community, the seed supplies for the NPGS accessions must be regenerated and increased relatively frequently. Consequently, a NPGS priority is to modify or develop new seed increase methods that are more cost-effective, but still preserve the genetic integrity and original genetic profile of the accessions. For example, ARS researchers in Pullman, Washington, developed and implemented more effective cultivation practices, pollen isolation distances, increased populations sizes, and more efficient seed sampling strategies at harvest for temperate grass genera, such as *Agropyrum, Bromus, Elymus, and Lolium.* They also determined that flies were effective pollinators for the oilseed mustard *Lesquerella* and more effective and cost-efficient pollinators of leek (*Allium ampeloprasum*) than were honeybees. Observations of potential pollinators on field plantings of the legume genera *Astragalus* and *Onobrychis* resulted in modifications to field regeneration procedures that will reduce the risk of unwanted cross-pollination. ARS researchers at Griffin, Georgia, developed customized regeneration techniques for perennial *Hibiscus* species, which yield high-quality seed for conservation and distribution. ARS researchers in Ames, Iowa, found that windborne pollen from a *Brassica napus* variety could pass through the screen mesh of insect cages then in use for controlled pollination and cause a low level of inadvertent crossing. They also tested with genetic markers and statistical genetic analyses whether some accessions of *B. napus* were duplicates, and whether bulked samples of *B. rapa* contained the desired homogeneous genetic profiles. The preceding studies represent only a minor fraction of the many applied research investigations that strongly enhanced the efficiency of the NPGS crop genetic management operations during the last 5 years.

Bradley, V.L. 2010. Isolation distance, inflorescence sampling, and population size: maintaining genetic diversity in the U.S. temperate grass germplasm collection. International Symposium on Forage, Turfgrass and Biofuel Germplasm Research p.100-103.

Clement, S.L., Hellier, B.C., Elberson, L.R., Staska, R.T., Evans, M.A. 2007. Flies (Diptera: Muscidae, Calliphoridae) are efficient pollinators of Allium ampeloprasum L. (Alliaceae) in field cages. Journal of Economic Entomology. 100(1):131-135.

Cruz, V.V., Nason, J.D., Luhman, R., Marek, L.F., Shoemaker, R.C., Brummer, E., Gardner, C.A. 2006. Analysis of bulked and redundant accessions of Brassica germplasm using assignment tests of microsatellite markers. Euphytica. 152:339-349.

Morris, J.B., Wang, M.L. 2011. Evaluation for morphological, reproductive, anthocyanin index, and flavonol traits in ornamental and nutraceutical producing Hibiscus species. Ornamental Plants: Types, Cultivation and Nutrition. Hauppauge,NY:Nova Publishers. p.111-127.

Developing photoperiod control (day length reduction) methods for Zea mays and Helianthus. Exposure to light must be controlled to induce flowering and produce seeds from diverse genetic resources unadapted to the long summer days at higher latitudes. ARS researchers at Ames, Iowa, developed a shade cloth structure for field cultivation that successfully limits daylight exposure to 8 hours to induce earlier flowering in crops such as maize and sunflower. This has enabled day length-sensitive accessions of maize and sunflowers to be regenerated in Iowa, and also enabled those accessions to be crossed to adapted lines for germplasm enhancement purposes.

Gardner, C., T. More, F. Engstrom, M. Lively, A. Smelser, M. Millard, and M. Blanco. 2009. Maize photoperiod control: Part II. Abstract 55225, 2009 Crop Science Society of America Annual Meeting, 3 Nov. 2009, Pittsburgh, PA.

Marek, L. 2008. Promoting flowering in Helianthus argophyllus: manipulation day length in the field. National Sunflower Research Forum, January 2008. 7 pp.

Development and application of valuable molecular disease diagnostic tests for ensuring the health of plant germplasm. During the last 5 years, ARS researchers and collaborators developed and/or applied powerful molecular diagnostic tools for ensuring that germplasm in NPGS genebanks is free of key diseases. Examples include molecular diagnostic tests for: seedborne Stewart's wilt in maize and bacterial fruit blotch of melons (Ames, Iowa); Citrus leprosis virus (Riverside, California); alfalfa mosaic virus in crotalaria (Griffin, Georgia); avocado viral diseases (Miami, Florida) and cucumber mosaic, banana streak, and banana mild mosaic viruses (Mayagüez, Puerto Rico). These molecular diagnostic tests enable rapid, inexpensive, and accurate detection of key pathogens, which is critical for maintaining germplasm health and permitting international and domestic germplasm exchange. Ready access to vigorous and healthy plant germplasm is integral to accelerating progress in crop genetic resource conservation and breeding.

Block, C.C., Shepherd, L.M. 2008. Long-term survival and seed transmission of Acidovorax avenae ssp. citrulli in melon and watermelon seed. Plant Health Progress. Available: www.plantmanagementnetwork.org/sub/php/brief/2008/melon/

Guerra-Moreno, A.S., Keremane, M.L., Brlansky, R.H., Lee, R.F. 2007. Citrus leprosis symptoms can be associated with the presence of two different viruses: cytoplasmic and nuclear, the former having a multipartite RNA genome. International Organization of Citrus Virologists Proceedings, 16th Conference, Pgs. 230-239.

Gillaspie Jr, A.G., Barkley, N.L., Morris, J.B. 2006. An unusual strain of alfalfa mosaic virus detected in Crotalaria spp. germplasm in Georgia. Plant Pathology Journal 5(3): 397-400.

Clonally propagated crops:

Despite the NPGS-wide emphasis on seed-propagated crops, one of the NPGS' priority germplasm maintenance goals has been to improve the management of clonally propagated germplasm. More than 26,000 of the approximately 40,000 clonal accessions (more than 60 percent of total) were vegetatively propagated during the last 5 years. Conserving genetic resources of perennial crops (especially trees) in field plantings is expensive and subjects the invaluable genetic materials to the continual threats of diseases, pests, and environmental extremes. Accessions were backed-up in duplicated field planting and, whenever feasible, in controlled environments via tissue culture or cryopreservation. Researchers and curators accomplished substantial progress with backing up some of those clonal accessions, and with developing new controlled storage techniques for some crops, which increased genebank operational effectiveness and efficiency.

New more effective approaches for conserving genetic resources of wild relatives of apples. ARS researchers at Fort Collins, Colorado, and Geneva, New York, applied statistical genetic models to identify a core subset of about 100 trees that captured most of the genetic diversity contained in a total of more than 1,000 wild apple trees. Similarly, for another species of wild apple, a core subset of about 30 trees encompassed most of the genetic diversity in 700 trees. These results enable apple curators to reduce the number of wild apple trees required for field plantings without significantly diminishing the total amount of genetic diversity which is conserved. They also enable apple breeders to more rapidly identify new sources of genetic variability for horticultural traits key to apple genetic improvement.

Richards, C.M., Volk, G.M., Reeves, P.A., Reilley, A., Henk, A.D., Forsline, P.L., Aldwinckle, H. 2009. Selection of stratified core sets representing wild apple (Malus sieversii). Journal of the American Society for Horticultural Science. 134:228-235.

Volk, G.M., Richards, C.M., Henk, A.D., Reilley, A.A., Reeves, P.A., Forsline, P.L., Aldwinckle, H.S. 2009. Capturing the diversity of wild Malus orientalis From Georgia, Armenia, Russia and Turkey. Journal of the American Society for Horticultural Science. 134:453-459.

Richards, C.M., Volk, G.M., Reilley, A.A., Henk, A.D., Reeves, P.A., Lockwood, D.R. and Forsline, P.L. 2009. Genetic diversity and population structure in Malus sieversii, a wild progenitor species of domesticated apple. Tree Genetics and Genomes 5: 339-347.

Long-term storage/safety duplication of NPGS and microbial germplasm:

As of June 2011, approximately 437,000 of the 540,000 NPGS accessions (about 81 percent) have been duplicated, with samples both at an active genebank and in the long-term collection at the National Center for Genetic Resources Preservation (NCGRP), Fort Collins, Colorado. This is an increase of about 10 percent from the number of NPGS accessions backed up 5 years ago. Most of these accessions are seed-propagated; effective methods for backing up many clonally propagated species have not yet been developed. Some NPGS accessions are also safeguarded in the Svalbard Seed Vault in Norway. The NCGRP also provides safety duplication for about 500,000 accessions from several CGIAR genebanks, and other national plant genetic resources programs, so that the Fort Collins genebank likely contains the largest collection of agricultural biodiversity in a single location in the world. Safety duplication is the most effective means of reducing the risk for catastrophic loss of accessions; consequently, storage at the NCGRP has enormous impact in reducing such risk on a global scale.

NPGS accessions backed-up in Svalbard Global Seed Vault. In January 2008, the NPGS sent seeds of approximately 11,000 accessions, including nearly 1,500 that originated in the United States, to the Svalbard Global Seed Vault in Norway for long-term preservation. In 2009 and 2010, 11,000 more accessions were sent. In addition to providing an additional level of safety, these exchanges demonstrated U. S. support (above and beyond the $25 million contributed to date by the United States to the Global Crop Diversity Trust) for the global multilateral system for plant genetic resource conservation and sustainable use.

Backup of plant genetic resources of temperate fruit, nut, and ornamental crops. If a plant germplasm accession is preserved only in one genebank, it is vulnerable to catastrophic loss. Maintaining healthy tree germplasm in an orchard is risky, especially over many years, and maintaining field plantings of clonal crop germplasm is relatively expensive. When feasible, cryopreservation of such germplasm can be more secure and many times more cost effective than backing up the germplasm in orchard plantings at a second site. ARS staff in Geneva, New York, and Fort Collins, Colorado, backed up more than 2,800 orchard accessions of the Geneva apple collection at the NCGRP through cryogenic preservation of dormant buds. Staffs of four NPGS genebanks have collaborated to back up genetic resources of *Corylus, Fragraria, Humulus, Mentha, Pyrus, Ribes, Rubus,* and *Vaccinium* in multiple sites in the form of plants in orchards and greenhouses, tissue cultures, and cryogenic preservation. North American ash trees (*Fraxinus*), important components of native U. S. forests and a frequently planted street and landscape tree, are imperiled by the emerald ash borer, a devastating introduced insect pest. ARS researchers at Ames, Iowa, and Fort Collins developed a reliable protocol for storing *Fraxinus* buds in genebanks, thereby protecting these precious materials for future use. ARS staff at Fort Collins also developed a cryopreservation system for *Juglans cineria*, a native walnut crop threatened by fungal disease. ARS researchers at Corvallis, Oregon, developed more reliable and effective techniques for cryopreserving shoot tips of brambles (*Rubus*) that extend the viability of these tissues in culture, and result in genetically stable, more vigorous shoots when grafted after recovery from preservation conditions. As a result of this and other research, many more of the NPGS clonally propagated materials are backed up at second locations and/or in long-term storage. The Fort Collins base collection now has over 4,350 vegetatively propagated accessions in long-term back-up storage. These materials, many of which are imperiled in the wild or maintained in orchards as single field plots, are now much less susceptible to loss from pests, diseases, and abiotic factors.

Reed, B.M., Castillo, N., Wada, S., Bassil, N.V. 2010. Genetic stability of cryopreserved shoot tips of Rubus germplasm. In Vitro Cellular and Developmental Biology - Plants. 46:246-256.

Volk, G.M., Bonnart, R.M., Waddell, J.W., Widrlechner, M.P. 2009. Cryopreservation of dormant buds from diverse Fraxinus species. CryoLetters. 30:262-267.

Volk, G.M., Waddell, J.W., Bonnart, R.M., Towill, L.E., Ellis, D.D., Luffman, M. 2008. High viability of dormant Malus buds after 10 years of storage in liquid nitrogen vapor. CryoLetters 29:89-94.

Back-up of NPGS tropical and subtropical fruit tree germplasm. Laurel wilt, a deadly fungal disease of avocado and other Lauraceae carried by a beetle, now threatens the field collection of avocado at the NPGS genebank in Miami, Florida. Curators and researchers developed innovative approaches for safeguarding the collection by injecting trees with the fungicide propiconazole in advance of any Laurel wilt infection. They developed a protocol for the safe movement of duplicate cuttings of avocado budwood to another ARS genebank in Hawaii that ensures that neither the fungus nor the beetles are introduced into Hawaii. This protocol involves an intermediate quarantine at the ARS facility in Fort Detrick, Maryland. Approximately 75 percent of the accessions have already been grafted onto budwood at the Fort Detrick facilities, and about a third is ready for introduction to Hawaii. Citrus varieties and genetic resources are often propagated as clones and maintained in field orchards and screen houses because long-term storage of citrus clonal vegetative tissue has not been feasible. ARS researchers at Fort Collins, Colorado, implemented new micrografting recovery methods that enable clonal citrus samples to be stored at the temperature of liquid nitrogen and successfully re-propagated. Storing duplicate clonal samples in secure genebank vaults safeguard that germplasm, and will enable the distribution to researchers more efficiently.

New models for predicting the life spans of stored plant germplasm. The life spans of stored germplasm are difficult to measure empirically because expected timeframes are impractically long. Nonetheless, this basic information is critical for successfully preserving germplasm. ARS scientists at Fort Collins, Colorado, developed tools to understand temperature effects on reaction kinetics at extremely low temperatures and applied them to model the time course of viability loss in plant germplasm. The model, validated by long-term storage experiments, demonstrate that, despite the extreme cold of liquid nitrogen, cryopreserved germplasm will likely still deteriorate over a period of a few thousand years. Furthermore, liquid nitrogen storage is not effective for preserving viability in germplasm that has been over-dried or slightly deteriorated. These researchers also developed a mathematical model to estimate the extent of random genetic shifts as a function of seed longevity and frequency of accession regeneration. The model shows the importance of maintaining original samples and increasing the seed supply from these samples in a parallel fashion. Finally, ARS staff at Ames, Iowa, and their collaborators developed a promising new method to model maize seed deterioration. This novel approach is based on a quadratic viability pattern – which can account for possible seed after-ripening followed by decline in viability – analyzed through Bayesian statistics which provide increased computational efficiency. This model predicts seed viability more effectively and, thus, increases the efficiency of scheduling maize seed testing. Collectively, this research is also valuable for developing management standards for genebanks internationally.

Richards, CM, DR Lockwood, GM Volk, C Walters. 2010. Modeling demographics and genetic diversity in ex situ collections during seed storage and regeneration. Crop Sci. 50: 2440-2447.

Walters, C., Ballesteros, D., and Vertucci, V.A. 2010. Structural mechanics of seed deterioration: standing the test of time. Plant Science 179: 565-57.

Trapp II, A., P. Dixon, M. P. Widrlechner, and D. A. Kovach (accepted for publication). Scheduling viability tests for seeds in long-term storage based on a Bayesian multi-level model. J. Agric., Biol., and Environ. Statistics.

Seed longevity is detectable through non-invasive assays. ARS scientists at Fort Collins, Colorado, developed assays that noninvasively detect very early signs of seed deterioration during storage. Assays are based on volatile emission from seeds detected through gas chromatography of the storage headspace; seed oil crystallization kinetics detected by differential scanning calorimetry; and molecular relaxations within seed structures detected from dynamic mechanical analysis. These sensitive non-destructive assays do not deplete samples of valuable germplasm that are normally consumed during viability monitoring tests. These assays help preserve seed supplies in long-term storage more efficiently and effectively than do traditional approaches.

Mira, S., Gonzalez-Benit, E., M., Hill, L.M., Walters, C.T. 2010. Characterization of volatile production during storage of lettuce (Lactuca sativa) seed. J. Experiment. Bot. 61:3915-3924.

Ballesteros, D. and Walters, C. 2011. Detailed characterization of mechanical properties and molecular mobility within dry seed glasses: relevance to the physiology of dry biological systems. The Plant J. (accepted July 15, 2011)

Microbes

Preservation of plant-associated and industrial microbes. During the last 5 years, about 25,000 of the approximately 93,000 cultures at the ARS Culture Collection in Peoria, Illinois, were sub-cultured and rejuvenated, and about 24,000 of those cultures were tested for viability. More than 90 percent of the accessions of this collection are backed-up off-site via duplicate vials of lyophilized cultures stored at the crop genebank at Ames, Iowa. Duplicate vials for a core subset of the *Rhizobium/Bradyrhizobium* collection at Beltsville, Maryland, are now housed at NCGRP in Fort Collins, Colorado. ARS researchers at Fort Collins determined that most of the strains of the fungus *Rhizoctonia solani*, an important pathogen for several crops, survived more than a decade of storage at -160°C in liquid nitrogen vapor phase. Thus, cryogenic storage methods, which enable ready, long-term storage of important reference isolates, are also well-suited for the preservation of *R. solani* culture collections. This will reduce the time and cost of maintaining these isolates.

Webb, K.M., Hill, A.L., Laufman, J., Hanson, L.E., Panella, L.W. 2011. Long-term preservation of a collection of Rhizoctonia solani, using cryogenic storage. Annals of Applied Biology. 158(3):297-304.

Anticipated Product 8: *Efficient and timely distribution increases the frequency and use of germplasm in research and in crop genetic improvement.*

The demand for accessions from the NPGS has never been higher. On average, more than one-third of the total number of accessions in the NPGS is accessed each year, and nearly every NPGS genebank experienced substantial increases in demand for genetic materials during the last 5 years. Thus, rather than serving as museums primarily for storing germplasm, the NPGS underpins crop research and breeding globally by making an enormous diversity of genetic materials available rapidly, and free of charge and restriction.

As an indication of the extremely strong demand for the soybean collection (Urbana, Illinois) and genetic stocks of maize (Urbana) and tomato (Davis, California), the number of accessions distributed each year from those collections, on average, surpassed the total number of unique accessions in the collection. These statistics do not mean that every single accession in each collection was distributed every year; rather, they illustrate the high volume of requests received for these materials. Among horticultural crop collections, the number of accessions distributed annually from the potato (Sturgeon Bay, Wisconsin), citrus (Riverside, California), tropical fruits (Hilo, Hawaii), apple (Geneva, New York), and grape (Geneva and Davis, California) collections ranged from 50 to 90 percent of the total holdings of unique accessions.

From the perspective of total numbers of accessions distributed annually, the NPGS genebanks with large collections (more than 20,000 accessions managed) of seed-propagated crops shouldered the heaviest burden. Aberdeen, Idaho; Ames, Iowa; Griffin, Georgia; Pullman, Washington; and Urbana, Illinois, handled about 75 percent of the almost 1 million NPGS accessions distributed during the last 5 years.

By percentage, the increase in the distribution volume was especially striking for horticultural, often clonally-propagated, crops. For example, the NPGS genebank in Davis, California, experienced a nearly 13 percent increase in distributions in 1 year. The NPGS genebank in Corvallis, Oregon, handled record demands for its germplasm during this period. Surprisingly, relatively minor horticultural crops were in proportionately greater demand (again on a percentage basis) than some more widely-important species.

The demand for microbial collections was also strong during the last 5 years, with more than 29,000 strains (about 30 percent of the total holdings) distributed from the Peoria microbial collection.

Anticipated Product 9: *High-quality, comprehensive characterization, evaluation, and genebank curatorial data are readily accessible, either from a transformed, upgraded, well-maintained GRIN, from databases housed at GRIN as the primary site, or at sites linked to GRIN.*

Access to inexpensive, vast information storage capacities; ever faster data transfer and retrieval through high-speed Internet connections; and innovative models for database architecture have revolutionized information management technologies within the NPGS. During the last 5 years, the NPGS delivered information 24/7 to an increased volume of requestors in the United States and internationally. With reliable and rapid access to the millions of data points in GRIN (www.ars-grin.gov/), researchers could tailor their requests to receive the accessions which best met their needs. As a result, the average number of accessions per request decreased, although the absolute number of requests increased, indicating that researchers were better able to identify needed materials.

Furthermore, as described below, the NPGS and international partners joined resources to transform GRIN into a flexible, modular, information management system (GRIN-Global) capable of meeting the exacting challenges of safeguarding and delivering invaluable passport and descriptive data associated with NPGS' and the global community's plant genetic resource collections.

Development of GRIN-Global, the successor to GRIN. ARS programmers, curators, bioinformaticists, and information managers, in partnership with the staff of Bioversity International, and with funding from the Global Crop Diversity Trust, developed a new genebank information management system that will succeed GRIN for managing information associated with their collections (see www.ars-grin.gov/npgs/gringlobal/training/What_is_GRIN-Global.pdf and www.grin-global.org/index.php/Main_Page). Innovations in software design were necessary to construct a powerful, flexible, and easy-to-use global plant genetic resource information system that can serve both curators (genebank managers) in less-developed nations who rely on small, legacy computers, and curators in large, technologically advanced multi-national systems, such as the CGIAR centers. The novel design of GRIN-Global enables it to be tailored to the needs of the curator or the genebank manager, and to the specifications of the curator's individual desktop or laptop computer, so that it can be readily customized for the typically idiosyncratic and specific workflows. It also generates custom reports that deliver information to external requestors and/or stakeholders most effectively.

The public Web site for GRIN-Global enables a germplasm requestor to identify and order desired accessions directly from the relevant genebank on-line. The Web site will then automatically notify the genebank responsible for distributing the germplasm, automatically adjust inventory levels when the order is shipped, and even notify the requestors by e-mail that the order has been fulfilled. The public Web site also permits users to register for a user account so they can manage their individual order histories and check the status of new order requests in real-time.

Importantly, a fledgling GRIN-Global user community was established, as a result of several "Train-the-Trainer" workshops. This community will be instrumental for the successful implementation, continued innovation, and vigorous evolution of GRIN-Global. As GRIN-Global matures and is adopted and deployed by genebanks, further community development and access to an open-source venue will maximize GRIN-Global's utility.

Arnaud, E., S. Dias, M. Mackay, P. Cyr, C. Gardner, P. Bretting, G. Kinard, L. Guarino, and S. Louafi. 2010. Chapter 11: A global portal enabling worldwide access to information on conservation and use of biodiversity for food and agriculture. Pp. 175-185 in L. Maurer and K. Tochtermann (eds.) Information and Communication Technologies for Biodiversity Conservation and Agriculture. Shaker Verlag, Aachen, Germany.

Postman, J.D., Bretting, P.K., Kinard, G.R., Cyr, P.D., Weaver, B., Millard, M.J., Gardner, C.A., Bohning, M.A., Emberland, G.P., Sinnott, Q.P., Ayala Silva, T., Hummer, K.E., Franco, T., Mackay, M., Guarino, L. 2010. GRIN-Global: An international project to develop a global plant genebank information management system. Acta Horticulturae 859:49-55.

PROBLEM STATEMENT 1B: *Assess the Systematic Relationships and Genetic Diversity of Crop Genetic Resources*

This Problem Statement focuses on characterizing the genetic relationships of the NPGS plant genetic resources and, together with Problem Statement 3B (see Component 3), evaluating the agricultural merit of those materials. To meet those key challenges, the NPGS must fulfill the demand for additional genetic marker characterizations, and for evaluations of high-value traits, such as superior adaptation, vigor, and nutritional value; enhanced productive potential, capacity, and efficiency; and/or improved resistance to environmental extremes, pests, and diseases. To fulfill the demand for characterization and evaluation information, the NPGS requires expanded capacity for high-throughput genotyping, and for conducting additional germplasm evaluations for priority traits. The expanded genotypic and phenotypic datasets for NPGS genetic resources must be readily accessible from GRIN.

The NP 301 Action Plan identified seven Anticipated Products which were expected to result from research addressing the needs expressed in Problem Statement 1B. The Anticipated Products now serve to help measure the national program's progress during the last 5 years with meeting the needs of crop researchers and producers. A sampling of accomplishments that illustrate those products for this problem statement follow the list of Anticipated Products.

ANTICIPATED PRODUCTS IN ACTION PLAN:

1. Poorly known crop genetic resources are accurately identified, phenotyped, and classified.
2. Knowledge of the genetic variation within and between crop accessions, species, and genera is expanded.
3. Genetic marker systems are developed for efficiently assessing genetic diversity and genotyping genebank accessions, especially for poorly understood specialty crops.
4. New marker genotype and genetic profile databases for germplasm collections serve as tools for genetic resource management.
5. Duplicate accessions, as well as novel genetic variability in collections, are identified.
6. Genetic gaps in collections are detected, thereby aiding in setting acquisition priorities.
7. New statistical genetic approaches for optimizing the efficiency and effectiveness of germplasm sampling, maintenance, and regeneration are developed.

PROBLEM STATEMENT 1B: ACCOMPLISHMENT OVERVIEW

During the last 5 years, NPGS genebanks and cooperators captured genotypes and phenotypes for tens of thousands of accessions to serve as tools for more efficient and effective curation, as well as to ascertain taxonomic identity, systematic relationships, and utility of accessions for crop research and breeding. Across the NPGS, more than 450,000 new morphological and trait evaluation data points for hundreds of crops and crop wild relatives were added to the GRIN database. These data have revealed genetic profiles – patterns of genetic diversity and divergence – which are so important for devising strategies for germplasm acquisition, defining optimal core subsets, and maximizing the genetic diversity and minimizing genetic redundancy within genebank collections. The genetic markers and new statistical genetic approaches also provided effective means for monitoring accessions' genetic integrity, especially for outcrossing crops, so that they can be maintained true-to-type.

NPGS has substantially expanded its capacity to characterize the genetic variation for crops and their wild relatives through high through-put genetic marker analyses, especially SSRs (simple sequence repeats) and SNPs (single nucleotide polymorphisms) markers. Across the NPGS, more than 46,000 different plant accessions were genotyped by genetic markers, mainly generated by the preceding two technologies. Genetic profiles were generated for accessions of crop and model plant genera from A to Z, including *Allium, Arachis, Brachypodium, Capsicum, Carthamus, Carya, Chionanthus, Citrus, Cocos, Corylus, Cucumis, Ficus, Fragraria, Glycine, Gossypium, Ipomoea, Juglans, Lespedeza, Malus, Olea, Oryza, Persea, Prunus, Pyrus, Rheum, Rubus, Sorghum, Solanum (including tomato), Theobroma, Triticum, Ulmus, Vaccinium, Vicia, Vitis*, and *Zea*. Additional ornamental and tropical fruit tree genera were also characterized. Further, thousands of accessions of plant-associated or industrial microbes were also genotyped. These new genetic marker technologies and molecular genotypic information provide rapid and accurate assays of crop genetic variability, yielding critical information for designing efficient and effective curatorial programs and for novel crop breeding strategies (see Components 2 and 3 for additional related accomplishments in this area).

Briefly, some of the highlighted accomplishments for this Problem Statement include:

- NPGS researchers and collaborators describing a new species of melon (*Cucumis*) from existing genebanks collections;
- Utilizing thousands of genetic markers to genotype nearly every accession in the entire NPGS grape collections (*Vitis*; 1,000+ accessions), and more than 25 percent of the cotton collection (*Gossypium*; 2,500+ accessions);
- Reassessment of the center of origin for domesticated potato (Solanum), coconut (*Cocos*), and related plants;
- Developing markers for identifying high-value traits, such unsaturated fatty esters in peanuts (*Arachis*), cytological information that revealed gaps in the genetic coverage for collections of elms (*Ulmus*), whereash genetic markers uncovered similar gaps for the tomato (*Solanum*) collection;
- Applying genetic marker genotypes to enhance managerial efficiencies for the rice (*Oryza*) and hazelnut (*Corylus*) collections; and
- Detecting mislabeled and unwanted duplicate accessions in NPGS and international germplasm collections of cacao (*Theobroma*) with new genetic markers.

Notably, the Genomics and Bioinformatics Research Unit (GBRU) at Stoneville, Mississippi, (see www.ars.usda.gov/main/site_main.htm?modecode=64-02-50-00) played a major role in developing and applying genetic marker systems for many specialty crops (see related accomplishments under Component 2). Developing and validating genetic markers for these diverse plant genetic resources were so complicated that their costs were prohibitive for many smaller laboratories, such as those in many NPGS genebanks. The GBRU developed a more efficient and effective approach for identifying SSR markers to overcome some of the obstacles in previous procedures, and afford an economical method of marker development. The GBRU also wrote bioinformatic programs to help identify the markers which yield the most useful/diverse genetic information. These methods have already been applied successfully to numerous genera, especially for tropical tree crops at NPGS genebanks in Miami, Florida; Hilo, Hawaii; and Mayagüez, Puerto Rico. These marker systems have helped confirm the identity of inadvertently duplicated germplasm accessions and determined the relative genetic diversity conserved by those collections.

Arias De Ares, R.S., Ballard, L.L., Scheffler, B.E. 2009. UPIC: how many SSR markers to run. Bioinformation 3(8):352-360.

Techen, N., Arias, R.S., Glynn, N.C., Pan, Z., Khan, I.A., Scheffler, B.E. 2010. Optimized construction of microsatellite-enriched libraries. Molecular Ecology Resources Vol. 10(3), pp. 508-515

As a cautionary note, ARS researchers found that the genetic barcoding approach was not effective for identifying different potato (*Solanum*) taxa, demonstrating that genetic markers do not constitute a universally applicable remedy for ameliorating all challenges facing NPGS curators and germplasm users.

In addition to the preceding results, and those detailed under Component 3, Problem Statement 2, the publications listed in Appendix 2 document analyses of other genera and crops that have not been described in the present narrative. When possible, contributions to additional specific Anticipated Products are identified following the narrative text.

PROBLEM STATEMENT 1B: SELECTED ACCOMPLISHMENTS

Anticipated Product 1: *Poorly known crop genetic resources are accurately identified, phenotyped, and classified.*

Identification of a new wild *Cucumis* species from the NPGS melon collection. Six accessions of wild *Cucumis* (melon) from Zambia conserved in the NPGS collection at Ames, Iowa, were described and identified as a new species, *Cucumis zambianus*, based on molecular and phenotypic data. Correct taxonomic identification of genetic resources ensures that research results are repeatable, and that results can be related to those from prior research. [Also contributes to Anticipated Product 2.]

Widrlechner, M.P., Kirkbride, J.H., Ghebretinsae, A.G., Reitsma, K.R. 2008. Cucumis zambianus (Cucurbitaceae): a new species from northwestern Zambia. Systematic Botany. 33:732-738.

Genetic relationships among coconut germplasm and varieties. ARS researchers at Miami, Florida, found that the genetic relationships among coconut varieties are more complicated than previously supposed. The results of SSR genetic markers genotyping for more than 230 coconut trees representing 10 varieties suggest that prior assessments of genetic diversity and the center of origin for this important tropical crop will require reappraisal. Earlier analyses failed to test for hybrids among the samples and applied only one type of statistical analysis to the data. The improved understanding of coconut systematic relationships can more accurately highlight gaps in the genetic coverage for palm germplasm collections, and help maintain these materials true-to-type. [Also contributes to Anticipated Products 2 and 5.]

Mauro-Herrera, M., Meerow, A.W., Perera, L., Russell, J., Schnell II, R.J. 2009. Ambiguous genetic relationships among coconut (Cocos nucifera L.) cultivars: the effects of outcrossing, sample source and size, and method of analysis. Genetic Resources and Crop Evolution. 57:203-217.

Meerow, A.W., Borrone, J.W., Couvreur, T.L., Mauro-Herrera, M., Hahn, W.J., Kuhn, D.N., Nakamura, K., Schnell II, R.J. 2009. Phylogenetic analysis of seven WRKY genes across the palm subtribe Attaleinae (Areceaceae) identifies Syagrus as sister to the coconut. PLoS One. DOI http://dx.plos.org/10.1371/journal.pone.0007353

Determining the center of origin and correct scientific names for domesticated potato. Indigenous landrace potatoes (*Solanum tuberosum*) in South America are used by potato breeders to improve the crop. ARS researchers at Madison, Wisconsin, enlisted patterns in SSR genetic markers to show that the domesticated potato originated from wild relatives in the Andes mountain range in Peru, rather than much farther south in lowland Chile, as had been hypothesized earlier. This information has helped curators reassess managerial priorities, and potato breeders choose the optimal germplasm from among closely related wild species. It also led to a reevaluation of the historical importance of Andean potatoes for the production of potatoes outside of the tropical latitudes of the Peruvian Andes. Furthermore, ARS researchers and colleagues compiled, for the first time, 602 scientific names of cultivated potatoes, and associated these names with the modern taxonomy of cultivated potatoes. They summarized the history of cultivated potato taxonomy, developed identification tools (keys) for cultivated potatoes, and described four cultivated species. This comprehensive work provides a reference to genebank managers and breeders for the more efficient curation and use of cultivated potato genetic resources. [Also contributes to Anticipated Products 2 and 4.]

Ovchinnikova, A., Krilova, E., Gavrilenko, T., Smekalova, T., Dorofeev, V., Zhuk, M., Knapp, S., Spooner, D.M. 2011. Taxonomy of cultivated potatoes (Solanum Section Petota: Solanaceae). Botanical Journal of the Linnaean Society. 165:107-155.

Ghislain, M., Nunez, J., Trujillo, G., Herrera, M., Guzman, F., Spooner, D.M. 2007. Extensive SSR genotyping of potato landraces supports a drastic revaluation of their gene pool structure and classification. Proceedings of the National Academy of Sciences. 104:19398-19403.

Molecular characterization of priority microbial genetic resources. The microbial genetic diversity of many strains included in the ARS Culture Collection in Peoria, Illinois, has not been well characterized, making it difficult to assess the breadth of genetic coverage and its real or potential value to agricultural and biotechnology research. ARS researchers and colleagues characterized the genetic diversity of thousands of those strains based on the nucleotide sequence variability for key marker genes. For example, the partial sequences of four different protein-coding genes for 10 plant pathogenic *Streptomyces* species and several other putatively closely related species demonstrated that the pathogenic species can be discriminated and identified based on these gene sequences. Additionally, similar sequences are being determined for all streptomycetes in the collection. ARS researchers and colleagues at Peoria, Illinois, developed a comprehensive Web-accessible multilocus DNA sequence database which allows identification of *Fusarium* pathogens for the first time. This database provides the only means by which these pathogens can be accurately identified and reported. This new information provides the means for discerning potential biotechnological applications as well as their utility for disease control, toxin reduction, and prevention of food spoilage. [Also contributes to Anticipated Product 5.]

Tamura, T., Ishida, Y., Otoguro, M., Hatano, K., Labeda, D.P., Price, N.P., Suzuki, K. 2008. Reclassification of Streptomyces caeruleus as a synonym of Actinoalloteichus cyanogriseus and reclassification of Streptomyces spheroides and Streptomyces laceyi as later synonyms of Streptomyces niveus. International Journal of Systematic and Evolutionary Microbiology. 58(12):2812-2814.

Park, B., Park, J., Cheong, K., Choi, J., Jung, K., Lee, Y., Ward, T.J., O Donnell, K., Geiser, D.M., Kang, S. 2010. Cyber-infrastructure for Fusarium (CiF): Three integrated platforms supporting strain identification, phylogenetics, comparative genomics, and knowledge sharing. Nucleic Acids Research. doi:10.1093/nar/gkq1166.

Phenotyping and genotyping chile peppers. A key factor for selecting vegetable crop germplasm for research is the extent and accuracy of the morphological descriptions, but at present relatively few are available. ARS researchers at Griffin, Georgia, measured morphological traits and captured digital images for key characteristics of 600 accessions of chile pepper (*Capsicum annuum*) grown at two locations. Several thousand data points and hundreds of digital images were uploaded into the GRIN database. These data will enable a more accurate assessment of the extent of morphological diversity in the collection, and will help the scientific user community select material most appropriate for specific research projects. Despite this morphological variability, certain *Capsicum* species can be difficult to identify solely by their morphological characteristics. ARS researchers in Griffin found that DNA sequence changes within a particular gene (the “waxy locus”) can help to identify these species. Variability in the sequence of this gene was determined for approximately 500 genebank accessions of *Capsicum* (*C. annuum*, *C. chinense*, and *C. frutescens*) and in most instances those data supported the currently accepted classification. In some instances, it resulted in a reclassification of the accessions. Accurately classified and identified accessions help ensure that this germplasm can meet researchers’ needs.

Jarret, R.L., Berke, T. 2008. Variation for fruit morphological characteristics within a germplasm collection of Capsicum chinense Jacq. HortScience. 43:1694-1697.

Jarret, R.L. 2008. DNA barcoding in a crop genebank: resolving the Capsicum annuum species complex. The Open Biology Journal. 1:35-42.

High through-put phenotyping of Brachypodium. A simple model for studying grass cell walls is needed to accelerate progress in understanding the potential to alter the properties of cellulosic biomass. ARS researchers from Albany, California, and their collaborators applied a high-throughput phenotyping platform (phenomics) to characterize more than 100 accessions of *Brachypodium*, a model species for facilitating genetic analyses of wheat, barley, and some biofuel crops. Extensive natural variation was detected for several traits relevant to biofuels including, cell wall composition, stem density, and fermentability. These findings will enable the identification of genes which might be manipulated in bioenergy crops to improve cell wall properties for biofuel production. [Also contributes to Anticipated Product 1.]

Vogel, J.P., Tuna, M., Budak, H., Huo, N., Gu, Y.Q. and Steinwand, M.A. 2009 Development of SSR markers and analysis of diversity in Turkish populations of Brachypodium distachyon. BMC Plant Biology. 9: Article No. 8.

Phenotyping and genotyping poorly known ornamental genetic resources. *Chionanthus*, the Chinese fringe tree, an attractive, spring-flowering tree or shrub, was poorly known genetically. ARS researchers in Washington, D.C.; Poplarville, Mississippi; and Stoneville, Mississippi, developed and applied more than 250 SSR markers to genotype the races of Chinese fringe trees now in the United States. Identifying these genetic races will accelerate their conservation and use by clearly distinguishing the genetic types and eliminating duplication in collections and crop improvement programs. [Also contributes to Anticipated Product 2.]

R. Arias, R.S., N. Techen, T.A. Rinehart, R.T. Olsen, J.H. Kirkbride, Jr., and B.E. Scheffler. 2010. Development of simple sequence repeat markers for Chionanthus retusus (Oleaceae) and effective discrimination of closely related taxa. HortScience 46(1):23-29.

Anticipated Product 2: *Knowledge of the genetic variation within and between crop accessions, species, and genera is expanded.*

Genetic characterization of the NPGS cotton germplasm collection. ARS researchers at College Station, Texas, and Stoneville, Mississippi, and their collaborators genotyped more than 2,300 accessions, or approximately 25 percent of the total NPGS cotton collection, with 105 SSR markers. They determined species introgression, redundancy among accessions, and within-accession variation with the markers. These data provide valuable information on the patterns and distribution of genetic diversity within the collection to guide curatorial decision-making, and reveal sources of unique genetic variation to exploit for cotton improvement. [Also contributes to Anticipated Products 4 and 5.]

Percy, R. G., Fang, D.D., Hinze, L., Yu, J.Z., Frelichowski, J., Scheffler, B., and Gore, M. 2011. Preliminary assessment of the efficacy of a core marker set in revealing genetic diversity In the U.S. germplasm collection [abs]. *In* Proceedings of the National Cotton Council Beltwide Cotton Conferences, January 4-7, 2011, Atlanta, GA. p. 717.

Yu, J., Fang, D.D., Ulloa, M., Percy, R.G., Kohel, R.J., Hinze, L.L., Frelichowski, J.E., Cho, J., Campbell, B.T., Chee, P., Zhang, J., Abdurakhmonov, I., Abdukarimov, A., Jones, D.C. 2010. Development of core SSR markers for Gossypium germplasm characterization [abstract]. In: Proceedings of the National Cotton Council Beltwide Cotton Conferences, January 4-7, 2010, New Orleans, Louisiana. p. 811. CDROM.

SSR markers clarify genetic relationships among *Vaccinium* accessions. ARS researchers at Corvallis, Oregon, generated SSR marker genotypes which clarified the genetic relationships among genebank accessions of blueberries, cranberries, and other species of *Vaccinium*. A diagnostic set of SSR markers were developed for distinguishing different blueberries, and also cranberry accessions and cultivars. More than 300 genebank accessions of *Vaccinium* L. section *Myrtillus* were characterized. The native Hawaiian ‘Ohelo’ berry (*V. reticulatum*), under development as an ornamental, was found to be genetically distinct from the other members of the section *Myrtillus.* A suite of genetic markers for *V. reticulatum* is potentially useful for maintaining Ohelo berry cultivars true-to-type. These data also highlighted the need for incorporating more accessions into NPGS genebanks to represent adequately the genetic diversity within these *Vaccinium* species and for developing additional markers for more precise taxonomic resolution in the genus *Vaccinium.* [Also contributes to Anticipated Products 1, 4, and 6.]

Bassil, N.V., Bunch Jr, T.R., Nyberg, A.M., Zee, F.T., Hummer, K.E. 2010. Microsatellite markers distinguish Hawaiian Ohelo from other *Vaccinium L*. Section *Myrtillus* species. Acta Horticulturae 859:81-88.

Hinrichsen, P., M. Herminia, C., Ravest, G., Rojas, G., Mendez, M., Bassil, N.V., Munoz, C. 2009. Minimal microsatellite marker panel for fingerprinting blueberry cultivars. Acta Horticulturae 810:173-180.

Bassil, N.V., Oda, A.K., Hummer, K.E. 2009. Blueberry microsatellite markers identify cranberry cultivars. Acta Horticulturae 810:181-186.

Characterizing a core subset of *Triticum* germplasm resistant to stripe rust. ARS researchers at Pullman, Washington, identified more than 18,000 accessions of *Triticum* with tolerance or resistance to stripe rust. ARS researchers at the Aberdeen, Idaho, genebank and their colleagues then analyzed the accessions for ecogeographical, agronomic, and morphological traits to identify a core subset of stripe rust-resistant germplasm. In addition to facilitating use by researchers and breeders, the core subset will help identify unique new resistance genes and locate ecogeographical gaps in the collection which can be targeted for future germplasm acquisition. [Also contributes to Anticipated Products 1, 4, and 6.]

Bonman, J.M., Bockelman, H.E., Jin, Y., Hijmans, R.J., Gironella, A. 2007. Geographic distribution of stem rust resistance in wheat landraces. Crop Sci. 47:1955-1963.

Identifying key sorghum lines for bioethanol production. Sorghum with high sugar content (sweet sorghum) is an important biofuel crop, but its genetic content must be characterized before it can be bred and incorporated into genetic research for bioethanol production efficiently. ARS researchers analyzed 96 sweet sorghum samples with DNA genetic markers to determine their genetic variability and population structure. This new genetic information enables curators to manage sweet sorghum accessions more effectively and accelerates breeding of superior sweet sorghum cultivars for bioethanol production**.** [Also contributes to Anticipated Products 2 and 7.]

Wang, M.L., Zhu, C., Barkley, N.L., Chen, Z., Erpelding, J.E., Murray, S., Tesso, T., Pederson, G.A., Yu, J. 2009. Genetic diversity and population structure analysis of accessions in the US historic sweet sorghum collection. Theoretical and Applied Genetics. Online DOI 10.1007/s00122-009-1155-6. 120:13-23.

Olive and walnut germplasm characterized. Genotypic analyses of walnut (*Juglans*) genetic structure and differentiation with 6,000 SNP markers revealed wide genetic variation in the NPGS walnut germplasm collection at Davis, California. The data will be merged with 25 economic phenotypes collected across 2 years for further genetic analysis. Genetic characterization of the NPGS olive (*Olea*) collection (more than 120 accessions) at Davis also indicated extensive polymorphisms with relatively simple genetic structure that enabled the identification of many duplicate samples in the collection. These data will enhance the managerial efficiency for this collection and enable requestors to readily identify the most appropriate material for their research. [Also contributes to Anticipated Products 1 and 5.]

Gunn, B.F., Aradhya, M.K., Salick, J.M., Miller, A.J., Yongping, Y., Lin, L. 2010. Genetic variation in walnuts (Juglans regia and J. sigillata; Juglandaceae): Species distinctions, human impacts, and the conservation of agrobiodiversity in Yunnan, China. American Journal of Botany 97:660-671.

Soleri, D., Koehmstedt, A.M., Aradhya, M.K., Polito, V., Pinney, K. 2010.Comparing the historic olive trees (Olea europaea L.) of Santa Cruz with contemporaneous trees in the Santa Barbara, CA area: a case study of diversity and structure in an introduced agricultural species conserved in situ. Genetic Resources and Crop Evolution 57:973-984.

Identification of unique apple accessions with traits potentially important for mechanical harvest. Retention of ripe apples on the tree would be critical for mechanical harvesting, but this trait is generally absent from breeding stocks. Some wild apple species, especially the small-fruited crabapple, do retain fruit. ARS researchers identified six unique accessions that retained fruit that was overripe, with relatively high internal ethylene concentrations, low starch content, low firmness, and high coloration in the NPGS apple germplasm collection at Geneva, New York. These unique non-abscising genotypes are currently undergoing more detailed anatomical, morphological, physiological, and functional genomic analyses to determine their utility as sources of this key trait**.** [Also contributes to Anticipated Product 1.]

Sun L, Bukovac MJ, Forsline PL and van Nocker S. 2009. Natural variation in fruit abscission-related traits in apple (Malus). Euphytica 165(1): 55-67

Genetic relationships of citrus wild relatives. ARS scientists at Riverside, California, and their cooperators analyzed the genetic relationship of 61 species of 35 genera of the Rutaceae related to *Citrus* via the variability in the nucleotide sequence of a specific gene. The phylogenetic information generated provides a source of SNP markers for rapid and accurate assays of crop genetic variability needed to design efficient and effective curatorial programs and novel breeding strategies. [Also contributes to Anticipated Product 4.]

Ramadugu, C., K. L. Manjunath, R. F. Lee, and M. Roose. 2011. Single nucleotide polymorphisms in Citrus and members of Aurantioideae. Plant and Animal Genomes XIX Conference Proceedings. W147 www.intl-pag.org/19/abstracts/W21_PAGXIX_147.html

Genetic markers for breeding and managing genetic resources of tomato. The paucity of detectable genetic variation in the cultivated genepool for tomato has impeded genetic resource management and crop improvement. ARS researchers at Geneva, New York, discovered 188 mutations in 50 genes from NPGS genebank accessions that can serve as genetic markers for mapping and gene selection during crop improvement. This variability also indicated that the tomato genebank accessions might include some new sources of diversity crucial for continued progress with identifying yield genes and alleles that confer protection against novel diseases, pests, or environmental challenges. The greatest genetic diversity in a set of 30 tomato accessions and one commercial type resided in accessions that originated in Chile, Ecuador, and Peru, identifying this region as a priority for collecting germplasm and for in situ conservation of wild and cultivated populations. The genetic variability in a wild relative of tomato revealed that intercrossing had occurred among tomato domesticates and that wild tomato relative. These patterns of gene diversity will assist genebank curators with setting managerial priorities, and breeders with improving the tomato crop. [Also contributes to Anticipated Products 3 and 4.]

Labate, J.A., Robertson, L.D., Baldo, A.M. 2009. Multilocus sequence data reveal extensive departures from equilibrium in domesticated tomato (Solanum lycopersicum L.). Heredity 103:257-267.

Labate, J.A., Robertson, L.D., Wu, F., Tanksley, S.D., Baldo, A.M. 2009. EST, COSII, and arbitrary gene markers give similar estimates of nucleotide diversity in cultivated tomato (Solanum lycopersicum). Journal of Theoretical and Applied Genetics. 118:1005-1014.

Anticipated Product 3: *Genetic marker systems are developed for efficiently assessing genetic diversity and genotyping genebank accessions, especially for poorly understood specialty crops.*

Genotyping the NPGS grape collection with thousands of genetic markers. ARS researchers in Ithaca, New York, and at the NPGS genebanks in Davis, California, and Geneva, New York, genotyped much of the NPGS grape (*Vitis*) collection (more than 1,000 accessions) for variability at more than 5,000 SNP genetic markers. The enormous data set provides genebank curators, grape geneticists, and breeders with an extremely powerful tool for managing grape germplasm, understanding its evolutionary history, and accelerating the pace of grape breeding. Those data have led to additional diversity analysis, core collection selection, identification of possible speciation patterns, and detection of taxonomically misidentified accessions. In earlier research, 340 NPGS accessions representing 52 taxa of *Vitis* were analyzed with SSR markers to unravel the genetic structure and phylobiogeography of grape – knowledge key for optimal grape genetic resource management. A morphometric analysis of 25 nominal, ordinal, and metric horticultural traits from 500 cultivated grape NPGS accessions including wine, table, and dual-use type enabled curators to better manage this collection, and geneticists and breeders to choose optimal accessions for their research. [Also contributes to Anticipated Products 1, 2, 4, 5, and 6.]

Myles, S. M. et al. 2011. Genetic structure and domestication history of the grape. Proc. U. S. Natl. Acad. Sci. March 1, 2011 vol. 108 no. 9 3530-3535.

Bautista, J., Dangl, G., Yang, J., Reisch, B., Stover, E.W. 2008. Use of genetic markers to assess pedigrees of grape cultivars and breeding program selections. American Journal of Enology and Viticulture 59:248-254

Arroyo-Garcia, R., Ruiz-Garcia, L., Bolling, L., Octete, R., Lopez, M., Arnold, C., Ergul, A., Soylemezoglu, G., Uzun, H., Cabello, F., Ibanez, J., Aradhya, M.K., Antanassov, A., Balint, S., Cenis, J., Constantini, L., Gorislavets, S., Grando, M.S., Klein, B.Y., Mcgovern, P.E., Merdinoglu, D., Pejic, I., Pelsy, F., Primikirios, N., Risovannaya, V., Roubelakis-Angelakia, K.A., Snoussi, H., Sotiri, P., Tamhankar, S., This, P., Troshin, L., Malpica, J.M., Lefort, F., Martinez-Zapater, J.M. 2006. Multiple origins of cultivated grapevine (Vitis vinifera L. ssp. sativa) based on chloroplast DNA polymorphisms. Molecular Ecology 15: 3707-3714

Genotyping assay developed for high oleic acid peanuts. ARS researchers in Griffin, Georgia, developed a real-time polymerase chain reaction genotyping assay for alleles of genes governing the high oleic acid trait in peanuts. This assay enables curators to identify particularly valuable accessions, and breeders to test seed or leaf tissue in initial crosses rather than via destructive chemical analyses of ground seeds. This rapid assay will help identify key genotypes linked to important agronomic traits, improve curatorial efficiency, and decrease the time and effort required to characterize the progeny of breeding crosses by eliminating undesirable seedlings. [Also contributes to Anticipated Products 1 and 2.]

Barkley, N.L., Wang, M.L., Pittman, R.N. 2011. A real-time PCR genotyping assay to detect FAD2A SNPs in peanuts (Arachis hypogaea L.). Electronic Journal of Biotechnology. 14(1).

Chen, Z., Wang, M.L., Barkley, N.L., Pittman, R.N. 2010. A simple allele-specific PCR assay for detecting FAD2 alleles in both A and B genomes of the cultivated peanut for high oleate trait selection. Plant Molec. Biol. Reporter. 28:542-548.

Maize genetic diversity characterized. Maize is the most diverse crop in the world, but it was nearly impossible to characterize adequately that diversity across the genome without recently developed DNA sequencing technology. ARS scientists at Ithaca, New York, characterized diversity across the genome for 27 maize germplasm lines that are key parents for maize genetic analyses, and found over 1.6 million variable regions in the genome. Patterns in the variability in these regions can guide curators in making decisions and setting priorities. They also provide researchers with molecular tools for discovering key genes controlling agronomic traits, which will accelerate corn improvement.

Gore, M.A., Chia, J., Elshire, R.J., Sun, Q., Ersoz, E.S., Hurwitz, B.L., Peiffer, J.A., McMullen, M.D., Grills, G.S., Ross-Ibarra, J., Ware, D., Buckler IV, E.S. 2009. A first generation haplotype map of maize. Science. 326(5956):1115-1117.

New genetic markers for faba beans. Faba bean (*Vicia faba*) is the sixth most important of the world's legume food crops. ARS researchers genotyped 155 of 750 accessions of faba in the NPGS collection with target region amplification polymorphism (TRAP) markers to assess the collection's genetic diversity. The subset of 155 samples included a relatively high level of genetic diversity, which was associated with the geographic origins of the accessions. These results have assisted with setting priorities for managing this collection and have promoted the utilization of these materials in breeding programs. [Also contributes to Anticipated Products 1, 2, and 4.]

Kwon, S.J., Hu, J., Coyne, C.J. 2010. Genetic diversity and relationship among faba bean (Vicia faba L.) germplasm entries as revealed by TRAP markers. Plant Genetic Resources: Characterization and Utilization. 8(3); 204-213.

Anticipated Product 4: *New marker genotype and genetic profile databases for germplasm collections serve as tools for genetic resource management.*

New genetic marker data section for GRIN. A new genetic marker data section was added to the GRIN database to manage and deliver the wealth of data now available for NPGS germplasm. To date, nine genetic marker datasets have been added for *Vaccinium, Theobroma,* *Rheum, Pisum, Humulus, Pyrus, Malus, Corylus,* and *Vitis*. GRIN is now capable of storing and presenting datasets with both phenotypic (trait) and genotypic (marker) information. Links were established between genetic marker analyses of NPGS accessions with data deposited in the National Center for Biological Information's GenBank. Users searching GenBank can link to GRIN and vice versa. [www.ars-grin.gov/cgi-bin/npgs/html/croplist.pl and the genetic marker data for cacao at www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?256 are particularly notable.]

Volk, G.M., Richards, C.M. 2008. Availability of genotypic data for USDA-ARS National Plant Germplasm System accessions using the Genetic Resources Information Network (GRIN) database. HortScience 43:1365-1366.

Multilocus sequence typing for rhizobial symbionts of *Medicago* and annual medics. Two large plasmids comprise about half the DNA content of *Medicago*-nodulating rhizobia, with one plasmid carrying all the genetic information necessary for forming the nitrogen fixation symbiosis with the host plant. More than 230 strains in the ARS national collection of rhizobia were characterized by multilocus sequence typing with six genes, and patterns of variability in the plasmids were analyzed to determine their divergence. The entire sequence for one of the nodulation genes and one of the nitrogen fixation genes in the rhizobia were determined and revealed that more diversity occurred in each of the two plasmids than in the bacterial chromosome. As they evolved, the two plasmids shared genes extensively. Infection and establishment of an effective symbiosis with alfalfa was associated with specific alleles of two rhizobial genes for symbiosis. In addition to their importance for characterizing the genetic diversity within ARS's rhizobial collection, these findings might have utility for managing the symbiosis in alfalfa forage and organic production fields. [Also contributes to Anticipated Products 2 and 3.]

Van Berkum, P.B., Elia, P.E., Eardly, B.D. 2010. Application of Multilocus Sequence Typing to study the genetic structure of megaplasmids in Medicago-nodulating rhizobia. Applied and Environmental Microbiology. 76:3967-3977.

Van Berkum, P.B., Elia, P.E., Eardly, B.D. 2006. Multi-locus sequence typing (mlst), a basis for the population analysis of medicago-nodulating rhizobia. Journal of Bacteriology. 188:5570-5577.

Anticipated Product 5: *Duplicate accessions, as well as novel genetic variability in collections, are identified.*

Determining redundancy within the NPGS onion germplasm collection with genetic markers. Conserving genetically redundant accessions can constitute a suboptimal allocation of genebank curatorial resources and can reduce the efficiency of crop germplasm characterization, evaluation, and enhancement. ARS researchers at Pullman, Washington, and their collaborators found that patterns of genetic variability in two different types of genetic markers (SSR and targeted region amplified polymorphisms or TRAP) led to sometimes different conclusions regarding the genetic identity and divergence among a set of 35 short-day onion accessions. These results underscored the importance of combining a sufficiently large genetic marker data set with passport information, and of patterns in morphological markers when examining genetic redundancy of problematic samples. [Also contributes to Anticipated Products 3 and 4.]

Kisha, T.J., Cramer, C.S. 2011. Determining redundancy of short-day, onion accessions in a germplasm collection using microsatellite and targeted region amplified polymorphic markers. J. Amer. Soc. Hort. Sci. 136(2):1-6.2011.

Developing new genetic stocks. When genebanks such as the NPGS Maize Genetic Stock Center at Urbana, Illinois, acquire new maize genetic stocks, the curators must determine if the mutant phenotypes result from defects in known genes, or if they are novel traits. The collection curators analyzed several categories of mutants with similar effects on the maize plant and/or located in a similar position on a chromosome, and identified additional mutant variants of five genes. These data, together with additional mutant traits that affect kernel germination and yellow pigment intensity, will yield new well-characterized stocks. These stocks are valuable tools for understanding the genetic function underlying key agronomic traits.

Stinard, P.S., Kermicle, J.L., Sachs, M.M. 2009. The maize enr system of r1 haplotype–specific aleurone color enhancement factors. Journal of Heredity. 100(2):217-228.

Genetic markers identify hazelnut clonal germplasm. Crop germplasm maintained in long-term orchards are particularly prone to misidentification or mislabeling. ARS researchers at the Corvallis, Oregon, genebank applied SSR genetic markers to clonal hazelnut (*Corylus*) varieties maintained in the back-up orchard planting, and identified several instances where trees were mislabeled or where rootstock shoots had overtaken the desired scion shoot. These genetic marker data were key for helping to maintain these back-up collections true-to-type. [Also contributes to Anticipated Product 5.]

Bassil, N.V., Postman, J.D., Hummer, K.E., Sezer, A., Botu, M. 2009. SSR fingerprinting panel verifies identities of clones in backup hazelnut collection of USDA genebank. Acta Horticulturae 845:95-102.

Anticipated Product 6: *Genetic gaps in collections are detected, thereby aiding in setting acquisition priorities.*

New genetic marker data reclassify the infraspecific variability in cacao and enhance the quality of cacao genebank collections. ARS researchers in Miami, Florida, and their collaborators analyzed the genetic diversity in more than 1,200 geographically diverse samples of Latin American cacao (*Theobroma cacao*) with more than 100 powerful new genetic markers. They found that cacao actually includes 10 genetically distinct subgroups, rather than the two to four subgroups of traditional classifications. In contrast to the commonly accepted notion that the ancient Criollo type from Central American is a primary ancestor for Trinitario cacao from Trinidad (an importance source of fine flavor cacao), ARS researchers at Beltsville, Maryland, and Miami found only small genetic contributions of Criollo cacao to the contemporary Trinitario genotype. These new findings are important both for deciding which cacao landraces from Central America and Caribbean region should be priorities for genebank and in situ conservation, and for tracking the origin of fine-flavored cacao. ARS researchers from Mayagüez, Puerto Rico, Miami, and Beltsville, and their cooperators analyzed the genetic profiles in the germplasm collection of about 1,000 cacao trees maintained at the NPGS genebank at Mayagüez. That collection consists of trees from diverse genetic backgrounds that represent the geographic origins of cacao. Misidentification, mislabeling, and gaps in genetic diversity were identified and new germplasm will be introduced to include underrepresented germplasm. This refined knowledge of cacao genetic relationships will improve the quality of cacao genebank collections, and accelerate the ability of cacao breeders to identify genetically distinct materials with desirable traits for genetic improvement. [Also contributes to Anticipated Products 1, 2, and 5.]

Motamayor, J.C., Lachenaud, P., Wallace, J., Loor, G., Kuhn, D.N., Brown, J.S., Schnell II, R.J. 2008. Geographic and genetic population differentiation of the Amazonian chocolate tree. PLoS One. Available: DOI: 10.1007/s12042-008-9011-4

Johnson, E.S., Bekele, F., Brown, J.S., Song, J.H., Motamayor, J., Zhang, D., Schnell II, R.J., Meinhardt, L.W. 2009. Population structure and genetic diversity of the Trinitario cacao (Theobroma cacao L.) from Trinidad and Tobago. Crop Science. 49:564-572

Irish, B.M., Goenaga Portela, R.J., Zhang, D., Schnell Ii, R.J., Brown, J.S., Motamayor, J. 2010. Microsatellite fingerprinting of the USDA-ARS Tropical Agriculture Research Station cacao (Theobroma cacao L.) germplasm collection. Crop Science. 50:656-667.

Expanded knowledge of the genetic diversity among American elms. Despite its importance as a forest tree and major role in the American nursery industry, the American elm (*Ulmus americana*) was poorly known genetically and taxonomically. ARS scientists from Washington, D.C., determined through flow cytometry that this species includes both diploid and tetraploid chromosome races. The diploid race was previously unknown, suggesting that considerable untapped genetic variation remains in this species. ARS researchers also revised the taxonomy of the elm family (Ulmaceae) for The Jepson Manual to provide up-to-date information on the taxonomy, distribution, and ecological status for native elms of California and adjacent states. This genetic and taxonomic knowledge will assist curators in acquiring underrepresented elm genetic diversity and identify unknown populations in collections. [Also contributes to Anticipated Products 1 and 2.]

Whittemore, A. T. and R. Olsen. 2011. Ulmus americana is a polyploid complex. American Journal of Botany 98: 754-760.

Whittemore, A. T. In press. Ulmaceae. In press. The Jepson Manual: Higher Plants of California ed. 2. Berkeley: University of California Press. Prepublication online: http://ucjeps.berkeley.edu/tjm2/review/treatments/ulmaceae.html

Anticipated Product 7: *New statistical genetic approaches for optimizing the efficiency and effectiveness of germplasm sampling, maintenance, and regeneration are developed.*

Rice mini-core subset for identifying valuable genes. Because the NPGS rice collection is so large (18,000 accessions from 116 countries), it is difficult for scientists to choose the best materials for their research. ARS scientists in Stuttgart, Arkansas, applied statistical genetic methods to choose a mini-core subset from the NPGS rice collection which contained only 217 accessions, but which captured the full range in variation of 26 agronomic traits throughout the entire collection. Genotyping the mini-core subset with 70 SSR markers revealed over 900 alleles, indicating wide genetic diversity for the mini-core subset. The data are guiding rice curatorial decision-making and priority setting. Furthermore, researchers can analyze the accessions in the mini-core subset to mine important genes from the rice collection. [Also contributes to Anticipated Products 2, 3, and 4.]

Agrama H.A., W.G. Yan, F.N. Lee, R. Fjellstrom, M-H. Chen, M. Jia and A. McClung. 2009. Genetic assessment of a mini-core developed from the USDA rice genebank. Crop Science 49:1336-1346.

Li, X., Yan, W., Agrama, H., Hu, B., Jia, L., Jia, M.H., Jackson, A.K., Moldenhauer, K., McClung, A.M., Wu, D. 2010. Genotypic and phenotypic characterization of genetic differentiation and diversity in the USDA rice mini-core collection. Genetica. 138:1221-1230.

Limits to the utility of DNA barcoding for identifying highly diverse plant species. DNA barcoding seeks to develop simple, inexpensive, and rapid DNA assays that can be applied by non-experts to identify unknown species. ARS researchers at Madison, Wisconsin, tested the ability of three different DNA barcoding marker genes to determine accurately the species identity for a diverse set of 72 wild potato species. None of the three genes were very accurate at distinguishing or serving as markers for species boundaries for these wild potatoes. Consequently, these results serve as a warning against relying solely on DNA barcoding genes to identify highly diverse plant species successfully. [Also contributes to Anticipated Product 3.]

Spooner, D.M. 2009. DNA barcoding will frequently fail in complicated groups: an example in wild potatoes. American Journal of Botany. 96:1177-1189.

Testing the values of taxonomy and ecogeography for predicting the utility of germplasm. ARS researchers at Madison, Wisconsin, tested the ability of taxonomy and ecogeographical data to statistically predict the presence of a valuable trait (host plant resistance to white mold of potatoes) in collections of potato germplasm accessions. No consistent association was observed between white mold resistance and the taxonomy, place of collection, or environmental factors associated with potato accessions. Consequently, in this case, taxonomic relationships and ecogeographical data cannot reliably predict where additional sources of white mold resistance genes will be found in potato germplasm collections. These results alert curators regarding the limits to extrapolating from such data, and they also highlight the need for conducting additional tests of predictive value for other agronomic traits, and in other crops.

Spooner, D.M., Jansky, S.H., Simon, R. 2009. Tests of taxonomic and biogeographic predictivity: resistance to multiple disease and insect pests in wild relatives of cultivated potato. Crop Science. 49:1367-1376.

COMPONENT 2: Crop Informatics, Genomics, and Genetic Analyses

Crop genetics and genomic improvement today have been transformed by revolutionary developments in plant genomics, computational biology, high-speed Internet connections and the plummeting costs of data storage and retrieval. The genetic and genomic information and resources generated by ARS scientists and their collaborators, augmented by germplasm and associated information furnished by the National Plant Germplasm System and its GRIN database (See Component 1) have enhanced crop genetic research and breeding programs globally. This transformation is the product, in no small part, of the vision, innovation, and dedicated efforts of ARS scientists and staff.

The research accomplishments of NP 301 Component 2 between 2006 and 2011 have resulted in historic changes for crop research. Some of the historic contributions by ARS scientists, in collaboration with university and government laboratory scientists are highlighted below.

Figure 3. The publication of the corn genome sequence in Science was an historic accomplishment for plant science and agriculture.

- *Maize genome sequenced.* ARS scientists, together with researchers at 31 other institutions, assembled and annotated the first whole genome sequence for the maize inbred B73. This landmark accomplishment was supported by the National Corn Growers Association, National Science Foundation, NIFA, DOE, and ARS.
- *Soybean genome sequenced.* ARS scientists, together with the DOE Joint Genome Institute, the Hudson-Alpha Genome Sequencing Center, and 15 other institutions, assembled, and annotated the first whole genome sequence for soybean Williams 82. This historic achievement was supported by the United Soybean Board, DOE, NSF, and ARS.
- *Whole genome analysis of global germplasm diversity for major crops including rice, soybean, maize, grape, and others.* ARS researchers developed high throughput genotyping platforms – DNA arrays and genotyping by sequencing – to determine the genetic and molecular basis of genetic variation for target traits. (See also the examples in Component 1, Problem Statement 1B).
- *Crop databases connect crop research communities with rapidly expanding volume of information for crop traits, genetics, genes, and genomes.* ARS has developed “knowledgebase systems” for maize, soybean, wheat, rice, and other crops that link decades of historical and contemporary genetic and trait information with data generated by next-generation sequencing technologies.
- *New software tools for complex trait analysis.* New software and research tools were devised by ARS researchers and are accessible through the ARS crop genetics and genome databases for calculating, analyzing, and graphically visualizing DNA sequences, population structure, trait associations, and plant evolutionary patterns.

- *Genetic analysis of complex traits through genome wide association analysis and quantitative trait locus (QTL) mapping.* ARS genetic research has dramatically enlarged the breeder's toolbox of trait loci that can accelerate progress in crop improvement.
- *New resources for crop genetics and functional genomics.* ARS scientists generated new recombinant inbred lines (RILs), fast neutron mutant populations, and TILLING mutant populations that dramatically enhanced the discovery of new target trait loci and target trait genes.

In Component 2, the research accomplishments address two broad problem statements as outlined in the NP 301 Action Plan. Selected accomplishment highlights are described in greater detail below.

Problem Statement 2A: ***Genome Database Stewardship and Informatics Tool Development***

A genetic and genomic database system that serves individual crop research communities – as well as enable~~s~~ scientists to interrogate and analyze information across crops and model plants – is critical for accelerating and optimizing crop genetic improvement. As leaders in devising novel digital applications for crop genetics, ARS scientists have the responsibility to enhance the ARS crop databases and develop information toolboxes for breeders, geneticists, and genomicists through innovative and multidisciplinary collaborations. The portfolio of ARS crop genetics and genomic databases must integrate genetic, physical, and sequence maps and genetic diversity analyses, enabling rapid mapping of target traits. Maintained and enhanced by a dedicated community of ARS curators and external advisors, it must serve as a resource for constructing gene models, sequence annotations, and supporting experimental and expression data. Consequently, crop researchers can more efficiently and effectively identify gene and genome structures and functions, and apply that knowledge to crop improvement. This will enable researchers to build on genetic advances in one crop to accelerate genetic gain in others.

The research projects that contribute accomplishments to Problem Statement 2A must coordinate their work with research projects from other NP 301 research components, and also with the Interagency Working Group on Plant Genomes (IWG-PG) and the crop genomics research communities. The ARS crop databases for plant genetics and genomics must develop important new resources, powerful research tools, and the capacity to manage plant genetic, genomic, and trait information generated in the field, in the laboratory, or in silico. In addition, these projects have supported the training of many students and postdoctoral fellows who have continued to conduct research in industry, academia, and national laboratories.

The NP 301 Action Plan included six anticipated products for this problem statement, which are focused on the needs and priority problems of crop researchers and producers. In this problem statement, the accomplishments are not assigned to a specific anticipated product because many of them contribute simultaneously to several products.

Anticipated Products in Action Plan:

1. Interconnected and interoperable databases.
2. Single points of access (portals) to multiple databases.
3. Long-term maintenance of reliable genetic, genomic, and phenotypic description data sets achieved via active data curation.
4. Easy-to-use public interfaces to data repositories.

5. Facile private interfaces to data repositories to aid curators in their work.
6. Software and data analysis tools that enable the analysis of genetic and genomic data sets.

PROBLEM STATEMENT 2A: ACCOMPLISHMENT OVERVIEW

ARS scientists have made significant contributions in elucidating (via genome sequencing, mapping, and identification methods) the structure and function of a broad spectrum of crop genomes. These contributions have also enhanced the U.S. national capacity for crop genomic and bioinformatic research. Whenever genetic repertoire, gene order, gene content, and gene function are conserved across crops, genomic information and technology developed for one crop can be applied to simultaneously improve closely or distantly related crops. Some of the major scientific impact and benefits of the ARS' contributions under this problem statement include:

- The ARS portfolio of crop genetics and genomics databases has provided long-term support for the collection, maintenance, and analysis of reliable genetic, genomic, and phenotypic description datasets.
- ARS crop databases have been heavily subscribed resources, domestically and globally, and have benefitted and enhanced basic and applied research programs, whether government, university, or private.
- The ARS crop databases have contributed key information and analytical tools for genetic improvement of crops and fundamental plant science research.
- Information and data are linked across the crop databases via trait and phenotype ontologies, germplasm identifiers, genotype data, and expression data.
- Information and tools have been professionally curated and made accessible to the research community without restriction.

PROBLEM STATEMENT 2A: SELECTED ACCOMPLISHMENTS

ARS researchers have served as chief architects for the U.S. portfolio of crop genome databases. The ARS crop databases have played a key role in providing universal access to crop genome sequences, gene models and annotations, and advanced software for analyzing public domain genomic datasets. Crop breeding has benefited from ARS strategic investments in genetic resources, the ready availability of genome sequences, and the development of analytical tools to understand how gene sequence variation determines variation in crop traits. As summarized in Table 1, the ARS crop databases are on track to host more than 500,000 visitors in 2011. For the purpose of this report, a visitor is a repeat Web site visitor who accesses multiple pages of the database. The system is highly utilized internationally; 56 percent of visitors originate from outside the United States. However, it is also a broad-based resource for the U.S. research community as well (44 percent of visitors).

ARS Genomics and Genetics Databases	Projected total visitors for 2011 based on January - July statistics
Visitors per Year	545,649
Domestic Visitors	242,595
Other Visitors	303,052

Table 1. Combined visitor statistics for the ARS genetics and genome databases including SoyBase, MaizeGDB, Gramene, GrainGenes, Legume Information System (LIS), and PLEXdb indicate a strong international interest.

The six major ARS databases – SoyBase, MaizeGDB, Gramene, GrainGenes, Legume Information System, and PLEXdb – and the maize-specific database Panzea provide critical support for genetic research and germplasm improvement (Table 2).

ARS Database Name and Web link	Primary Crop Research Communities Served	Interlinked To The Databases Listed Below
SoyBase	Soybean, Legumes	LIS; PlantGDB; Phytozome
Legume Information System (LIS)	Soybean, Legumes	SoyBase; JCVI Medicago truncatula genome browser; Noble Foundation Mt hapmap browser; Lotus japonicus genome browser (Miyakogusa); Phytozome; Uniprot
MaizeGDB	Maize	MaizeSequence; Maize Coop ; Gramene; PlantGDB; GRIN; Comparative Genomics database
Gramene	Rice, Maize, Grape, Sorghum, Arabidopsis, Brachypodium	MaizeSequence; MaizeGDB; GRIN; MIPS; 9 plant metabolic pathway databases;
PLEXdb	Wheat, Barley, Rice, Maize, Sugarcane, Soybean, Tomato, Poplar, Grape, Citrus, Cotton, Brachypodium, Medicago, Arabidopsis	Gramene; TAIR; SoyBase; MaizeGDB; Phytozome; MSU_RGAP
GrainGenes	Wheat, Oats, Barley, Brachypodium	WheatDB (UC Davis); PLEXdb; WGGRC (KSU); CORE;
Panzea[†]	Maize	Gramene; GRIN

Table 2. The ARS crop genetics and genomics databases are increasingly interlinked to crop genetics and genomic resources distributed globally. Information and data for traits, pathways, and genes for a growing number of crops are interlinked and include new genome sequences, new ontologies, and improved genetic maps. [†]Intended as a project specific database.

SoyBase and the Soybean Breeder's Toolbox

Integrating Genetics and Molecular Biology for Soybean Researchers

The resources and tools developed during 2006-2011 by the staff of SoyBase and the Soybean Breeder's Toolbox provide easy-to-use single points of access to multiple databases, software, and data analysis tools, the reliable, long-term maintenance of data with expert curation, and private, as well as, public data interfaces. They serve as the central repositories for genetic and genomic data and related resources for soybean, as well as an initial access point for laboratory specific Web pages and specialized data sets.

Approximately 28,000 visitors utilize the resources at SoyBase (http://soybase.org/) each year. A major update for SoyBase was completed in 2009, primarily to afford public access to the Williams 82 soybean genome sequence. The data and analytical tools are regularly updated by the Soybase curatorial staff. Access to the full genome sequence via the easy-to-use soybean genome browser interface has enabled more rapid progress for soybean breeding, soybean genetics, and functional genomics

During the last 5 years, SoyBase added many new or enhanced features, including:

- Improved maps and genome sequences – Genetic maps, genomic resources, and trait mapping results are now anchored to the soybean physical map and whole William 82 genome sequence and annotations (see http://soybeanbreederstoolbox.org/).
- SoyCyc – This module contains the first Soybean Metabolic Pathway Database that enables researchers to apply *ab-initio* methods to locate soybean genes mapped to metabolic reactions and pathways.
- Soybean Ontologies – SoyBase Soybean Ontologies (http://soybase.org/ontology.php) were developed with the soybean research community to furnish a controlled vocabulary for soybean field growth stages (Whole Plant Growth Ontology), plant structure names (Structure Ontology), development (Development Ontology), and plant traits (Trait Ontology), thereby enhancing the interconnections and interoperability of SoyBase with other plant genome databases.
- SoySeq – This soybean RNA sequence atlas (http://soybase.org/soyseq/index.php) enables high resolution analysis of whole genome-level gene expression thoughout 14 stages of soybean growth and development.
- Analytical Tools – SoyBase users can now readily search *Glycine max* and *Glycine soja* genome sequences, soybean EST library sequences, SoyChip annotations, and SoyCyc for sequence information (http://soybase.org/tools.php).
- QTL – 30 years of soybean trait information, including 1,423 QTL records, have been described in SoyBase, linked to the soybean genome sequence, genetic maps, genetic marker sets (33,000 SSR markers), and fast neutron mutants that are mapped to the genome sequence.

Grant, D.M., Nelson, R., Cannon, S.B., Shoemaker, R.C. 2009. SoyBase, The USDA-ARS Soybean Genetics and Genomics Database. Nucleic Acids Research. Doi: 10.1093/nar/gkp798.

Gessler, D.D., Schiltz, G.S., May, G.D., Avraham, S., Town, C.D., Grant, D.M., Nelson, R. 2009. SSWAP: A Simple Semantic Web Architecture and Protocol for Semantic Web Services. BMC Bioinformatics. 10:309doi:10.1186/1471-2105-10-309.

MaizeGDB
Maize Genetics and Genomics Database

A key resource for maize researchers, MaizeGDB is a long-term repository and resource for genetic, genomic, and phenotypic information and datasets that are carefully curated by an expert team of ARS staff, with input from the maize communities' working group. During the last 5 years, MaizeGDB (www.maizegdb.org) has become an increasingly interconnected and interoperable database system for delivering genetic, molecular, and biological information about maize (corn). The improvements developed by ARS scientists (Maize GDB and Gramene, see below) and Washington University Genome Center has yielded easy-to-use single points of access to multiple databases, software, and data analysis tools, and has combined expert curation with reliable, long-term maintenance of data. An average of about 42,000 visitors has utilized resources at MaizeGDB each year. These scientists are located in more than 100 countries and access.

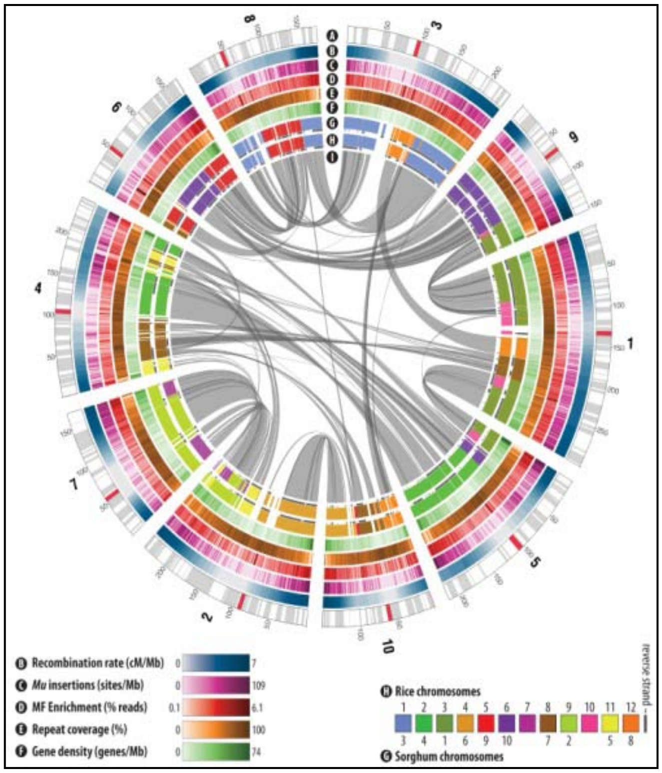


Figure 4. An illustration of the maize B73 genome showing several aspects of the genome, including gene content, repeat content, genetic recombination, methyl-filtration, cross-species orthology, and centromere localization.

Some of the new and enhanced features at MaizeGDB developed during the last 5 years include:

- Maize B73 genome sequence – The most recently annotated corn B73 genome reference sequence was released and has been delivered by MaizeGDB (www.maizesequence.org). MaizeGDB enables the research community to contribute genome annotations, and delivers tutorials instructing maize researchers on applying MaizeGDB tools and resources.
- Maps – Maize genetic and physical maps are integrated with the whole genome sequence for B73.
- Mutants – Catalog of diverse maize mutants (interconnected with GRIN; www.ars-grin.gov).
- MaizeCyc – This biochemical pathway database links maize gene sequences to metabolic enzymes and pathways.
- Ontologies – This database facilitates data searches across plant genome databases.
- Analytical Tools – New ZeAlign tool enables researchers to map query sequences as a batch onto maize chromosomes; the POPcorn portal (http://popcorn.maizegdb.org/main/index.php) provides single point access and data retrieval from 109 searchable projects and 134 searchable resources. The Locus Lookup Tool will identify the best available chromosomal coordinates and hence the candidate genes for a given locus name.
- Information about the holdings of the maize stock center, mutant collections, and phenotype descriptions (including linkage to ARS' germplasm database GRIN; see Component 1).
- An international repository for breeding data (e.g. from CIMMYT).

Lawrence, C.J., Harper, E.C., Schaeffer, M.L., Sen, T.Z., Seigfried, T.E., Campbell, D.A. 2008. MaizeGDB: The Maize Model Organism Database for Basic, Translational, and Applied Research. International Journal of Plant Genomics. 2008:496957.

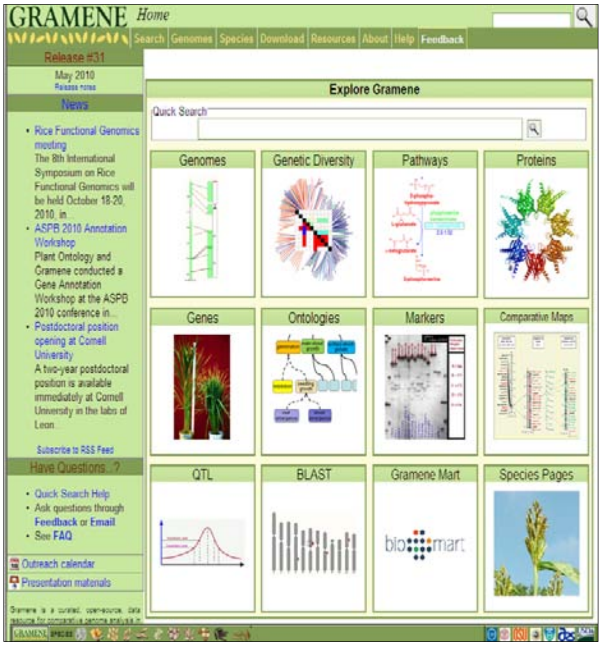
Sen, T.Z., Andorf, C.M., Schaeffer, M.L., Harper, E.C., Sparks, M., Duvick, J., Brendel, V., Cannon, E., Campbell, D.A., Lawrence, C.J. 2009. MaizeGDB Becomes Sequence-centric. Database: The Journal of Biological Databases and Curation. doi: 10.1093/database/bap020. p.1.

Sen, T.Z., Harper, E.C., Schaeffer, M.L., Andorf, C.M., Seigfried, T.E., Campbell, D.A., Lawrence, C.J. 2010. Choosing a genome browser for a model organism database (MOD): surveying the Maize Community. Database: The Journal of Biological Databases and Curation. doi: 10.1093/database/baq007. p.1.

GRAMENE

Gramene (www.gramene.org), a key public database for comparative plant genomics, emphasizes cereal species. It has enabled plant researchers to better understand how crop genes and genomes determine crop phenotypes and traits, by creating automated and curated relationships between genome sequences and biological information, thereby facilitating access to and analysis of information within and across crops and model species. It was developed and has been maintained by ARS scientists and staff, together with collaborators from Cornell University, Cold Spring Harbor Laboratory, Oregon State University, and Ensembl Genomes, Cambridge, United Kingdom. In addition to support from ARS, funding from NSF and NIFA has been critical for Gramene's evolution into a preeminent bioinformatic tool during the last 5 years.

During this period, Gramene has developed into an exceptionally interconnected and interoperable database system that provides easy-to-use single points of access to multiple databases, software, and data analysis tools. Approximately 135,000 visitors utilize the resources at Gramene each year. It has become an indispensable information resource for research on crops such as maize, sorghum, poplar, and grape, plus model plants *Arabidopsis* and *Brachypodium*. It has enabled cross-species homology relationships to be determined, drawing on information derived from genomic and EST sequencing projects; protein structure and function analysis; genetic and physical mapping; interpretation of biochemical pathways, gene, and QTL localization; and descriptions of phenotypic characters and mutations.



- Rice Functional Genomics meeting
  The 8th International Symposium on Rice Functional Genomics will be held October 18-20, 2010, in...
- ASPB 2010 Annotation Workshop
  Plant Ontology and Gramene conducted a Gene Annotation Workshop at the ASPB 2010 conference in...
- Postdoctoral position opening at Cornell University
  A two-year postdoctoral position is available immediately at Cornell University in the labs of Leon...

- Quick Search Help
- Ask questions through Feedback or Email
- See FAQ

Gramene is a curated, open-source, data resource for comparative genome analysis in...

Figure 5. The Home page of the Gramene Web site offers easy access to the various modules.

A key to Gramene's success has been strong partnerships with plant- and crop-specific databases, including The Arabidopsis Information Resource (TAIR), Legume Information System (LIS), SoyBase and the Soybean Breeders Toolbox (see information above), Munich Information Center for Protein Sequences (MIPS), and GRIN.

Gramene currently supports 12 database modules – Genomes, Genetic Diversity, Pathways, Proteins, Genes, Ontologies, Markers, Comparative Maps, QTL, Blast, Gramene Mart, Species Pages, and associated tools – for 20 plant species. The most widely used modules include:

- Genetic Diversity – The GD module has stored information regarding genotypes, phenotypes, and test environments. It has served as a launch point for analyzing the preceding data sets. New analytical tools provided by this module include SNP Query, TASSEL, GDPC browser, and Flapjack (from the Scottish Crops Research Institute)
- Pathways – This module have housed the pathway databases RiceCyc, MaizeCyc, SorghumCyc, and BrachyCyc that has enabled genes to be mapped to respective reactions and pathways, interspecific comparisons between the pathways. Omics Viewer and Comparative Analysis tools have been delivered, and mirrors of pathway databases for nine other species have also been provided.
- Ontologies – Gramene is a member of the Gene Ontology Consortium (www.geneontology.org/). The ontologies at Gramene have grown to now include Plant Ontology (PO), Trait Ontology (TO), Gene Ontology (GO), and Environmental Ontology (EO). Gramene's taxonomy ontology (GR tax) has provided a collective resource of structured and controlled vocabularies for associating crop and plant knowledge sets with plant biological structures, processes, phenotypes, and traits.
- Gene – A curated resource, this module has provided information about a broad spectrum of plant genes. It includes descriptions of genes and their alleles associated with morphological, developmental, and agronomically important phenotypes, variants of physiological characters, biochemical functions, and isozymes.
- Genomes – A joint project between Gramene and Ensembl Genomes at the European Bioinformatics Institute (EBI), Cambridge, United Kingdom, this module contains 20 genome databases including rice, corn, sorghum, grape, poplar, *Brachypodium*, *Arabidopsis*, and *Physcomitrella* with updated assembly and gene annotations from Gramene and the community.

Researchers can access whole genome alignments through Gramene to explore the conservation of chromosomal structure, gene structure, and gene function. Specifically, Gramene provides pre-computed whole genome and gene–gene alignments through a BLASTZ-net (www.gramene.org/info/docs/compara/analyses.html#blastz) pairwise whole genome alignment method implemented by Ensembl.

Gramene delivers a Java Web Start-enabled version of the TASSEL (Trait Analysis by Association, Evolution and Linkage) software program for evaluating trait associations, patterns of linkage disequilibrium, and genetic diversity. TASSEL was originally developed by the Maize Diversity project (see Panzea). All diversity data are available in various download formats including HapMap and PLINK, to enable users to analyze those data with their own bioinformatic tools.

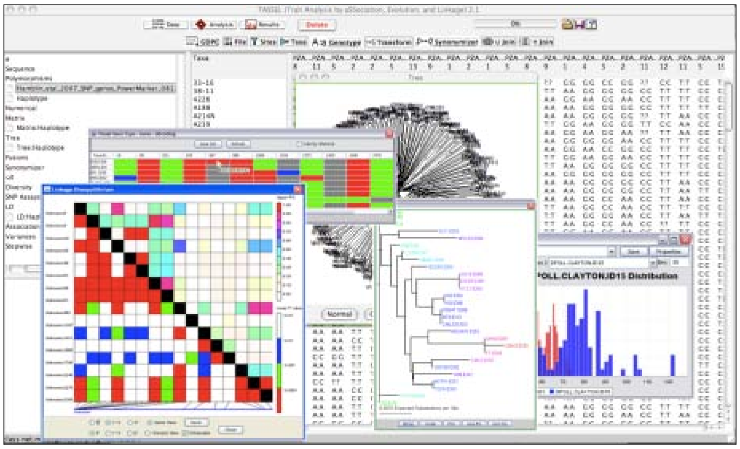


Figure 6. TASSEL is a software package for evaluating trait associations, evolutionary patterns, and calculating and graphically visualizing linkage disequilibrium.

Liang,C., Jaiswal,P., Hebbard,C., Avraham,S., Buckler,E.S.,Casstevens,T., Hurwitz,B., McCouch,S., Ni,J., Pujar,A. et al. (2008). Gramene: a growing plant comparative genomics resource. Nucleic Acids Res., 36 (Database issue), D947–D953.

Youens-Clark, K., Buckler, E., Casstevens, T., Chen, C., DeClerck, G., Derwent, P, Dharmawardhana, P., Jaiswal, P., Kersey, P., Karthikeyan, A., Lu, J., McCouch, S., Ren, L., Spooner, W., Stein, J., Thomason, J., Wei, S., and Ware, D. 2010. Gramene database in 2010: updates and extensions. Nucleic Acids Research, 39 (Database issue). D1085–D1094.

Youens-Clark, K., Faga, B., Stein, L., Ware, D. 2009. CMap 1.01: a comparative mapping application for the internet. Bioinformatics. 25(22):3040-3042.

GrainGenes (http://wheat.pw.usda.gov/) hosts a suite of datasets and bioinformatic tools that serve the Triticeae (barley and wheat) and oat research communities. An average of approximately 300,000 visitors each year access GrainGenes for its databases, publications, bioinformatic tools, Web sites, community announcements, and curatorial assistance. GrainGenes delivers genetic and physical maps; genome and gene transcript sequences and annotations; data analysis and data mining tools; and data and community resources, as does SoyBase and MaizeGDB.

- Maps – Collated interactive genetic maps for wheat (diploid, tetraploid, hexaploid), barley, oat, rye, rice, and sugarcane. The Wheat Composite map integrates 12 genetic maps and 3,700 loci (www.genica.net.au/cmap/crcmpb-live/cgi-bin/cmap/viewer). Information browsers include CMap, GBrowse, and Binmap Viewer.
- Wheat DB – A physical map of the *Aegilops tauschii* genome, the diploid wheat progenitor that contributed the wheat D genome, is being constructed (http://wheatdb.ucdavis.edu/wheatdb/). The physical map of *Ae. tauschii* has been integrated through a common set of markers with other wheat genetic maps in GrainGenes. Wheat DB has also facilitated genome-wide SNP discovery for *Ae. Tauschii*.
- SQL Interface – This module provides direct access to the GrainGenes Database via SQL queries.
- Genetic Markers – Sequence probes, primers, and genes with links to genetic maps. GrainGenes is home to new SNP and SSR genotyping and phenotyping data for wheat, barley, and oats, including new SNP and SSR genotyping data for five wheat mapping populations from the Wheat CAP project.
- Genotyping and Phenotyping Toolbox – GrainGenes is host to The Hordeum Toolbox (http://hordeumtoolbox.org /) and the The Avena Toolbox (TAT) (http://avena.pw.usda.gov/tat/) ensuring long-term availability of resources and information for barley and oat breeders and researchers. TAT is the Web portal for data generated by the Cooperative Oat Research Enterprise (CORE) (see Component 3).
- Trait Data – GrainGenes has housed 15 years of crop evaluation results including the Uniform Early and Midseason Oat Performance Nurseries, 1994 – 2007; and Quaker Uniform Oat Nurseries, 1997 – 2005.

- GrainGenes provides access to 5X wheat genome sequences.

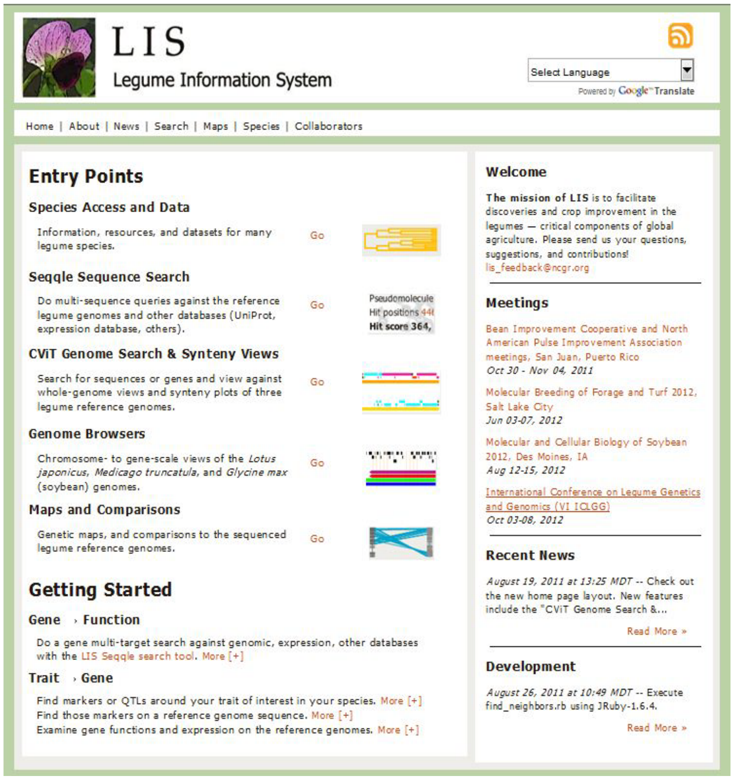
You, F.M., Huo, N., Gu, Y.Q., Luo, M., Ma, Y., Hane, D., Lazo, G.R., Dvorak, J., Anderson, O.D. 2008. BatchPrimer3: A high throughput web application for PCR and sequencing primer design. BMC Bioinformatics. 9:253.

You, F.M., Luo, M.C., Gu, Y.Q., Lazo, G.R., Thomas, C., McGuire, P.E., Dvorak, J., and Anderson, O.D. 2007. Genoprofiler: Automated processing of high throughput capillary fingerprinting data. Bioinformatics. 23:240-242.

LIS
Legume Information System

Legume crops – soybean, alfalfa, common bean, lentils, chickpea, pigeonpea, fava bean, and more – are crucial for global food security and human nutrition. Yet, few legume crop researchers have access to the genomic information required to sustain a modern breeding program. In response to that need, ARS and collaborators at the National Center for Genome research (NCGR) have developed the Legume Information System (LIS) (www.comparative-legumes.org/) – which serves as both a long-term data repository and a portal that interlinks the genetic and genomic resources for important legume crops and model species.

The enhancements to LIS during the last 5 years have resulted in a dramatic increase in usage to 17,000 visitors in 2011. The legume research community has availed itself of the genome browsers for the model legumes *Lotus japonicus* and *Medicago truncatula*, the "Seqqle" sequence search tool, comparative genetic/genomic map viewers, and a powerful new search tool that integrates results from three reference legume species and from other genomic resources.



Entry Points

Species Access and Data

Information, resources, and datasets for many legume species.

Seqqle Sequence Search

Do multi-sequence queries against the reference legume genomes and other databases (UniProt, expression database, others).

CViT Genome Search & Synteny Views

Search for sequences or genes and view against whole-genome views and synteny plots of three legume reference genomes.

Genome Browsers

Chromosome- to gene-scale views of the *Lotus japonicus*, *Medicago truncatula*, and *Glycine max* (soybean) genomes.

Maps and Comparisons

Genetic maps, and comparisons to the sequenced legume reference genomes.

Getting Started

Gene › Function

Do a gene multi-target search against genomic, expression, other databases with the LIS Seqqle search tool. More [+]

Trait › Gene

Find markers or QTLs around your trait of interest in your species. More [+]
Find those markers on a reference genome sequence. More [+]
Examine gene functions and expression on the reference genomes. More [+]

Welcome

The mission of LIS is to facilitate discoveries and crop improvement in the legumes — critical components of global agriculture. Please send us your questions, suggestions, and contributions!
lis_feedback@ncgr.org

Meetings

Bean Improvement Cooperative and North American Pulse Improvement Association meetings, San Juan, Puerto Rico
Oct 30 - Nov 04, 2011

Molecular Breeding of Forage and Turf 2012, Salt Lake City
Jun 03-07, 2012

Molecular and Cellular Biology of Soybean 2012, Des Moines, IA
Aug 12-15, 2012

International Conference on Legume Genetics and Genomics (VI ICLGG)
Oct 03-08, 2012

Recent News

August 19, 2011 at 13:25 MDT -- Check out the new home page layout. New features include the "CViT Genome Search &...

Development

August 26, 2011 at 10:49 MDT -- Execute find_neighbors.rb using JRuby-1.6.4.

Figure 7. The mission of LIS is to facilitate discoveries and crop improvement in the legumes — critical components of global agriculture.

With the advent of cheaper sequencing technologies, legume research has increasingly focused on generation of full genome sequences (available now for three legume genomes, with more to follow in 2012). Other sequence-based datasets are expected to include transcriptome assemblies, low-coverage genome re-sequencing efforts, and large SNP datasets. These datasets, from many diverse legumes, are often analyzed in comparison to the sequenced reference genomes. In 2009-2010, LIS was redesigned in anticipation of increased demand for resources and tools to analyze new legume datasets, including three reference genome sequences (soybean, *Medicago truncatula*, and *Lotus japonicus*).

LIS now provides powerful tools for retrieving, comparing, analyzing, and visualizing legume genetic and genomic data, gene sequence annotations, which has been collated from over 18 legume species (three with complete quality genome sequences) to serve as genomic resources for the genetic improvement of other legume crops.

Specific information tools delivered by LIS include:

- Species Access and Data – Provides information and datasets for many legume species.
- Maps and Comparisons – Genetic maps and comparisons to the sequenced legume reference genomes.
- CViT Genome Search and Synteny Views – Search for sequences or genes and view against whole-genome sequences and synteny plots of three legume reference genomes.
- Genome Browsers – Provides chromosome-to-gene-scale views of the *Lotus japonicus*, *Medicago truncatula*, and *Glycine max* (soybean) genomes.
- Gene to Function/Seqqle Sequence Search – Enables multi-sequence queries to be performed against the reference legume genomes and other databases (UniProt, expression database, and others).
- Trait to Gene – Find markers or QTLs associated with a trait of interest in a specific species; locate those markers on a reference genome sequence; and examine information for gene functions and expression from the reference genomes.

Severin, A.J., Peiffer, G., Xu, W.W., Hyten, D.L., Bucciarelli, B., O'Rourke, J.A., Grant, D.M., Farmer, A.D., May, G.D., Vance, C.P., Shoemaker, R.C., Stupar, R.M., Bolon, Y. 2010. An integrative approach to genomic introgression mapping. Plant Physiology. DOI:10.1104/pp.110.158949.

Cannon, S.B, May, G. D., and Jackson, S.A. 2009. Three Sequenced Legume Genomes and Many Crop Species: Rich Opportunities for Translational Genomics. Plant Physiol. 151: 970-977. doi:10.1104/pp.109.144659

New high-throughput sequencing technologies have transformed how scientists survey gene expression, discover genes, and annotate genomes. Nonetheless, there are few easy-to-use bioinformatic tools to analyze and share gene expression data with the research community following publication, or to update gene annotations. ARS scientists in Ames, Iowa, and colleagues at Iowa State University (with funding from ARS and NSF), created PLEXdb (Plant Expression Database) (www.plexdb.org), a unified gene expression resource for plants and plant pathogens, to meet the need for advanced bioinformatic tools.

The primary goal of PLEXdb is to integrate under one Web site genomics data and bioinformatics tools that have been accessible only from disparate sources. Without this integration, gene expression data would need to be downloaded from an independent repository site; checked for quality; mapped to genes; and those genes would need to be mapped to genomic locations and orthologs in other species. Software for expression data analysis and for viewing associated data would need to be installed locally; and methods to post-process results would be required. PLEXdb accomplishes these many different tasks through a single Web interface that is easily accessible to any researcher within two or three clicks from the PLEXdb front page. Most students or even faculty lack the resources and expertise to develop their own comparative analysis pipelines, and have relied on PLEXdb for gene expression annotation and analysis tools.

With PLEXdb, researchers can apply insights from model plants like *Arabidopsis* or *Brachypodium*, to genomic analyses of crops such as barley, wheat, rice, corn, poplar, soybean, grape, tomato, and others (14 in total, so far). Researchers can also investigate "incompletely annotated" genes in less well-characterized genomes to quickly determine how the homologs in model plants behave under diverse experimental conditions.

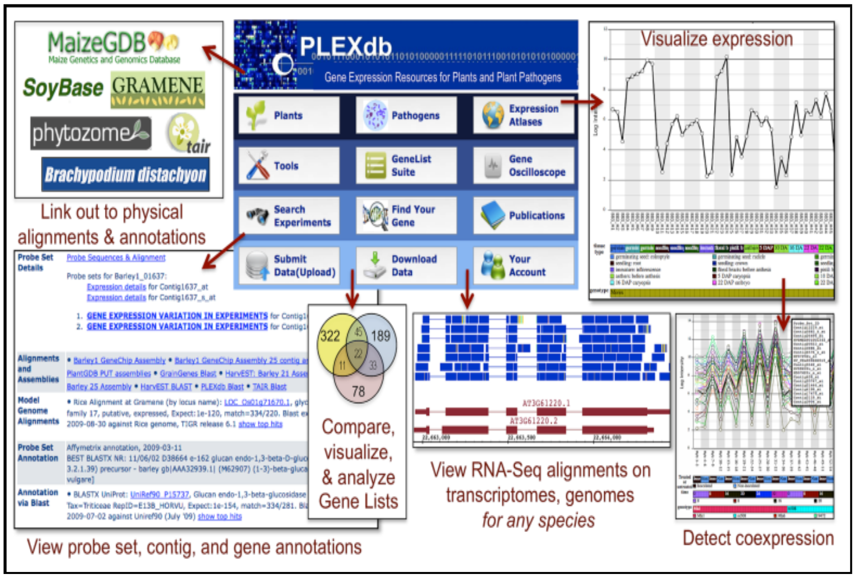


Figure 8. PLEXdb Expression Based Annotation Resource features one-stop shopping. Shown here are just a few of the example linkouts to community genome browsers, and visualization and annotation resources within two to three clicks from the PLEXdb entry portal.

PLEXdb, in partnership with crop community databases, is currently the only public site that supports comparisons of gene expression patterns across multiple plant species. It enables individual users and/or consortia to upload their own data sets and then compare them to previously published results prior to publication to interpret the structure, function, and regulation of genes in economically important plants. Thus, PLEXdb serves as an interconnected and interoperable database system with easy-to-use single points of access to multiple databases, software, and data analysis tools; reliable, long-term maintenance of data with expert curation; and that provides for private data, as well as public data interfaces.

Since the end of 2006, the number of public experiments in PLEXdb whose results are publicly available grew 10-fold to 390 (446 total) and hybridizations grew 7-fold to 8,850 (11,103 total). Each hybridization experiment contains from 20 to 50,000 genes per organism.

More than 1,600 registered users from more than 60 countries have downloaded data over 26,000 times for analysis, including more than 15,000 in 2010. The number of new registered users has increased steadily: 240 joined in 2007, 250 more joined in 2008, and 300 signed up in 2009 and again in 2010. There also have been many anonymous (unregistered) users – typically 500-800 visits per day – demonstrating the value the scientific community places on this Web resource.

The Panzea Web site (www.panzea.org) has hosted databases and bioinformatic tools associated with the Maize Diversity Project, bioinformatics tools from the Genetic Architecture of Maize and Teosinte Project, and TASSEL, a Java software package to evaluate trait associations, evolutionary patterns, and linkage disequilibrium. The Panzea Web site (with funding from ARS and NSF) had over 454,671 page requests from August 2010 to July 2011. Roughly 65 percent of the database users are domestic and 35 percent international, and more than one-third of domestic users, roughly 19 percent of the total, are from seed industry. Finally, 200 users have regularly downloaded the entire database and have implemented it locally, so that usage is not reflected in these statistics. The Maize Diversity Project database, constructed by ARS researchers and colleagues from the University of Wisconsin and Cornell University, contains information for more than a million genetic markers developed by the project and for custom maize germplasm resources developed for QTL, association, and fine mapping analyses. Panzea also includes phenotypic data for that germplasm.

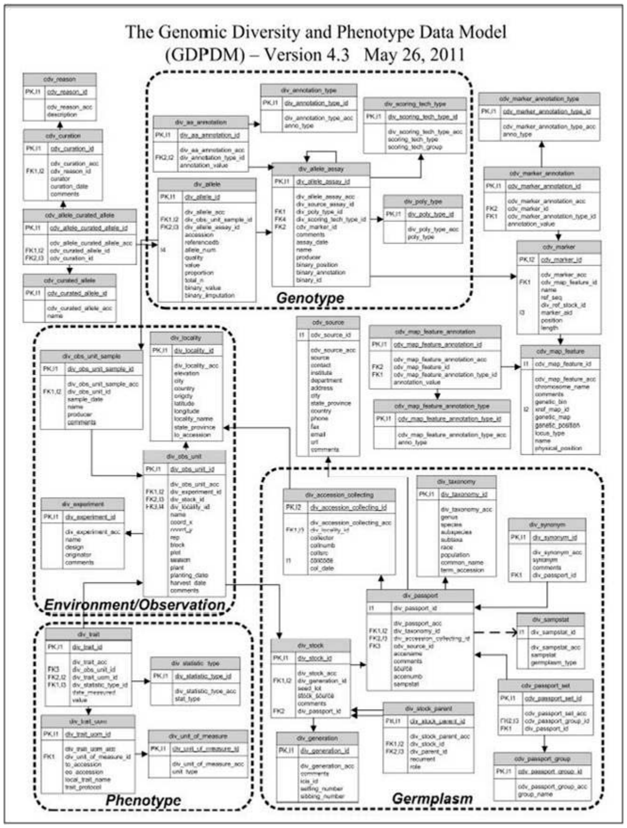


Figure 9. Genomic Diversity and Phenotype Data Model (GDPDM) used to store information on phenotypes, genotypes, germplasm enhancement, and association data.

Panzea also houses bioinformatics tools generated by the Genetic Architecture of Maize and Teosinte Project which has dissected the genetic architecture of complex traits in maize – such as flowering time, plant height, and kernel quality – to characterize allelic series at these genes and examine their epistatic and environmental interactions, with the ultimate goal of predicting phenotype from genotype.

As mentioned earlier, Panzea is also home to TASSEL, a public Java software package to evaluate trait associations, evolutionary patterns, and linkage disequilibrium. Since its release, TASSEL has been downloaded over 21,000 times (different versions) and for one recent 14-month period there were 3,000 different users of the compiled version of the program.

Thus, Panzea is an interconnected and interoperable database system that has provided easy-to-use single points of access to multiple databases, software, and data analysis tools; the reliable, long-term maintenance of data with expert curation; and private, as well as public data interfaces. Information and resources delivered by Panzea enable researchers to advance the molecular breeding of maize by facilitating detailed genetic dissection of key agronomic traits and/or genomic selection.

Zhang, Z., Buckler IV, E.S., Casstevens, T., Bradbury, P. 2009. Software engineering the mixed model for genome-wide association studies on large samples. Briefings in Bioinformatics. 10(6):664-675.

Buckler IV, E.S., Myles, S., Peiffer, J., Brown, P., Ersoz, E., Zhang, Z., Costich, D. 2009. Association Mapping: Critical Considerations Shift from Genotyping to Experimental Design. The Plant Cell. 10.1105/tpc.109.068437

Tomato Functional Genomics Database. The Tomato Functional Genomics Database (TFGD) enables tomato researchers to store, query, analyze, visualize, and integrate large-scale tomato functional genomic data sets. During the last 5 years, this database has been substantially expanded from the previous Tomato Expression Database by including metabolite profiles as well as large-scale tomato small RNA (sRNA) data sets. Computational pipelines have been developed to process and deliver microarray, metabolite, and sRNA data sets archived in the database. The TFGD has also enabled efficient information retrieval through a set of query interfaces and analytical tools, including improved array probe annotations, tools to identify co-expressed genes, and associated biological processes and biochemical pathways. The suite of tools and interfaces in TFGD enable more efficient data mining of recently released, and continually expanding, large-scale tomato functional genomics data sets. Funding for the TFGD comes from ARS and NSF.

Fei, Z., Joung, J., Tang, X., Zheng, Y., Huang, M., Lee, J., Tieman, D., Alba, R., Klee, H., Giovannoni, J.J. 2010. Tomato Functional Genomics Database (TFGD): a comprehensive collection and analysis package for tomato functional genomics. Nucleic Acids Research. D1156-1163.

Joung, J., Corbett, A., Fellman, S., Tieman, D., Klee, H., Giovannoni, J.J., Fei, Z. 2009. Plant MetGenMAP: an integrative analysis system for plant systems biology. Plant Physiology. 151:1758-1768.

PROBLEM STATEMENT 2B: *Structural Comparison and Analysis of Crop Genomes*

The research conducted by ARS to address this problem statement has focused on understanding how genetic variation is determined by crop genome, sequence variation, and by genome structure. Rapid improvements in DNA sequencing technology and technologies for the detection of sequence variants such as single nucleotide polymorphisms (SNPs), copy number variants (SSRs), and insertion/deletions have resulted in dramatic improvements in plant genotyping. These tools in turn enabled ARS scientists and collaborators to analyze crop diversity and population structure, genetically map and analyze target traits, and determine the molecular basis for genetic variation. The benefit of the accomplishments in Problem Statement 2B include new knowledge, tools, and strategies for improved utilization of genetic diversity, improved crop germplasm and varieties, and genetically-improved populations for breeding.

The NP 301 Action Plan included six anticipated products for this problem statement that are focused on the needs and priority problems of crop researchers and producers. Accomplishments that illustrate those products for this problem statement are described following the list of anticipated products.

ANTICIPATED PRODUCTS IN ACTION PLAN:

1. Detailed genetic maps and markers for important agronomic genomes.
2. Integrated genetic, physical, and cytogenetic maps.
3. Improved tools for genetic and genomic analysis of complex genomes, including polyploid species.
4. BAC libraries with extensive ordered and oriented contig assemblies.
5. Annotated gene sequences for candidate gene location.
6. Custom designed oligonucleotide microarrays for alignment of genomic structure.

PROBLEM STATEMENT 2B: ACCOMPLISHMENT OVERVIEW

ARS scientists have been at the forefront of developing key genetic tools for crop genomics and genetics and breeding. The following examples represent a sampling of their accomplishments, including vastly improved information and tools for genetic and genomic analyses; SNP chips and DNA microarrays; and annotated sequences for candidate gene discovery that have yielded, and are expected to continue to yield, landmark advances toward improving disease and pest resistance, increased tolerance to abiotic stresses, and improved end-product quality in varieties and germplasm for U.S. agriculture. The integration of genetic, physical, and cytogenetic maps for rice, maize, wheat, and soybean, which have been key to many of these accomplishments, has been discussed previously in Problem Statement 2A.

Notable selected highlights for this problem statement include:

- China rice germplasm genotyped with second-generation DNA sequencing;
- USDA soybean germplasm genotyped with a new generation of SNP detection platforms;
- New markers and genetic maps developed for specialty crops;
- Oat SNP marker toolkit that drove breakthrough achievements for breeding; and
- Identification of plant regulatory sequences and regulatory networks.

PROBLEM STATEMENT 2B: SELECTED ACCOMPLISHMENTS

Anticipated Product 1: *Detailed genetic maps and markers for important agronomic genomes.*

Rice germplasm from China genotyped. Uncovering the genetic basis of agronomic traits in crop landraces that have adapted to various agro-climatic conditions is important to world food security. ARS scientists in Ithaca, New York, working with scientists from China, identified approximately 3.6 million SNPs by sequencing 517 rice landraces and constructed a high-density haplotype map of the rice genome through a novel data-imputation method. This information has served as a fundamental resource for rice genetics research and breeding. This accomplishment complements the ARS rice germplasm genotyping effort detailed in Problem Statement 1B, Anticipated Product 7.

Huang, X., Wei, X., et al. 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nature Genetics 42, 961–967.

Genetic map developed for melon (*Cucumis melo*). *Cucumis melo* L. (melon) is a highly diverse species. Melon fruits are highly variable in shape, size, rind form, firmness, color, and flavor. A genetic map of melon enriched for fruit traits was constructed, from a recombinant inbred population developed from a cross between representatives of the two subspecies of melon. Altogether, 44 fruit QTLs were identified including QTLs for fruit sugar content, particularly sucrose – the major sugar affecting sweetness in melon fruit. The recombinant inbred population provided an important new resource for further genomics and metabolomics studies in melon, as well as markers for breeding for fruit quality.

Harel-Beja, R., Portnoy, V., Fei, Z., Giovannoni, J.J., Ori, N., Lewinsohn, E., Tadmor, Y., Schaffer, A., Katzir, N. 2010. A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes. Theoretical and Applied Genetics 121, 511-533.

Clepet, C., Joobeur, T., Zheng, Y., Jublot, D., Huang, M., Truniger, V., Boualem, A., Hernandez-Gonzalez, M.E., Dolcet-Sanjuan, R., Portnoy, V., Mascarell-Creus, A., Caño-Delgado, A.I., Katzir, N., Abdelhafid Bendahmane, A., Giovannoni, J.J., Aranda, M.A., Garcia-Mas, J., and Fei, Z. 2011. Analysis of expressed sequence tags generated from full-length enriched cDNA libraries of melon. BMC Genomics 12, 252.

Sequencing the watermelon genome and development of new DNA markers for genetic improvement of watermelon. Years of selecting for fruit quality in watermelon has resulted in a narrow genetic base among cultivars that makes the crop vulnerable to diseases and pests. Thus, there is a need to develop discriminating genetic and genomic resources that facilitate the identification and incorporation of resistance genes into watermelon cultivars. The heirloom watermelon cultivar ‘Charleston Gray’ was selected for genome sequencing, 72 percent of which has been annotated. New DNA sequences that exist in high frequency in actively expressed genes of watermelon have proven highly efficient in distinguishing closely related watermelon cultivars and in the development of genetic linkage maps for watermelon. The developing watermelon gene sequence and information about the HFO-TAGs are available on the International Cucurbit Genomic Initiative Web site (www.icugi.org) and should aid in the overall effort to breed for improved watermelon cultivars.

Levi, A., Wechter, W.P., Massey, L.M., Carter, L., Hopkins, D. 2011. Genetic Linkage Map of Citrullus lanatus var. Citroides Chromosomal Segments Introgressed into the Watermelon Cultivar Crimson Sweet (Citrullus lanatus var. lanatus) Genome. American Journal of Plant Sciences. 2:93-110.

Levi, A., Wechter, W.P., Harris-Shultz, K.R., Davis, A.R., Fie, Z. 2010. High-frequency Oligonucleotides in Watermelon Expressed Sequenced Tag-unigenes Are Useful in Producing Polymorphic Polymerase Chain Reaction Markers among Watermelon Genotypes. Journal of the American Society for Horticultural Science. 135:369-378.

Anticipated Product 2: *Integrated genetic, physical, and cytogenetic maps.*

The ARS crop databases described in Problem Statement 2A provides extensive information and access to integrated map resources – genetic, physical and cytogenetic for a total of 36 crops.

Maize genome sequenced. ARS scientists, together with the Genome Center at Washington University and 31 other institutions, sequenced, assembled, and annotated the first whole genome sequence for the maize inbred B73. This was an historic achievement for the maize research community, for plant biology, and for the seed industry given the size (2.3 billion base pairs), complexity (32,000 genes), and highly repetitive nature (85 percent composed of transposable elements) of the maize genome. The B73 genome sequence has enhanced the power of maize genetics and maize breeding dramatically by enabling the discovery and mapping of several million SNP markers through second-generation DNA sequencing, and subsequent genome-wide analysis of highly divergent maize germplasm. At the same time, the maize genome sequence provided new and exciting insights into key agronomic traits and plant processes, including hybrid vigor, flowering time, drought tolerance, disease resistance, C4 carbon fixation, crop domestication, genome structure, and genome evolution, to name but a few. Moreover, the integration of the maize B73 whole genome sequence with maize genetic maps developed over several decades has enabled maize geneticists to identify many important new control genes and QTLs that have resulted in new tools for crop improvement. The impacts of this landmark accomplishment are also described in Component 1 and Component 3. The maize genome sequencing project was supported by ARS, the National Corn Growers Association, NSF, NIFA, and DOE.

Schnable, P.S., Ware, D., Fulton, R.S., Stein, J.C., Wei, F., Pasternak, S. et al. 2009. The B73 maize genome: complexity, diversity, and dynamics. Science 326, 1112-1115.

Soybean genome sequenced. ARS scientists, together with the DOE Joint Genome Institute, the Hudson-Alpha Genome Sequencing Center, and 15 other institutions, sequenced, assembled, and annotated the first whole genome sequence for soybean (variety Williams 82). This landmark achievement for the soybean research community has dramatically enhanced the power of soybean genetics, functional genomics, and soybean breeding by enabling the discovery and mapping of 46,000 protein-coding genes (70 percent more than *Arabidopsis)* and 50,000 SNP markers through subsequent genome-wide analysis of selected soybean varieties and wild accessions. Sequencing the soybean genome has resulted in new and exciting insights into the genetic bases for key soybean traits including resistance to disease and pests (soybean rust, soybean cyst nematode, and aphids), enhanced seed oil and protein composition, enhanced oxidative stress tolerance, and mineral deficiency tolerance. Moreover, the soybean genome sequence is now integrated with over 30 years of data covering 90 different traits through SoyBase and the Soybean Breeder's Toolbox that enable soybean geneticists to identify important new genes for disease resistance and quantitative trait loci that have resulted in important new tools for crop improvement. The various impacts of this landmark accomplishment are also described in Component 1 and Component 3. The soybean genome sequencing project was supported by ARS, United Soybean Board, DOE, NIFA, and NSF.

Schmutz, J., Cannon, S.B., Schlueter, J., Ma, J., Hyten, D.L., Song, Q., Mitros, T., Nelson, W., May, G.D., Gill, N., Peto, M.F., Shu, S., Goodstein, D., Thelen, J.J., Cheng, J., Sakurai, T., Umezawa, T., Shinozaki, K., Du, J., Bhattacharyya, M., Sandhu, D., Grant, D.M., Joshi, T., Libault, M., Zhang, X., Hguyen, H., Valliyodan, B., Xu, D., Futrell-Griggs, M., Abernathy, B., Hellsten, U., Berry, K., Grimwood, J., Yu, Y., Wing, R.A., Cregan, P.B., Stacey, G., Specht, J., Rokhsar, D., Shoemaker, R.C., Jackson, S. 2010. Genome Sequence of the Paleopolyploid Soybean (Glycine max (L.) Merr.). Nature. 463:178-183.

Maps and genetic markers for breeding cacao (*Theobroma cacao L.*). Cacao, the source of chocolate, is a multi-billion dollar international commodity grown by several million small farmers in tropical developing nations. Threatened by several virulent diseases and damaging pests, cacao requires new tree types with inherent resistance to pests and diseases, plus high yields and fine cocoa quality. Cacao breeders have lacked the number of genetic markers required to rapidly select trees with desired traits at the seedling stage, rather than at maturity. ARS and Mars, Inc. researchers in Miami, Florida, and their cooperators, identified thousands of genetic markers and mapped them to their chromosomal locations. These new genetic tools for geneticists and breeders have increased the efficiency and effectiveness of cacao genetic improvement projects, to benefit farmers and cocoa processors globally. They also generated key information in support of the early stages of the recently completed cacao genome sequencing project. (See www.cacaogenomedb.org/main.)

Livingstone, D., Motamayor, J.C., Schnell II, R.J., Cariaga, K.A., Freeman, B.L., Meerow, A.W., Brown, J.S., Kuhn, D.N. 2010. Development of Single Nucleotide Polymorphism markers in Theobroma cacao and comparison to Simple Sequence Repeat markers for genotyping of Cameroon clones. Molecular Breeding. DOI 10.1007/s1 1032-010-9416-2

Brown, J.S., Phillips-Mora, W., Power, E.J., Krol, C.A., Cervantes Martinez, C., Motamayor, J., Schnell II, R.J. 2007. Mapping QTL for resistance to frosty pod and black pod diseases, and for horticultural traits in Theobroma cacao L.. Crop Science. 47:1851-1858.

Schnell II, R.J., Kuhn, D.N., Brown, J.S., Tondo, C.T., Motamayor, J.C. 2007. Development of marker assisted selection program for cacao. Journal of Phytopathology. 97(12):1664-1669.

Faleiro, F.G., Queiroz, V.T., Lopes, U.V., Guimaraes, C.T., Pires, J.L., Yamada, M.M., Araujo, I.S., Pereira, M.G., Schnell II, R.J., De Souza Filho, G.A. 2006. Mapping QTLs for Witches'Broom (Crinipellis perniciosa) Resistance in cacao (Theobroma cacao L.). Euphytica. 149:227-235

Genetic maps and markers for avocado (*Persea americana Mill.*) genetic improvement. Detailed linkage maps and genetic markers linked to key horticultural traits are especially valuable for accelerating genetic improvement of tree crops with long generation times and protracted non-reproductive juvenile phases. During the last 5 years, ARS researchers from Miami, Florida, and their cooperators, developed genetic markers for identifying the parentage and maintaining the integrity of key avocado breeding populations. They also generated one of the most detailed linkage maps assembled for this crop. These new genetic tools have already significantly accelerated progress with characterizing the genetic control of key horticultural traits for avocado breeding.

Borrone, J., Brown, J.S., Tondo, C.L., Mauro-Herrera, M., Kuhn, D.N., Violi, H., Sautter, R.T., Schnell II, R.J. 2009. An EST-SSR based linkage map for Persea americana Mill. (avocado). Tree Genetics and Genomes. 10.1007/s11295-009-0208-y

Ying, Z., Davenport, T.L., Zhang, T., Schnell II, R.J., Tondo, C.L. 2009. Selection of Highly Informative Microsatellite Markers to Identify Pollen Donors in 'Hass' Avocado Orchards. Plant Mol Biol Rep 27:374-380

Violi, H.A., Brown, J.S., Tondo, C.L., Borrone, J.W., Ploetz, R., Schnell II, R.J. 2009. Microsatellite markers reveal low breeding system efficacy and pollen contamination can limit production of full-sib avocado progeny. Scientia Horticultureae. doi:10.1016/j.scienta.2008.11.011

Cucumber mitochondrial genome sequence and genetics. Cucumber and melon are unusual in plants because the mitochondria are paternally transmitted rather than maternally transmitted as in the vast majority of plants. The mitochondrial genome sequence of cucumber, generated by ARS scientists in Madison, Wisconsin, together with university scientists from Krakow, Poland, is also one of the largest among all eukaryotes (1.5 million basepairs), due in part to the accumulation of short 20 to 60 bp repetitive DNA motifs, resulting in frequent genome rearrangements affecting the organization and expression of mitochondrial genes. The researchers discovered that passage of cucumber through cell cultures produces mtDNA rearrangements that affect expression of specific genes and allow for mutational analyses of the mtDNA. Additionally, a unique nuclear locus (Psm) was identified that controls the sorting of paternally transmitted mtDNAs. Recently, ARS scientists in Madison, Wisconsin, and Ithaca, New York, in collaboration with scientists at 16 other institutes, released a draft sequence of the cucumber genome. Comparative sequencing and mapping of cucumber and melon placed Psm on linkage group R of cucumber and G10 of melon. Eventual cloning of Psm should provide insights on the role of nuclear genes in controlling the prevalence of specific mtDNAs, which are imperative for optimal plant performance.

Huang, S., Li, R., Zhang, Z., Li, L., Gu, X., et al. 2009. The Genome of the Cucumber, Cucumis Sativus L. Nature Genetics 41, 1275-1281.

Bartoszewski, G., Gawronski, P., Szklarczyk, M., Verbakel H, Havey MJ. 2009. A one-megabase physical map provides insights on gene organization in the enormous mitochondrial genome of cucumber. Genome 52, 299-307.

Anticipated Product 3: *Improved tools for genetic and genomic analysis of complex genomes, including polyploid species.*

Identification of regulatory sequences in plant genomes. ARS scientists at Cold Spring Harbor, New York, together with scientists from Duke University, University of California-Davis, University of Massachusetts, and the University of Cambridge, United Kingdom, combined three approaches: high spatial resolution gene expression experiments; yeast one-hybrid (Y1H); and yeast two-hybrid (Y2H) assays to delineate a subset of interactions occurring within a plant gene regulatory network that determines tissue-specific transcription factor and microRNA expression in plants. Their data showed that upstream transcription factors are expressed in more diverse cell types than are their targets, and that promoters that are bound by a relatively large number of transcription factors correspond to key developmental regulators. Although molecular phenotypes were identified for 65 percent of the transcription factors, morphological phenotypes were associated with only 16 percent. Thus, gene regulatory network activity is robust, and phenotype level changes may be canalized or buffered.

Brady, S.M., Zhang, L., Megraw, M., Martinez, N.J., Jiang, E., Yi, C.S., Liu, W., Zeng, A., Taylor-Teeples, M., Kim, D., Ahnert, S., Ohler, U., Ware, D., Walhout, A.J.M., Benfey, P.N. 2011. Molecular Systems Biology 7, 459.

Anticipated Product 4: *BAC libraries with extensive ordered and oriented contig assemblies.*

The ARS crop databases highlighted in Problem Statement 2A provide extensive access to ordered and oriented sequence contigs and sequence scaffolds, including BAC libraries. For example, researchers can access scaffolds, contigs, and ordered and oriented BACs for the maize and soybean genomes via the genome browsers in SoyBase and MaizeGDB, and that information has been incorporated into the maize and soybean genetic, physical, and DNA sequence maps on the Web sites. Information on sequence scaffolds, contigs, and BACs for rice, *Medicago truncatula*, and *Lotus japonicus* are accessible via Gramene and the Legume Information System as well.

Anticipated Product 5: *Annotated gene sequences for candidate gene location.*

The ARS crop databases highlighted in Problem Statement 2A contain excellent examples of annotated gene sequences ARS researchers have generated for a growing number of crops. They link back to the highly annotated and updated reference genome sequences for corn, soybean, rice, *Arabidopsis*, sorghum, poplar, *Medicago truncatula*, and lotus. The type of information available includes gene structure, protein structure and function, gene expression, linked traits, metabolic pathways, and orthologous and paralogous genes.

Anticipated Product 6: *Custom designed oligonucleotide microarrays for alignment of genomic structure.*

Universal soybean SNP linkage panel. ARS scientists in Beltsville, Maryland; Raleigh, North Carolina; and Ames, Iowa, together with collaborators from university and international laboratories, generated 1,536 genetically mapped SNP DNA markers, referred to as the Universal Soy Linkage Panel 1.0 (USLP 1.0), for genotyping soybeans rapidly. More than 6,000 soybean plants from populations developed by soybean breeders in 12 states were analyzed with these markers. Genotype data from those samples have defined the genome positions of genes controlling resistance to soybean rust, soybean cyst nematode, iron deficiency chlorosis, level of oleic and linolenic acid, and seed protein concentration in soybean oil. The markers were very important for anchoring sequence scaffolds during the assembly of the soybean genome DNA sequence. USLP 1.0 markers can be viewed in soybean map version 4.0 at SoyBase.

Hyten, D.L., Choi, I., Song, Q., Specht, J.E., Carter Jr, T.E., Shoemaker, R.C., Hwang, E., Matukumalli, L.K., Cregan, P.B. 2010. A high density integrated genetic linkage map of soybean and the development of a 1,536 Universal Soy Linkage Panel for QTL mapping. Crop Science. 50:960-968.

SoySNP50 chip for analyzing soybean germplasm diversity. 50,000 soybean SNPs were selected from the genome sequences of six cultivated soybean varieties and one wild soybean accession by ARS scientists in Beltsville, Maryland, with collaborators at the University of Maryland, College Park, to develop the Illumina Infinium iSelect SoySNP50 chip. Each SNP was mapped onto a soybean chromosome by comparison to the Williams 82 whole genome sequence. ARS researchers are genotyping with the SNP chip more than 10,000 accessions from the ARS Soybean Germplasm Collection representing 30 years of soybean trait analysis and development, genotypic variations has been associated with variations in yield, seed composition, nutrient use efficiency, flowering time, abiotic stress tolerance, pest and disease resistance (85 traits total). This information is being deposited in SoyBase as the analysis is completed.

Song, Q., Quigley, C.V., Jia, G., Cregan, P.B., Hyten, D.L. 2011. Development and evaluation of a SoySNP50 iSelect Infinium assay. Plant & Animal Genomes XIX Conference.

Genomic variation in soybean Williams 82. ARS scientists in Beltsville, Maryland, and St. Paul, Minnesota, with scientists from the University of Minnesota and Roche Nimblegen, Inc., analyzed the genetic basis of intracultivar variation for Williams 82. Williams 82 individuals exhibited variation in the number and size of several regions of the William 82 reference genome that originated from Kingwa, a donor parent used to introduce root rot resistance into the variety. The documentation of structural differences, at the genome sequence level, between individuals that belong to a soybean inbred crop accession is consistent with observed phenotypic variation. This information is useful for interpreting soybean genomic data sets and highlights important considerations for research communities that are developing or utilizing a reference genome sequence.

Haun, W.J., Hyten, D.L., Xu, W.W., Gerhardt, D.J., Albert, T.J., Richmond, T., Jeddeloh, J.A., Jia, G., Springer, N.M., Vance, C.P. and Stupar, R.M. 2011. The composition and origins of genomic variation among individuals of the soybean reference cultivar Williams 82. Plant Physiology 155, 645-655.

The Collaborative Oat Research Enterprise for the genetic improvement of oats. ARS scientists from Aberdeen, Idaho, enlisted scientists from Australia, Brazil, Canada, Israel, Norway, Spain, Sweden, United Kingdom, and the United States to found the Collaborative Oat Research Enterprise (CORE), a new global research partnership, committed to the genetic improvement of oats. (Web site: www.ars.usda.gov/Main/docs.htm?docid=20666) The scientists assayed 6,000 validated oat SNP markers to estimate the genetic diversity in 34 diverse oat genotypes. Shared genetic and molecular resources led to the identification of genes associated with beta-glucan content and crown rust resistance in North America, and for Fusarium head blight resistance in Norway. The added value from marker-assisted breeding will benefit oat growers, millers, and food processors.

Oliver, R.E., G.R. Lazo, J.D. Lutz, M.J. Rubenfield, N.A. Tinker, J.M. Anderson, N.H. Wisniewski-Morehead, D. Adhikary, E.N. Jellen, P.J. Maughan, G.L. Brown-Guedira, S. Chao, A.D. Beattie, M.L. Carson, H.W. Rines, D.E. Obert, J.M. Bonman, E.W. Jackson. 2011. Model SNP development for complex genomes based on hexaploid oat using high-throughput 454 sequencing technology. BMC Genomics 12, 77.

Sorghum recombinant inbred mapping population. ARS scientists in Lubbock and College Station, Texas, and Griffin, Georgia, together with university scientists, developed and released a sorghum recombinant inbred mapping population derived from a cross between IS3620C and

BTx623. 137 members of this 430 member mapping population served as the basis for the construction of landmark sorghum high-density genetic and physical maps, with the integrated genome map subsequently used to aid in assembly of the sorghum genome sequence. Since its public release in 2009, this population has served as an important resource for mapping key agronomic traits in sorghum including tillering, floral architecture, plant height, flowering date, grain size, and abiotic stress tolerance. [This accomplishment also contributes to Anticipated Product 1]

Burow, G.B., Klein, R.R., Franks, C.D., Klein, P.E., Schertz, K.F., Pederson, G.A., Xin, Z., and Burke, J.J. 2011. Registration of the BTx623/IS3620C recombinant inbred mapping population of sorghum. Journal of Plant Registrations. 5(1): 141-145.

PROBLEM STATEMENT 2C: *Genetic Analyses and Mapping of Important Traits.*

The goal of this problem area is to translate knowledge of how key crops traits are genetically controlled to accelerate the crop breeding needed to ensure an ample and high quality supply of food, feed, fiber, and feedstocks for renewable energy. The comparison and analysis of crop genomes through the ARS portfolio of crop databases and genetic resources have resulted in innovations in molecular marker development and marker detection technologies by ARS scientists and their collaborators for many major crops.

The NP 301 Action Plan included three anticipated products for this problem statement that are focused on the priority needs of crop researchers and producers. A sample of the accomplishments that have contributed to addressing those challenges follows the list of anticipated products.

ANTICIPATED PRODUCTS IN ACTION PLAN:

1. New theory and strategies for mapping traits.
2. Trait mapping populations and genetic stocks for gene and allele identification, such as near-isogenic lines, recombinant inbred lines, transposon knockouts, and targeting induced local lesions in genomes (TILLING) resources.
3. Genes controlling key traits and associated markers will be identified.

PROBLEM STATEMENT 2C: ACCOMPLISHMENT OVERVIEW

Teams of ARS scientists and their collaborators made significant advances during 2006-2011 with plant genotyping, trait gene identification, and genetic analysis and mapping of complex traits. In particular, genome-wide association studies (GWAS) and QTL mapping combined with second generation DNA sequencing – strategies developed and refined by ARS scientists and their collaborators – revolutionized the genetic dissection of plant complex traits. As a consequence, previously untapped sources of genetic diversity, developed and maintained by the National Plant Germplasm System described in Component 1, can be better incorporated into crop breeding programs. Furthermore, more precise knowledge of crop genetic architecture can accelerate progress in breeding programs.

ARS scientists also expanded the variety of genetic tools available for basic research and breeding. For example, new mutant populations were generated and made publicly available for soybean, rice, and sorghum. By combining mutant populations, such as the soybean fast neutron mutants, with genome-wide genotyping technologies, such as comparative genome hybridization or second generation sequencing, and incorporating the phenotypic and genotypic information into the ARS crop databases, researchers can more easily determine if a mutant exists for their candidate genes of interest, and that mutant’s altered phenotype.

Those new integrated genetic and genomics resources enabled ARS to discover important new genes that control high-value traits during 2006-2011. For example, new genes were discovered that coordinate plant growth and development by microRNAs, control cell wall production and composition, and can enable crop production to expand northward by controlling physiological responses to photoperiod.

PROBLEM STATEMENT 2C: SELECTED ACCOMPLISHMENTS

Anticipated Product 1: *New theory and strategies for mapping traits.*

Genome-wide association studies combined with next-generation sequencing for complex rice trait analysis. ARS scientists at Ithaca, New York, together with scientists from Cornell University, Michigan State University, and from six research institutes in China, combined next-generation sequencing and genome-wide association studies to analyze 14 agronomic traits in rice including tiller number, heading date, drought tolerance, seed shattering, grain size, spikelet number, and amylose content for rice subspecies that have adapted to various agro-climatic conditions. The loci identified approximately 36 percent of the phenotypic variance, on average, to demonstrate that integrating second-generation genome sequencing and genome-wide association studies represents a complementary strategy to classical biparental cross-mapping for dissecting complex traits in rice.

Huang, X., Wei, X., et al. 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nature Genetics 42, 961–967.

Genome-wide association studies combined with QTL locus mapping reveal genetic architecture for rice aluminum tolerance. ARS scientists in Ithaca, New York, in collaboration with Cornell University scientists, genetically characterized aluminum tolerance in rice with genome-wide association studies and QTL mapping combined. Forty eight regions associated with aluminum tolerance were identified by genome-wide association studies analysis, many of them new, most of which were subpopulation-specific. QTL mapping identified rare and/or subpopulation-specific alleles not detected by genome-wide association studies analysis. This work demonstrates the potential for exploring new statistical genetic approaches for exploiting the potential of phenotypically inferior lines in trait development.

Famoso, A.N., Zhao, K., Clark, R.T., Tung, C.W., Wright, M.H., Bustamante, C., Kochian, L.V., Susan R. McCouch, S.R. 2011. Genetic Architecture of Aluminum Tolerance in Rice (*Oryza sativa*) Determined through Genome-Wide Association Analysis and QTL Mapping. PLoS Genet 7, e1002221.

Anticipated Product 2: *Trait mapping populations and genetic stocks for gene and allele identification, such as near-isogenic lines, recombinant inbred lines, transposon knockouts, and targeting induced local lesions in genomes (TILLING) resources.*

Identifying rare mutations in populations. ARS researchers in Davis, California, working with scientists at the University of California, Davis, developed a new method for detecting rare mutations in mutagenized populations of rice and wheat based on second-generation DNA sequencing of target genes amplified from pooled template DNAs. Discovery of rare mutant alleles was efficient both in rice and wheat providing high sensitivity and specificity.

Tsai, H., Howell, T., Nitcher, R., Missirian, V., Watson, B., Ngo, K.J., Lieberman, M., Fass, J., Uauy, C., Tran, R.K., Khan, A.A., Filkov, V., Tai, T.H., Dubcovsky, J. and Comai, L. 2011. Discovery of rare mutations in populations: TILLING by sequencing. Plant Physiology 156, 1257-1268.

20,000 fast neutron mutants for soybean functional genomics. Soybean deletion mutants that display phenotypic variation in seed composition, maturity, morphology, pigmentation, roots, and nodulation are now available from ARS researchers in St. Paul, Minnesota. Fatty acid composition data are available for 5,000 lines. This mutant collection is an important addition to the resources available through SoyBase and Soybean Breeder's Toolbox.

Bolon, Y.E., Haun, W.J., Xu, W.W., Grant, D.M., Stacey, M.G., Nelson, R., Gerhardt, D.J., Jeddeloh, J.A., Stacey, G., Muehlbauer, G.J., Orf, J.H., Naeve, S.L., Stupar, R.M., Vance, C.P. 2011. Phenotypic and genomic analyses of a fast neutron mutant population resource in soybean. Plant Physiology 156, 240-253.

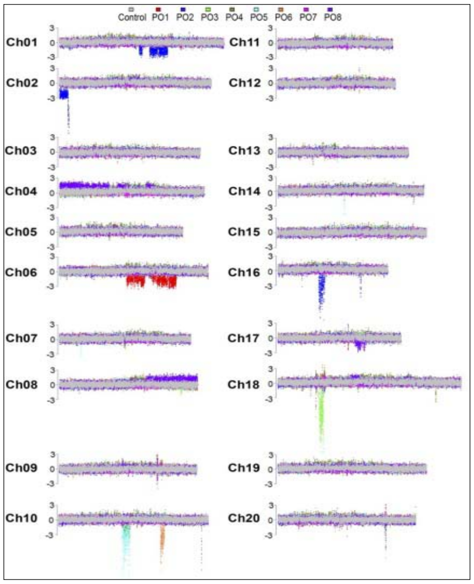


Figure 10. Analysis of copy number variation in eight soybean fast neutron mutants that display altered seed protein and oil mutants (PO) by comparative genomic hybridization.

Anticipated Product 3: *Genes controlling key traits and associated markers will be identified.*

Sorghum maturity locus gene cloned. *maturity1 (ma1)* is the major gene that permits the transition from vegetative growth to flowering under long-day conditions in grain sorghum. ARS scientists in College Station, Texas, together with university and industry scientists, cloned the wild-type (tropical) *ma1* gene, along with naturally occurring sequence variants that enable flowering under long-day conditions in temperate latitudes. These sequences are now applied to molecular and genetic screening of tropical sorghum germplasm for photoperiodic response, and for marker-assisted selection for this important trait in sorghum grain and biofuel research programs. ARS breeders are utilizing m*a1* as a marker for conventional breeding to adapt ARS tropical sorghum germplasm accessions to temperate latitudes, and thus enhance its utility to sorghum breeders seeking desirable traits.

Murphy, R.L., Klein, R.R., Morishige, D.T, Brady, J.A., Rooney, W.L., Miller, F.R., Dugas, D.V., Klein, P.E., and Mullet, J. E. 2011. Coincident light and clock regulation of pseudoresponse regulator protein 37 (PRR37) controls photoperiodic flowering in sorghum. Proc. Natl. Acad. Sci. USA. Accepted for publication**.**

Race specific resistance to rice blast decoded. Blast disease is a major yield limitation for rice production worldwide. ARS researchers in Stuttgart, Arkansas, discovered that in 15 rice cultivars one domain of the Pi-km gene is consistent across cultivars, and another domain that is highly variable and associated with resistance to different races of the blast pathogen. DNA markers for this domain have been exploited by breeders to stack resistance genes in new cultivars, thus reducing the need for applying fungicides.

Costanzo, S. and Jia, Y. 2010. Sequence variation at the rice blast resistance gene *Pi-km* locus: Implications for the development of allele specific markers. Plant Science 176, 523-530.

Sorghum gene identified that enhances biofuel production. The production of cellulosic biofuels from sorghum is restricted, in part, by technical barriers in breaking down the lignin. ARS researchers in Lincoln, Nebraska, and their colleagues, have identified a key gene for lignin synthesis, Bmr6, that encodes the enzyme cinnamyl alcohol dehydrogenase (CAD) in sorghum. Protein models for CAD helped explain how the mutations impact enzyme structure and thus lignin production in sorghum.

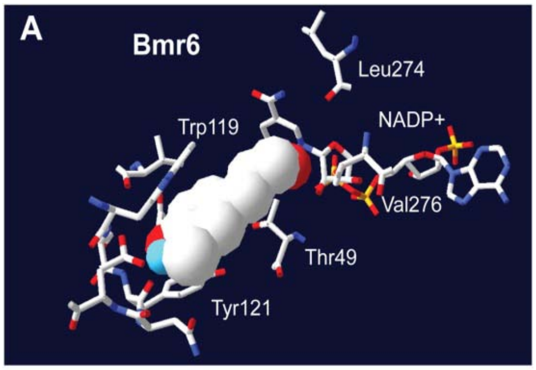


Figure 11. Predicted protein structure of sorghum (CAD) cinnamyl alcohol dehydrogenase protein Bmr6 containing the bound substrate coniferyl aldehyde.

Sattler, S.E., Saathoff, A.J., Haas, E.J., Palmer, N.A, Funnell-Harris, D.L., Sarath, G., and Pedersen, J.F. 2009. A nonsense mutation in a cinnamyl alcohol dehydrogenase gene is responsible for the sorghum *brown midrib6* phenotype. Plant Physiology150, 584–595.

Vacuolar acid invertase gene activity determines potato tuber quality. Cold storage of potatoes, followed by high-temperature processing, can result in elevated amounts of acrylamide, a neurotoxin and potential carcinogen. ARS scientists at Madison, Wisconsin, with international and university collaborators, demonstrated that silencing the potato vacuolar acid invertase gene *VInv* prevents reducing sugar accumulation in cold-stored tubers. Thus, processing quality and acrylamide problems in potato can be controlled effectively by suppression of the *VInv* gene through biotechnology or by targeted breeding.

Bhaskar, P.B., Wu, L., Busse, J.S., Whitty, B.R., Hamernik, A.J., Jansky, S.H., Buell, C.R., Bethke, P.C. and Jiang, J. 2010. Suppression of the vacuolar invertase gene prevents cold-induced sweetening in potato. Plant Physiology 154, 939-948.

MicroRNAs govern maize ear and tassel production. ARS scientists in Albany, California, together with scientists from industry, cloned *corngrass1* (c*g1*), the maize gene that governs the switch from juvenile to reproductive development in maize, and showed that it encodes two tandem *miR156* encoding genes. MicroRNA 156, in turn, regulates the maize domestication genes *tga1* and *mir172*, a microRNA gene that controls juvenile development. The *cg1* gene can either prolong or shorten juvenile development in maize, and thus vegetative biomass production.

Chuck, G., Cigan, M., Saeteurn, K., and Hake, S. 2007. The heterochronic maize mutant . results from overexpression of a tandem microRNA. Nature Genetics 39, 544-549.

Chuck, G., Meeley, R., Irish, E., Sakai, H. and Hake, S. 2007. The maize *tasselseed4* microRNA controls sex determination and meristem cell fate by targeting *Tasselseed6/indeterminate spikelet1.* Nature Genetics 39, 1517 – 1521.

Sex-chromosome evolution analyzed in strawberry. ARS researchers in Beltsville, Maryland, and colleagues at the University of Pittsburgh, applied QTL analysis to identify, in a wild progenitor of the cultivated strawberry, genomic regions associated with sex determination and other traits associated with femaleness and maleness. Their findings suggest that an autosomal chromosome is destined to become the sex-determining chromosome, simply because of the co-location on that chromosome of genes controlling many different reproductive functions, such as pollen number. This research demonstrated that traits associated with maleness and femaleness, but not necessary to actual reproductive function, have been associated through evolution, almost by chance, on the same genomic region. This study reshapes accepted dogma and will be of value for understanding genome evolution, particularly sex evolution, as well as the genetic control of horticultural traits, such as disease resistance, of strawberry and other crops with similarly highly polyploid genomes.

Spigler, R.B., Ashman, T., Lewers, K.S. 2010. Genetic architecture of sexual dimorphism in a subdioecious plant with a proto-sex chromosome. Evolution. DOI: 10.1111/j.1558-5646.2010.01189.x.

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COMPONENT 3: Genetic Improvement of Crops

Crop production in the United States is challenged by constraints to production efficiencies; emerging threats of diseases, pests, and environmental extremes; rapidly shifting consumer needs and preferences; expanding demand for biofuel feedstocks; and the need to ensure that crop products are healthy and of the highest quality. Research under this component must integrate genetic raw materials (Component 1) and new genetic and genomic knowledge (Component 2) with traditional and novel genetic enhancement and breeding approaches to produce the genetic stocks, enhanced germplasm, and superior crop varieties that underpin U. S. agriculture. The integrated research conducted under this component must transform unadapted, unimproved germplasm into more usable forms essential for breeders to rapidly generate superior cultivars and advanced breeding stocks. It must devise new ways to access genes from these unadapted sources by developing novel fundamental knowledge bases and new methods for genetic analyses to reduce variety development time.

The research conducted under this component has generated evaluation information to help commercial breeders deploy genes from ancestral and crop wild relatives by pre-breeding to eliminate undesirable traits. In doing so, ARS scientists have developed new genomic tools, such as more effective marker-assisted breeding approaches and revolutionary new statistical genetic and genomic methods. ARS scientists have put these tools and information to good use by developing a host of new varieties and germplasm lines for a full spectrum of U.S. crops – from *Allium* (onions, garlic) to *Zea* (maize). For a complete list of germplasm and variety releases, see Appendix 3.

In Component 3, the research accomplishments address three broad problem statements as outlined in the NP 301 Action Plan. Selected accomplishment highlights are described in greater detail below.

PROBLEM STATEMENT 3A: ***Genetic Theory and Methods of Crop Improvement***

This problem statement focuses on developing a fundamental knowledge base about various plant genetic mechanisms, and finding efficient ways to apply them in crop improvement, including developing and/or improving the ability to use marker-assisted breeding. ARS researchers focused on new gene discovery methods which could provide better understanding of fundamental genetic mechanisms and enable all plant breeders to work more efficiently and effectively. As plant breeders searched for ways to develop varities more quickly, the need for methods to reduce number of generations by identifying genes without visual observation in the field became a top priority in most breeding programs. This triggered a need for ARS to develop techniques to assess genes at the molecular level before flowering takes place, providing information that allowed a shift in breeding programs from a purely applied mode to marker-assisted breeding.

The Action Plan identified three anticipated products which were expected from ARS research addressing the needs expressed in Problem Statement 3A. Accomplishments that illustrate those products for this problem statement follow the list of anticipated products.

ANTICIPATED PRODUCTS IN ACTION PLAN:

1. Greater knowledge of fundamental plant genetic mechanisms and how they can be applied effectively to crop genetic analyses and crop improvement.
2. Improved experimental design and enhanced ability to identify, validate, and utilize QTLs in a broad range of crop species.
3. Reliable molecular marker-assisted methods and procedures.

PROBLEM STATEMENT 3A: ACCOMPLISHMENT OVERVIEW

During the last 5 years, ARS researchers and their colleagues transformed the “genetic toolkit” available to plant breeders by developing ground-breaking, new approaches that integrate traditional genetic analyses with leading edge genomic analytical capacities. New approaches to mapping complex traits have combined association mapping with genetic mapping methods. ARS researchers developed new statistical methods that more reliably linked phenotypic genetic variation to variability in molecular markers. They developed the largest and most powerful genetic resource ever developed for a crop species (Nested Association Mapping: (NAM) population) that captures much of the diversity in the highly polymorphic maize species. That new tool and other innovations determined how genetic variation produces trait variation in maize, and has also been applied to other crops.

The preceding and many other NP 301 research accomplishments during the last 5 years have been pivotal in making marker-assisted selection practical and effective for many other crops, including soybeans, sunflowers, cucumbers, lettuce, dry beans, potatoes, and peanuts. ARS scientists have made significant advances in QTL mapping for complicated polyploid genomes, such as wheat and barley, enabling the genomic mapping for the genes conferring Ug99 stem rust resistance. Their research has also provided the basis for genomic selection, which combines powerful statistical methods with new DNA marker and sequencing technologies to predict and select the optimal genotypes from among untested germplasm lines.

From the perspectives of both accelerating the progress of genetic improvement and reducing the costs of crop breeding, ARS research has generated significant impact on fruit and tree nuts, including:

- Utilizing DNA marker (SSRs and SNPs) analyses to uncover the genetic bases for key traits of fruit rootstocks and grape vines;
- Identification of powerful genetic markers for the high-value traits of seedlessness and powdery mildew resistance in grapes, and for the stoneless trait in plums; and
- Introgression of a gene from a poplar that promoted early flowering and fruiting into plum that reduced the juvenile stage from 3-4 years to less than 1 year. This early flowering and fruiting trait has already accelerated genetic progress with breeding of plum and other tree fruits, as well as forest and woody ornamental species.

PROBLEM STATEMENT 3A: SELECTED ACCOMPLISHMENTS

Anticipated Product 1: *Greater knowledge of fundamental plant genetic mechanisms and how they can be applied effectively to crop genetic analyses and crop improvement.*

New method for dissecting the genetic bases of multi-genetic traits combines association mapping with genetic mapping approaches and genome sequencing. ARS scientists overcame the limitations to the resolution of available genetic maps by developing novel genetic resources for quantitative trait analysis – designated as nested association mapping (NAM) populations – and combining these with improved statistical approaches. The amount of genetic diversity captured by NAM populations makes them well suited for assaying the full range of phenotypes and agronomic traits (See Figure 3 on page 6). ARS researchers from Ithaca, New York; Raleigh, North Carolina; and Columbia, Missouri, with their collaborators, intercrossed diverse maize lines to produce the NAM populations that serve as a community genetic resource for analysis of genetically complex traits. The NAM approach has combined extensive phenotyping of the population in the field with genome sequencing of the parents to develop the highest resolution genetic map for any plant species. Furthermore, these researchers developed a maize haplotype map through discovering and genotyping several million sequence polymorphisms among 27 diverse maize lines. Other ARS researchers have already applied these approaches to many other plant species including wheat, barley, oats, rice and Arabidopsis.

These innovating research approaches have already led to a more comprehensive understanding of plant genetic mechanisms. For example, flowering time in maize was revealed to be controlled by 50 to 100 genes, which has important implications for incorporating the genetic diversity of tropical maize into breeding programs for temperate-adapted maize. In addition, ARS researchers found that hybrid vigor results in part because superior hybrids carry optimal gene combinations that are unlikely to occur from current breeding methods. Novel breeding schemes designed to exploit this new knowledge of gene combinations have already begun to accelerate plant breeding worldwide. [Also contributes to Anticipated Products 2 and 3.]

McMullen, M.D., Kresovich, S., Sanchez-Villeda, H., Bradbury, P., Li, H., Sun, Q., Flint Garcia, S.A., Thornsberry, J., Acharya, C., Bottoms, C., Brown, P., Browne, C.J., Eller, M.S., Guill, K.E., Harjes, C., Kroon, D., Lepak, N.K., Mitchell, S., Peterson, B.E., Pressoir, G., Romero, S.M., Oropeza Rosas, M., Salvo, S.A., Yates, H., Hanson, M., Jones, E., Smith, S., Glaubitz, J., Goodman, M., Ware, D., Holland, J.B., Buckler IV, E.S. 2009. Genetic Properties of the Maize Nested Association Mapping Population. Science. 325:737-740.

Buckler IV, E.S., Holland, J.B., McMullen, M.D., Kresovich, S., Acharya, C., Bradbury, P., Brown, P., Browne, C.J., Eller, M.S., Ersoz, E., Flint Garcia, S.A., Garcia, A., Glaubitz, J.C., Goodman, M., Haries, C., Guill, K.E., Kroon, D., Larsson, S., Lepak, N.K., Li, H., Mitchell, S.E., Pressoir, G., Peiffer, J., Oropeza Rosas, M., Rocheford, T., Romay, C., Romero, S., Salvo, S.A., Sanchez Villeda, H., Sun, Q., Tian, F., Upadyayula, N., Ware, D., Yates, H., Yu, J., Zhang, Z. 2009. The Genetic Architecture of Maize Flowering Time. Science. 325(5941):714-718.

Gore, M.A., Chia, J., Elshire, R.J., Sun, Q., Ersoz, E.S., Hurwitz, B.L., Peiffer, J.A., McMullen, M.D., Grills, G.S., Ross-Ibarra, J., Ware, D., Buckler IV, E.S. 2009. A first generation haplotype map of maize. Science. 326(5956):1115-1117.

New methods for predicting the breeding values of genotypes aid plant breeders. ARS researchers in Ithaca, New York, with colleagues at Cornell University developed new methods to strengthen and accelerate marker-assisted genetic improvement. DNA marker data from across the genome can predict agronomic performance of genotyping more accurately than use of marker genotypes alone. Acceleration of genetic improvement occurs in part through the improved prediction accuracy, and in part through reducing the breeding cycle time by avoiding the time and cost of phenotyping. This new method has spurred research into the application of genomic selection to breeding barley, wheat, oat, and maize, and feasibility studies for rice and sugarcane breeding. [Also contributes to Anticipated Products 2 and 3.]

Heffner, E.L., Lorenz, A.J., Jannink, J., Sorrells, M.E. 2010. Plant breeding with genomic selection: potential gain per unit time and cost. Crop Science. 50:1681-1690.

Zhong, S., Dekkers, J., Jannink, J. 2009. Association-Based Genomic Selection in Cultivated Barley. Genetics. 182:355-364.

Heffner, E., Jannink, J., Sorrells, M. 2011. Genomic selection accuracy using multi-family prediction models in a wheat breeding program. The Plant Genome. 4:65-75.

Early flowering genes accelerates tree fruit breeding. Tree fruit breeding is a long-term and expensive process due to the long juvenile non-flowering stage. ARS scientists from Kearneysville, West Virginia, discovered that introducing a gene from the poplar tree, which-promotes early flowering and fruiting, shortened the juvenile stage in plum from 3-4 years to less than 1 year. Trees with the genetically engineered early flowering trait are culled during the last generation of breeding, and the mature trees without the transgene are selected for further breeding. This method for producing early flowering and fruiting has been termed “FasTrack,” and can significantly accelerate the breeding of new and improved varieties of plum and other fruits, and forest and woody ornamental species. (See Figure 2 on page 5.)

Srinivasan, C., Callahan, A.M., Dardick, C.D., Scorza, R. 2010. Expression of the poplar Flowering Locus T1 (FT1) gene reduces the generation time in plum (Prunus domestica L.) [abstract]. International Horticultural Congress. p. 103.

Anticipated Product 2: *Improved experimental design and enhanced ability to identify, validate, and utilize QTLs in a broad range of crop species.*

Master regulator of enhanced Ug99 stem rust resistance discovered in barley. ARS researchers in Ames, Iowa, and their university cooperators (with support from the NSF) analyzed resistance to the Ug99 stem rust strain in barley though genome-wide expression profiling, combined with expression Quantitative Trait Locus (eQTL) mapping. They identified a “master switch” that regulates the expression of hundreds of Ug99-responsive genes and demonstrated that the genetic location of the switch also confers enhanced adult plant resistance to Ug99. Further characterization of the master switch and development of allele-specific markers can mitigate the threat of Ug99.

Moscou, M.J., N. Lauter, B. Steffenson, and R.P. Wise. 2011a. Quantitative and Qualitative Stem Rust Resistance Factors in Barley Are Associated with Transcriptional Suppression of Defense Regulons. PLoS Genetics 7(7):DOI: 10.1371/journal.pgen.1002208.

Identification and mapping of new sources of resistance to aflatoxin accumulation in maize. Maize susceptibility to ear rot and aflatoxin accumulation by Aspergillus flavus causes significant economic losses for U. S. farmers. ARS researchers from Starkville, Mississippi, identified QTLs for resistance of aflatoxin accumulation on most maize chromosomes. Individual QTLs explained 1 - 12 percent of the phenotypic variation of the trait, and markers to these QTLs were developed for marker-assisted breeding.

Warburton, M.L., Brooks, T.D., Krakowsky, M.D., Shan, X., Windham, G.L., Williams, W.P. 2009. Identification and mapping of new sources of resistance to aflatoxin accumulation in maize. Crop Science. 49:1403-1408.

Williams, W.P., Windham, G.L. 2006. Registration of maize germplasm line Mp717. Crop Science. 46:1407-1408

Finding the gene associated with sheath blight disease resistance in rice. Developing sheath blight-resistant cultivars has been difficult because complete resistance is unknown, and because resistance is conditioned by genes modulated by the environment. ARS researchers in Stuttgart, Arkansas, identified DNA markers associated with sheath blight resistance. A major QTL for sheath blight resistance was found on chromosome 9. Markers closely linked to these sheath QTLs have accelerated introduction of sheath blight resistance into new cultivars.

Liu, G., Jia, Y., Correa-Victoria, F.J., Prado, G.A., Yeater, K.M., McClung, A.M., Correll, J.C. 2009. Mapping quantitative trait loci responsible for resistance to rice sheath blight disease using greenhouse assays. Phytopathology. 99(9):1078-1084.

Jia, Y., Coarrea-Victoria, F., McClung, A.M., Zhu, L., Liu, G., Wamishe, Y., Xie, J., Marchetti, M.A., Pinson, S.R., Rutger, J.N., Correll, J.C. 2006. Rapid determination of rice cultivar responses to the sheath blight pathogen Rhizoctonia solani using a micro-chamber screening method. Plant Disease. 91:485-489.

Anticipated Product 3: *Reliable molecular marker-assisted methods and procedures.*

Discovery and evaluation of 33,065 Simple Sequence Repeat (SSR) DNA markers in soybean. ARS researchers in Beltsville, Maryland, and Ames, Iowa, screened the DNA sequences for the 20 soybean chromosomes and identified more than 33,000 SSR markers for DNA marker-assisted soybean breeding and for mapping genes on the soybean chromosomes. The researchers created a soybean SSR database (BARCSOYSSR_1.0) with the genome position and primer sequences for SSRs shown on one of the 20 soybean chromosomes. The SSR markers are accessible from SoyBase. Soybean breeders and soybean geneticists have been applying this information to select DNA markers to facilitate gene cloning or marker-assisted soybean breeding. The researchers also identified a set of 23 SNP DNA markers, located on 19 of the 20 soybean chromosomes, which rapidly and inexpensively distinguish soybean cultivars.

Song, Q., Jia, G., Zhu, Y., Grant, D., Nelson, R.T., Hwang, E., Hyten, D.L., and Cregan, P. B. 2010. Abundance of SSR Motifs and Development of Candidate Polymorphic SSR Markers (BARCSOYSSR_1.0) in Soybean. Crop Science, vol. 50:1950-1960.

M. S. Yoon, Q. J. Song, I. Y. Cho, i J. E. Specht, D. L. Hyten, and P. B. Cregan. 2007. BARCSoySNP23: a panel of 23 selected SNPs for soybean cultivar identification.

SNP discovery and assay development in common bean. Next generation sequencing has significantly increased the speed at which SNPs can be discovered and applied as molecular markers for breeding. For minor crop species, such as common bean (*Phaseolus vulgaris L.*) a whole genome sequence is not yet available and next generation sequencing for SNP discovery has been difficult and costly. ARS researchers in Beltsville, Maryland, with cooperators from the University of Maryland and Seoul (South Korea) National University, developed a method that combines sequences obtained from the Roche 454-FLX system (454) with the Illumina Genome Analyzer (GA) to obtain high-throughput SNP discovery. This method led to the discovery of 3,487 SNPs of which 2,795 contained sufficient flanking genomic sequence for SNP assay development. Eight-six percent proved to be true SNPs and were applied to design a GoldenGate assay with 1,050 of the 3,487 predicted SNPs. Combining two next-generation sequencing techniques produced a method that now enables high-throughput SNP discovery in any diploid organism without the need for a whole genome sequence or creation of normalized cDNA libraries.

Hyten, D.L, Song, Q, Fickus, E.W., Quigley, C.V., Lim, J.-S., Choi, I.-Y., Hwang, E.-Y., Pastor-Corrales, M., Cregan, P.B. 2010. High-throughput SNP discovery and assay development in common bean. BMC Genomics 2010, 11:475, doi:10.1186/1471-2164-11-475.

Genetic regions affecting critical apple rootstock traits. The long evaluation time needed to select individuals for a positive apple rootstock trait represents a major limitation to progress in apple rootstock breeding. ARS researchers in Geneva, New York, identified chromosomal regions that control important apple rootstock traits and mapped genes for resistance to fire blight and powdery mildew and for root architecture to known regions of the apple genome. They discovered four regions modulating fire blight resistance; three regions that modulate powdery mildew resistance; four regions associated with number of internodes produced each season (a component of dwarfing); and three regions that modulate fine root formation – a trait that may be connected to replant disease tolerance. This information has been incorporated into marker-assisted breeding programs to enable selection of traits early before planting, thus increasing the efficiency of the breeding program.

Russo, N., Aldwinckle, H., Robinson, T., Fazio, G. 2008. Budagovsky 9 rootstock: uncovering a novel resistance to fire blight. Acta Hort. 793:321-324.

Beers, E., Cockfield, S., Fazio, G. 2007. Biology and management of woolly apple aphid, Eriosoma lanigerum (Hausmann), in Washington State. In: Proceedings of the IOBC, 30(4):37-42.

PROBLEM STATEMENT 3B: *Capitalizing on Untapped Genetic Diversity*

Research conducted under this problem statement sets a new standard for the development of new genetic sources for crop improvement, elucidating the genetic mechanisms and determining the level of diversity for key crop traits. ARS germplasm collections (page 12) have been vigorously evaluated for genes to solve emerging challenges. To this end, ARS researchers have made genes accessible, without restriction, to address the challenges of lack of resistance or tolerance to abiotic and biotic stresses. As new invasive pests or diseases enter the United States, new genes need to be discovered to develop resistant varieties. New resistance sources have been found for pests such as the soybean aphid and soybean rust among ARS germplasm collections, and researchers have attempted to introgress resistance genes from unadapted sources into more adapted backgrounds.

The NP 301 Action Plan has identified five anticipated products which were expected from ARS research to address the needs outlined by Problem Statement 3B. Anticipated products 4 and 5 presented significant overlapping results, so selected achievements will be combined in this report. Accomplishments that illustrate those products follow the list of anticipated products.

ANTICIPATED PRODUCTS IN ACTION PLAN:

1. Development of genetic stocks that serve as research tools and potential sources of genes for crop improvement.
2. Level of diversity determined for key traits in crops.
3. Germplasm with identified resistance (tolerance) to biotic and abiotic stresses.
4. Identification of germplasm with novel traits and properties that will enable development of new varieties for new uses and agricultural products.
5. Evaluation information that contributes to developing crops containing new sources of diverse traits derived from ancestral and wild relatives.

PROBLEM STATEMENT 3B: ACCOMPLISHMENT OVERVIEW

Maintaining consistent genetic gain for yield, resistance to biotic and abiotic stresses, and product quality in cultivar development programs requires that genetic diversity for key traits also be maintained. New germplasm lines derived from wild relatives of numerous crop species have been released by NP 301 researchers and cooperators to address this challenge.

A notable example in this area has been the ARS response to the challenge of wheat stem rust Ug99, first reported in Uganda in 1991, and the most virulent wheat stem rust strain in the past 60 years. The potential global dispersal of this uniquely virulent pathogen makes breeding resistant cultivars an urgent goal. During the last 5 years, ARS has redirected multiple laboratories to combat Ug99 and is a founding partner in the Borlaug Global Rust Initiative. ARS wheat geneticists and pathologists and university and private-sector colleagues were mobilized to assess the vulnerability of U.S. wheat and barley cultivars, to identify new sources of genetic resistance in U.S. lines and wild relatives, to develop genetic stocks with resistance genes, and to develop markers and genotyping tools to deploy genetic resistance into U.S. wheat and barley lines.

Genetic stocks also were developed for many crops to address the critical needs for plant genes that control the expression of key traits. For example, there is concern about how crops will adapt to the accelerating rate of global change. ARS has launched the first efforts to address this, and has found diversity for traits like soybeans adapted to elevated CO2 levels that can be incorporated into breeding programs. The success of ARS scientists in developing heat-tolerant legume varieties is important to a crop produced primarily under hot, dry conditions, and is extremely important for food-challenged nations.

PROBLEM STATEMENT 3B: SELECTED ACCOMPLISHMENTS

Anticipated Product 1: *Development of genetic stocks that serve as research tools and potential sources of genes for crop improvement.*

New genetic stocks with minimal *Aegilops speltoides* chromosome segments with Ug99 resistance. In wheat, the stem rust resistance gene Sr39, derived from the goatgrass species Aegilops speltoides, is highly resistant to multiple stem rust races, including Ug99. However, the gene has not been incorporated into wheat breeding programs because of undesirable genes associated with the large grass chromosomal segment on which Sr39 is located in the original wheat-goatgrass chromosome translocation. To make this valuable gene usable for wheat breeding, ARS researchers reduced the size of the alien chromatin surrounding Sr39 through chromosome engineering, in conjunction with stem rust testing and molecular marker analysis. Four wheat lines that carry Sr39 on a minimal *A. speltoides* chromosome segment were identified and verified by fluorescent DNA hybridization and by testing with eight races of stem rust. These researchers also developed three new molecular markers tightly linked to Sr39 on the short goatgrass chromosomal segment. The new wheat genetic stocks and linked molecular markers are important resources for breeding commercial wheat cultivars resistant to Ug99. **[**Also contributes to Anticipated Products 3 and 4.]

Niu, Z., Klindworth, D.L., Friesen, T.L., Chao, S., Jin, Y., Cai, X., Xu, S.S. 2011. Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering genetics. Genetics. 187(4):1011-1021.

Rice genetic stocks developed. ARS researchers in Stuttgart, Arkansas, and colleagues from the University of Arkansas, developed five rice genetic stocks – designated as RIL103, RIL158, RIL186, RIL220, and RIL221 – that contain major and minor genes linked with sheath blight resistance. These genetic stocks have good grain quality traits, early maturity, and glabrous leaves and hulls, traits which would be valuable for improving sheath blight resistance in U.S. rice cultivars, while maintaining product quality.

Prasad, B., Eizenga, G.C. 2008. Sheath blight disease screening methods to identify resistant Oryza spp. accessions. Plant Disease. 92:1503-1509.

Anti Jia, Y., Coarrea-Victoria, F., McClung, A.M., Zhu, L., Liu, G., Wamishe, Y., Xie, J., Marchetti, M.A., Pinson, S.R., Rutger, J.N., Correll, J.C. 2006. Rapid determination of rice cultivar responses to the sheath blight pathogen Rhizoctonia solani using a micro-chamber screening method. Plant Disease. 91:485-489.

Development of sorghum genetic stocks with reduced lignin and higher digestibility. Three sorghum genetic stocks – BN611, A/BN612, and RN613 – with the stacked brown midrib genes bmr-6 and bmr-12 were developed and made available for basic research on lignin synthesis, and for use as animal and bioenergy feedstocks. These brown midrib genes are known to be associated with reduced lignin and higher digestibility, making brown midrib sorghum better livestock feed and a better bioenergy feedstock. Previously, ARS researchers had developed and released genetic stocks containing the individual bmr-6 and bmr-12 genes, and demonstrated the effect these genes had on lignin content and other cell wall-related traits. Since these two genes affect different enzymes involved in lignin synthesis, it is expected their combined effects could result in even greater changes in lignin content and other traits. **[**Also contributes to Anticipated Product 4.]

Pedersen, J.F., Toy, J.J., Funnell-Harris, D.L., Sattler, S.E., Oliver, A.L. 2008. Registration of BN611, A/BN612, RN613 Sorghum Genetic Stocks with Stacked bmr-6 and bmr-12 Genes. Journal of Plant Registrations 2:258-262.

Sattler, S.E., Saathoff, A.J., Haas, E.J., Palmer, N.A., Funnell-Harris, D.L., Sarath, G., Pedersen, J.F. 2009. A nonsense mutation in a cinnamyl alcohol dehydrogenase gene is responsible for the sorghum brown midrib-6 phenotype. Plant Physiology 150 (2):584-95.

Sattler, S.E., Funnell-Harris, D.L., Pedersen, J.F. 2010. Efficacy of Singular and Stacked Brown Midrib 6 and 12 in Modification of Lignocellulose and Grain Chemistry. Journal of Agricultural and Food Chemistry. 58:3611-3616.

Sunflower genetic stocks released with disease resistance and oil traits. ARS scientists in Fargo, North Dakota, released several different sunflower genetic stocks to offer breeders materials to use in developing hybrid varieties:

- A genetic stock with traits of traditional high polyunsaturated sunflower with a seed oil composition of about 7.6 percent total saturated fatty acids, compared to about 13 percent for current conventional sunflower hybrids.
- Two genetic stocks with a high gamma-tocopherol content trait that allows another pathway through which to improve the stability of sunflower oil used in the food industry.
- Four restorer lines resistant to Sclerotinia head rot that provide diversity for agronomic characteristics. RHA 462 provides tolerance to Phomopsis stem canker, and RHA 464 has resistance to the most virulent North American races of rust and downy mildew.
- Three sunflower genetic stocks, each contributing a new gene available for incorporation of downy mildew resistance into a female parent for hybrid sunflower seed production. The genetic stocks also provide the genes for high oleic acid content and good agronomic and yield characteristics.

Hulke, B.S., Miller, J.F., Gulya, T.J., Vick, B.A. 2010. Registration of the Oilseed Sunflower Genetic Stocks HA 458, HA 459, and HA 460 Possessing Genes for Resistance to Downy Mildew. Journal of Plant Registrations. 4:93-97.

Anticipated Product 2: *Level of diversity determined for key traits in crops.*

Novel imaging and software platform for three-dimensional root phenotyping. ARS scientists in Ithaca, New York, together with Cornell University scientists, developed a high-throughput growth, imaging, and analysis platform to phenotype growing root systems in three dimensions. The high-throughput system, combined with an expanding array of germplasm resources, make it possible to explore the genetic components of root system architecture and physiology as they relate to both developmental processes and root traits associated with the acquisition of limiting resources (e.g. water and phosphorous).

> Clark, R.T., MacCurdy, R.B., Jung, J.K., Shaff, J.E., McCouch, S.R., Aneshansley, D.J., and Kochian, L.V. 2011. Three-dimensional root phenotyping with a novel imaging and software platform. Plant Physiol. 156, 455-465.

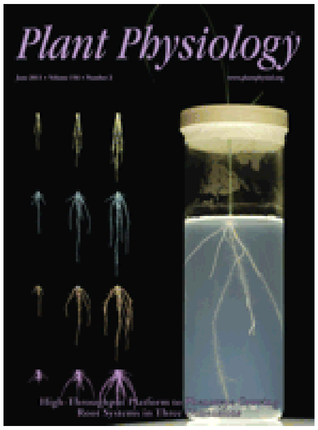

Figure 12. An innovative platform for analyzing crop root architecture using 3-D imaging and analysis software was highlighted as a breakthrough technology by Plant Physiology.

Identifying natural variation to achieve a 16-fold increase in maize Vitamin-A. Billions of people and livestock consume maize as major source of subsistence and nutrition. However, maize is generally not a good source of pro-Vitamin A, leading to childhood blindness and immune deficiencies especially in developing nations. Building on a prior discovery, ARS scientists and collaborators discovered that natural variation at two genes in maize, which can enable breeding for 16-fold higher levels of pro-Vitamin A than standard maize. Breeding programs for the developing world are already applying this information to breeding more nutritious varieties of maize.

> Buckler IV, E.S., Harjes, C., Rocheford, T., Bai, L., Brutnell, T., Kandianis, C.B., Sowinski, S.G., Stapleton, A.E., Vallabhaneni, R., Williams, M., Wurtzel, E., Jianbing, Y. 2008. Natural genetic variation in the lycopene epsilon cyclase gene can enhance provitamin A biofortification of maize. Science. 319(5861):330-333.

Diversity of traits influencing taste in peppers. ARS researchers from Griffin, Georgia, and their industry and university colleagues measured great variation for various sugars and acids that are believed to contribute to the sweetness and/or sourness of mature pepper fruit from 216 different varieties of pepper (*Capsicum chinense*). Analyses of these field-grown materials suggest that selection for varieties of pepper with defined sugar/acid profiles could result in new varieties with unique flavor attributes, and that germplasm analyzed could serve as a source of genes affecting fruit taste.

> Jarret, R.L., Berke, T., Baldwin, E.A., Antonious, G. 2009. Variability for Free Sugars and Organic Acids in *Capsicum chinense* Jacq.. Chemistry and Biodiversity. 6(2):138-145.

Anticipated Product 3: *Germplasm with identified resistance (tolerance) to biotic and abiotic stresses.*

Molecular markers for Ug99 resistance developed for genotyping and breeding. Molecular markers were identified to "tag" the stem rust resistance genes Sr24, Sr26, Sr36, and Sr1RSAmigo that confer resistance to Ug99. ARS researchers and their university colleagues screened a collection of 776 wheat cultivars and breeding lines from all U. S. growing regions with DNA assays to determine the frequencies of these genes in U.S. wheat germplasm. The precision of the DNA markers for predicting the presence of these genes was evaluated via comparison with data from infecting seedlings with the pathogen. This information has contributed to breeding wheat cultivars resistant to Ug99 by enabling the development of resistance "gene pyramids" for more durable stem rust resistance. [Also contributes to Anticipated Products 4 and 5.]

Olson, E.L., Brown Guedira, G.L., Marshall, D.S., Jin, Y., Mergoum, M., Lowe, I., Dubcovsky, J. 2010. Genotyping of U.S. Wheat Germplasm for Presence of Stem Rust Resistance Genes Sr24, Sr36 and Sr1RS Amigo. Crop Science. 50:668-675.

Mago, R., Brown Guedira, G.L., Dreisigacker, S., Breen, J., Jin, Y., Singh, R., Appels, R., Lagudah, E., Ellis, J., Spielmeyer, W. 2010. An accurate DNA marker assay for stem rust resistance gene Sr2 in wheat. Theoretical and Applied Genetics. 122:735-744.

Determination of genetic diversity for Russian wheat aphid resistance in barley. During the past 5 years, a substantial portion of U.S. barley production has shifted west into Russian wheat aphid prone areas, and all U.S. barley cultivars were susceptible to the aphid. In response, ARS researchers and their university colleagues screened for insect resistance and developed 50 Russian wheat aphid resistant germplasm lines, and have released four resistant cultivars.

Mornhinweg, D.W., Bregitzer, P.P., Porter, D.R., Peairs, F.B., Baltensperger, D.D., Hein, G.L., Randolph, T.A., Koch, M., Walker, T. 2009. Registration of 'Sidney' spring feed barley resistant to Russian wheat aphid. Journal of Plant Registrations. 3(3):214-218.

Disease resistance genes in small grains germplasm. ARS researchers determined that the fungus that causes the disease tan spot in wheat produces a host-selective toxin in wheat lines that carry the gene *Tsc2*. Therefore, elimination of the *Tsc2* gene from wheat lines results in a greater level of tan spot resistance. They identified molecular markers closely associated with *Tsc2* that wheat breeders and geneticists can apply to rapidly and efficiently screen wheat lines for the presence or absence of *Tsc2*. Other researchers identified molecular markers for adult resistance to stripe rust in wheat. These findings have accelerated the development of disease-resistant wheat. [Also contributes to Anticipated Product 2.]

Abeysekara, N.S., Friesen, T.L., Liu, Z., Mcclean, P.E., Faris, J.D. 2010. Marker Development and Saturation Mapping of the Tan Spot Ptr ToxB Sensitivity locus Tsc2 in Hexaploid Wheat. The Plant Genome. 3:179-189.

Zhang, Z., Friesen, T.L., Simons, K.J., Xu, S.S., Faris, J.D. 2009. Development,Identification and Validation of Markers for Marker-Assisted Selection Against the Stagonospora nodorum Toxin Sensitivity Genes Tsn1 and Snn2 in Wheat. Molecular Breeding,23:35-49.

Carter, A.H., Chen, X., Garland Campbell, K.A., Kidwell, K.K. 2009. Identifying QTL for high-temperature adult-plant resistance to stripe rust (Puccinia striiformis f. sp. tritici) in the spring wheat (Triticum aestivum L.) cultivar `Louise-. Theor. Appl. Genet. 119:1119-1128.

First ever drought-tolerant soybean germplasm. A new generation of soybean breeding lines was developed and tested for drought response by ARS researchers in Raleigh, North Carolina, and university colleagues after the discovery of two major QTLs that govern soybean wilting. Knowledge of these QTLs have provided a short cut for accelerating the incorporation of drought tolerant germplasm in breeding stock without resorting to extensive field testing in drought sites. In regional and local testing, two soybean lines – N01-11136 and N01-11771 – were slow wilting and with substantially better yield when grown under dry conditions. They also yielded reasonably well under optimal environments with minimal stress conditions. These germplasm lines have been made available to commercial breeders as parental stock.

Hufstetler, V.E., Boerma, R., Carter Jr, T.E., Earl, H.J. 2007. Genotypic variation for three physiological traits affecting drought tolerance in soybean. Crop Sci. 47:25-35.

Kidney bean germplasm with tolerance to high temperature stress. Two kidney beans (Phaseolus) – TARS-HT1 and TARS-HT2 – that are tolerant to high temperature conditions were bred cooperatively by ARS researchers in Mayagüez, Puerto Rico, and colleagues at the University of Puerto Rico, Cornell University, and the University of Tennessee. TARS-HT1 is tolerant to high day and night temperature stress and TARS-HT2 is tolerant to high day temperature stress and moderate night temperature stress. These lines can improve yield under hot summer conditions for farmers in regions prone to high temperature stress, and also can serve as sources for improved heat tolerance in other Phaseolus types.

Porch, T.G., Smith, J.R., Beaver, J.S., Griffiths, P.D., Canaday, C. 2010. Registration of TARS-HT1 and TARS-HT2 heat tolerant dry bean germplasm lines. HortScience. 1278-1280.

Porch, T.G., Ramirez, V.H., Santana, D., Harmsen, E.W. 2009. Evaluation of drought tolerance in common bean germplasm in Juana Díaz, Puerto Rico. Journal of Agronomy and Crop Science 195:1-7.

New germplasm source for downy mildew and leafminer resistance genes in lettuce. Downy mildew disease and leafminer insects reduce lettuce yield and quality. ARS researchers in Salinas, California, with additional industry funding, determined that a genebank accession PI509525, a weedy relative of lettuce, is both resistant to leafminers and does not host downy mildew. Experiments conducted during 4 years revealed that each type of resistance was controlled by an independent, single gene. Identification of the resistance genes enables the transfer of leafminers and downy mildew resistance from the wild species into cultivated lettuce.

Mou, B., Ryder, E.J. 2010. MU06-857, a Green Leaf Lettuce Breeding Line with Resistance to Leafminer and Lettuce Mosaic Virus. HortScience. 45: 666-667.

Sugar beet germplasm with multiple pest resistance. ARS researchers in Salinas, California, and Fort Collins, Colorado, bred two sugar beet multigerm germplasm lines which combine resistances to Rhizomania disease (caused by *Beet necrotic yellow vein virus*), Rhizoctonia root rot, Aphanomyces root rot, *Beet curly top virus*, sugarbeet root aphid, and Cercospora leaf spot. These lines have favorable yield characteristics when evaluated as lines or in experimental hybrids under Rhizomania-infested soil. Another important germplasm line combines Rhizoctonia resistance with higher sucrose and smooth roots, which reduces adhered soil by 50 percent, thus decreasing sugarbeet transportation and production costs.

> Panella, L.W., Hanson, L.E., Lewellen, R.T. 2008. Breeding for Multiple Disease Resistance in Sugarbeet: Registration of FC220 and FC221. Journal of Plant Registrations.

Disease-resistant, high-yielding sugarcane cultivars. New sugarcane cultivars are continuously needed for sustained or improved yields, resistance to intense disease pressures, and for improved adaptability to freezes, high water tables, and muck and sandy soils of Florida. ARS researchers in Canal Point, Florida, in cooperation with the University of Florida and the Florida Sugar Cane League, Inc., bred superior sugarcane varieties that are high-yielding in both soil types and with improved tolerance to limiting sugarcane diseases. These products of the ARS sugarcane program have contributed significantly to U.S. sugar production and to those of several developing countries.

> Davidson, R., Milligan, S.B., Glaz, B.S., Comstock, J.C., Hu, C., Glynn, N.C., Edme, S.J., Holder, D.G., Gilbert, R.A., Sood, S.G., Del Blanco, I.A., Zhao, D. 2011. Registration of 'CPCL 99-4455' Sugarcane. Journal of Plant Registrations. 5:54-61

Accomplishments contributing to Anticipated Products 4 and 5 have been combined because of overlapping products.

Accomplishment 4. ***Identification of germplasm with novel traits and properties that will enable development of new varieties for new uses and agricultural products.***

Accomplishment 5. ***Evaluation information that contributes to developing crops containing new sources of diverse traits derived from ancestral and wild relatives.***

New markers for and sources of Ug99 wheat stem rust resistance identifed. In a cooperative project with CIMMYT and the Kenyan Agricultural Research Institute in Njoro, ARS researchers evaluated Ug99 resistance levels in Kenya for 21,000 lines from U.S. wheat and barley breeders from 28 universities, 11 private sector companies, and ARS. New sources of Ug99 resistance were identified in wild wheat relatives, land races, and other accessions from the ARS National Small Grains Collection in Aberdeen, Idaho. Molecular markers that tag the stem rust resistance genes Sr24, Sr26, Sr36, and Sr1RSAmigo that confer resistance to the virulent Ug99 wheat stem rust strain were identified. A collection of 776 cultivars and breeding lines of wheat from all growing regions of the United States were screened with the DNA to determine frequencies of these genes in U.S. wheat germplasm. ARS researchers also identified Ug99 resistance in the A-genome diploid relatives of wheat after screening 1,061 accessions of *Triticum monococcum* and 214 accessions of *T. urartu* against race Ug99. The researchers found a large proportion of the accessions (79 percent of *T. monococcum* and 93 percent of *T. urartu*) were resistant to Ug99. These are previously uncharacterized resistance genes to Ug99 and provide critically needed to sources of Ug99 resistance in wheat. This information is contributing to development of wheat cultivars resistant to Ug99 by enabling the introgression of new sources of Ug99 resistance and development of resistance gene pyramids for more durable stem rust resistance. [Also contributes to Anticipated Products 1, 2, and 3.]

Bonman, J.M., Bockelman, H.E., Jin, Y., Hijmans, R.J., Gironella, A. 2007. Geographic Distribution of Stem Rust Resistance in Wheat Landraces. Crop Science. 47:1955-1963.

Olson, E.L., Brown Guedira, G.L., Marshall, D.S., Jin, Y., Mergoum, M., Lowe, I., Dubcovsky, J. 2010. Genotyping of U.S. Wheat Germplasm for Presence of Stem Rust Resistance Genes Sr24, Sr36 and Sr1RS Amigo. Crop Science. 50:668-675.

Rouse, M., Jin, Y. 2011. Stem Rust Resistance in A-Genome Diploid Relatives of Wheat. Plant Disease. 95:941-944.

Evaluation of pigmented rice bran for antioxidants with potential health benefits. Phytochemicals are antioxidants that may account for health-beneficial properties associated with eating whole grain brown rice. ARS scientists in Beaumont, Texas, determined the concentrations of phytochemicals, including vitamin E compounds as well as phenolics, flavonoids, anthocyanins, and proanthocyanins, in the bran of white, light brown, brown, purple, and red color rice cultivars. High antioxidant activity was found in some purple and red bran cultivars, but not in white. The information will be useful to rice breeders for enhancing rice health-beneficial components, and to the food industry interested in utilizing rice bran or whole grain as functional food.

Min, B., Mcclung, A.M., Chen, M.H. 2011. Phytochemicals and antioxidant capacity in rice brans of different color. Journal of Food Science. 76:C117-126.

Evaluating the arsenic content of rice grain. Arsenic is a common, natural element in agricultural soils, and although low concentrations occur in all food crops, including rice, high concentrations are undesirable because of the potential detrimental impact on plant growth and yield, and its potential toxicity to humans. ARS researchers at Stuttgart, Arkansas, and colleagues at Texas A&M University and the University of Arkansas, evaluated 25 cultivars from the ARS rice germplasm collection for arsenic content, and identified rice cultivars with 50 percent lower arsenic content than other cultivars. The researchers developed field management practices that can reduce accumulation of arsenic in rice grain. Breeders and researchers can use the identified cultivars with low arsenic concentrations as parents to develop new cultivars with improved nutritional value, and farmers can apply the new cultural management practices to assure high nutritional quality of the rice grain. [Also contributes to Anticipated Product 1.]

Pillai, T.R., Yan, W., Agarma, H.A., James, W.D., Ibrahim, A.M., Gentry, T.J., Mcclung, A.M., Loeppert, R.H. 2010. Total grain-arsenic and arsenic-species concentrations in diverse rice cultivars under flooded conditions. Crop Science. 50(1):2065-2075.

Yan, W., Agrama, H.A., Slaton, N.A., Gibbons, J.W. 2008. Soil and plant minerals associated with rice straighthead disorder induced by arsenic. Agronomy Journal. 100:1655-1661.

Identification of Sclerotinia stalk rot resistance in wild annual Helianthus species. Sclerotinia stalk rot is one of the major diseases affecting U.S. sunflower production, annually found in up to one-third of all fields and destroying up to 3 percent of the crop. ARS researchers developed an efficient method for screening for resistance to this disease in the greenhouse and evaluated more than 300 accessions of the 15 annual *Helianthus* species. They identified 20 accessions of seven annual species with stalk rot resistance superior to that in the best commercial hybrids.

> Miller, J.F., Gulya Jr, T.J., Vick, B.A. 2006. Registration of two maintainer (HA 451 and HA 452) and three restorer (RHA 453-RHA 455) Sclerotinia-tolerant oilseed sunflower germplasms. Crop Science. 46:2727-2728.

Discovery of spinach with low oxalic acid content and resistance to leafspot. Despite its high nutrient content, spinach (*Spinacia oleracea L.*) contains more oxalic acid than do most crops. Oxalic acid can reduce the bioavailability and absorption of calcium and iron, and calcium oxalate may deposit in the kidney of certain people as a form of kidney stone. The entire ARS spinach germplasm collection (338 accessions and 11 commercial cultivars) was screened for the first time, and accessions with low oxalate levels were identified. These new sources of low oxalate concentration might enable spinach to be bred for a low level of oxalate content.

> Mou, B. Evaluation of Oxalate Concentration in the U.S. Spinach Germplasm Collection. HortScience 43(6):1690-1693.2008.

Potato germplasm resistant to cold-induced sweetening. Potato tubers stored at cold temperatures accumulate glucose and fructose through the process of cold-induced sweetening. Such tubers produce dark colored, bitter tasting chips and fries with unacceptably high quantities of acrylamide, a neurotoxin and potential carcinogen. ARS researchers from Madison, Wisconsin, identified potato species with extreme resistance to cold-induced sweetening. Species with this key trait have contributed resistance to cold-induced sweetening for chipping potato breeding stock. Food scientists have analyzed them to better model the contribution of each biochemical constituent to chip color and acrylamide formation.

> Jansky, S.H., Hamernik, A.J., Bethke, P.C. 2011. Germplasm Release: Tetraploid Clones with Resistance to Cold-induced Sweetening. American Journal of Potato Research.

Bridging the gap between potato and its wild relatives. Some wild relatives of potato are exceptional sources of the key traits disease resistance, nutritional quality, resistance to cold sweetening, and high amylose content in tuber starch. ARS researchers in Madison, Wisconsin, developed a bridge crossing method in which these species are first crossed to a wild species *Solanum verrucosum*, that then enables subsequent hybrids to be crossed to cultivated potato, enabling introgression of this valuable trait wild potato in new commercial varieties. [Also contributes to Anticipated Product 3.]

> Jansky, S.H. 2006. Overcoming hybridization barriers in potato. Plant Breeding Conference Proceedings. 125:1-12.

Evaluating raisin grapes for drying time. Traditional hand harvest and drying grapes on trays are expensive and subject the grapes to damage by early fall rains. Cutting grape canes to dry raisins on the trellis requires special trellises and trained pruners. ARS researchers in Parlier, California, evaluated six raisin cultivars for their drying rates in three consecutive seasons and found three with relatively rapid drying. Growers can consider this trait in selecting which grape varieties to plant for raisins, which will benefit from more drying.

Ramming, D.W. 2009. Water loss from fresh berries of raisin cultivars under controlled drying conditions. American Journal of Enology and Viticulture. 60:2:208-214.

Leaf disk assay for identifying grapes with red flesh long before fruit production. Anthocyanins are considered an excellent source of antioxidant phytochemicals for health benefits, however, only a few wine grapes and no table or raisin grapes have anthocyanins in the flesh of their fruits. ARS researchers in Parlier, California, developed a leaf disk assay for early evaluation and detection of the red fruit flesh trait. This superior evaluation method enables plants with red fleshed fruit to be identified several years before they actually bear fruit, thereby accelerating the process of breeding of table and raisin grapes with red flesh and higher levels of antioxidants.

Ramming, D.W., Cousins, P.S. 2010. Development of Table and Raisin Grapes With High Anthocyanins Using a Leaf Disk Assay. International Society for Horticultural Science Meeting. P. 172.

Genetic markers for evaluating grape seedlessness and powdery mildew resistance. New cultivars of seedless table grape require embryo rescue by tissue culture, followed by several years of cultivation to evaluate whether they are indeed seedless. ARS researchers in Parlier, California, and their cooperators identified a new genetic marker for the seedless trait, which enable selection of seedless types early after embryo rescue and obviating the need for expensive seedling maintenance and evaluation. In addition, these researchers discovered a genetic marker for a broad spectrum powdery mildew resistance locus in the same breeding material, which will enable more efficient evaluation of breeding material to develop future cultivars which require fewer pesticide applications.

Ramming, D.W., Gabler, F., Smilanick, J.L., Cadle Davidson, M., Barba, P., Consolie, N.H., Mahanil, S., Cadle Davidson, L.E. 2011. A single dominant locus Ren4 confers non-race-specific penetration resistance to grapevine powdery mildew. Phytopathology. 101(4):502-508.

The stoneless trait in plum is identified. Pits (seed and the hard surrounding woody "stone") reduce the convenience of eating stone fruits (peaches, cherries, apricots, and plums). Removing the pits from stone fruits represents a major cost of processing, and generates much waste. ARS scientists found that the partially stoneless fruits developed in the early 1900's, although containing the gene that forms the woody stone tissue, have fewer cells that express those genes as compared to normal stone fruits. These data can accelerate breeding fruit without the stone and seed.

Callahan, A.M., Scorza, R., Dardick, C.D. 2009. Characterization of 'Stoneless': A naturally occurring, partially stoneless plum cultivar. Journal of the American Society for Horticultural Science. 134:120-125.

Descriptors developed for guayule germplasm. Commercialization of guayule as a domestic source of natural rubber is a strategic priority for the United States. A uniform set of germplasm descriptors is imperative to achieve uniform testing results among different research programs. ARS scientists in Maricopa, Arizona, and colleagues from Yulex Corp., developed and successfully applied a set of descriptors to evaluate breeding nurseries of both non-transgenic and transgenic guayule plants. The new set of descriptors was easy to use and required a minimum amount of time per plant, so that many lines could easily be evaluated. The descriptors will enable uniform comparisons of germplasm performance across experiments and environments, and will also assist regulatory agencies in decide whether to grant intellectual property rights for guayule varieties.

> Coffelt, T.A. and Johnson, L. (2010). A Set of Descriptions for Evaluating Guayule Germplasm. Industrial Crops and Products, doi.org/10.1016j.indcrop.2010.04.2004.

PROBLEM STATEMENT 3C: *Germplasm Enhancement/Release of Improved Genetic Resources and Varieties*

Across most, if not all crops, increasing the efficiency and effectiveness of breeding programs – especially by integrating genomics for marker-assisted breeding – has been a key need for crop breeders, researchers, and crop producers. More efficient breeding programs were imperative because of the increasingly high cost of field breeding trials, skilled labor, and the accelerated pace of change in markets. New and improved germplasm or cultivars with desired traits were vital for sustainable crop production. Where new cropping systems, consumer preferences, processing needs, or other alternative options emerged, new more effective breeding programs were needed to develop and deploy solutions. These take the form of new traits or finished varieties, and sometimes more efficient breeding processes or tools. Flexible new avenues for releasing materials were required so they could be commercialized more effectively.

The NP 301 Action Plan identified four anticipated products which would help meet the key needs of crop researchers, producers, processors, and consumers. Accomplishments that illustrate those products follow the list of anticipated products. Anticipated products 1 and 2 will be presented together because of significant overlap.

ANTICIPATED PRODUCTS IN ACTION PLAN:

1. Genomics-integrated, marker-assisted selection breeding programs.
2. New and improved germplasm and crop varieties containing desired traits.
3. New alternative crops for diverse management and market systems for crop producers.
4. Flexible, crop-specific procedures for releasing and protecting plant material.

PROBLEM STATEMENT 3C: ACCOMPLISHMENT OVERVIEW

During the last 5 years, ARS researchers released 545 new crop varieties and germplasm lines – more than 100 such releases each year (See a listing in Appendix 3). These new genetic materials deployed genes for more effective disease and insect resistance, as well as heat and drought stress tolerance. Some varieties delivered superior nutritional content and end-use qualities. Collectively, all of these new materials represent more effective genetic solutions to the most pressing challenges facing U. S. crop production. Their impact was particularly strong for crops such as small grains and horticultural, or “specialty crops,” where private-sector genetic improvement efforts were modest or even completely lacking.

For example, the varieties released included peanuts resistant to tomato spotted wilt and root knot nematode; legume cultivars and germplasm lines resistant to white mold, Ascochyta blight in chickpea, and Fusarium rot of pea; and many superior fruit and nut varieties. For tree fruits (citrus, pome, and stone fruits), these included not only scion varieties, but also highly valuable rootstock varieties that have substantially improved crop production. New genetic markers for key traits were especially important for enhancing the efficiency for breeding tree crops, and for traits, such as nematode resistance, where field assays are particularly expensive and complicated.

During the last 5 years, new forms of research partnerships with public and private sector partners enabled ARS research to leverage external resources to accelerate progress towards attaining many priority objectives. As a result, many of the varieties and enhanced germplasm discussed here were jointly developed and released with land-grant universities, commodity groups, and industry partners.

PROBLEM STATEMENT 3C: SELECTED ACCOMPLISHMENTS

Anticipated Product 1 and 2 were combined because of the extensive overlap of these achievements.

Anticipated product 1. *Genomics-integrated, marker-assisted selection breeding programs.*

Anticipated product 2. *New and improved germplasm and crop varieties containing desired traits*

Integrated breeding programs reduce losses to scab--Fusarium head blight (FHB)--in wheat and barley. The U.S. Wheat and Barley Scab Initiative (USWBSI), managed by ARS researchers from multiple locations in partnership with a coalition of university and industry partners, has supported an integrated small grains breeding and disease management program for reducing the severity of the scab disease (See a summary of Initiative accomplishments at http://www.scabusa.org). During the past 5 years, ARS researchers and university partners have released scab-resistant wheat varieties adapted to all the major U. S. wheat growing areas. New scab-resistant malting barley varieties will soon be released by the University of Minnesota and North Dakota State University scientists in partnership with ARS researchers. The deployment of scab resistance genes from wheat and barley has been accelerated by USWBSI-supported field screening and subsequent recombination into adapted genetic backgrounds. ARS genotyping laboratories in Raleigh, North Carolina; Pullman, Washington; Fargo, North Dakota; and Manhattan, Kansas, have identified genetic markers for screening for FHB-resistant genes in parental breeding stock. ARS researchers have coordinated scab disease nurseries and identified varieties at risk for losses. The Initiative also supports mycotoxin (DON; caused by scab fungi) testing for 67 wheat and barley breeders cooperators in 21 states. Growers have avoided substantial losses to scab by taking advantage of Initiative-funded prediction models, management advisories, and by avoiding vulnerable grain varieties.

Costa, J.M., Bockelman, H.E., Brown Guedira, G.L., Cambron, S.E., Chen, X., Cooper, A., Cowger, C., Dong, Y., Grybauskas, A., Jin, Y., Kolmer, J.A., Murphy, J.P., Sneller, C., Souza, E.J. 2010. Registration of the Soft Red Winter Wheat Germplasm MD01W233-06-

1 Resistant to Fusarium Head Blight. Journal of Plant Registrations. 4: 255-260.

Anderson, J.A., Chao, S., Liu, S. 2008. Molecular Breeding Using a Major QTL for Fusarium Head Blight Resistance in Wheat. Crop Science. 47(3):S112-S119.

Ug99-resistant and high quality wheat varieties. ARS researchers have released, or contributed to the release, of multiple new wheat varieties with disease resistance and new end-product qualities. These include two varieties with the first Ug99-resistance for the United States: Nueast (2009), a hard red winter wheat with Ug99 resistance, and Appalachian White (2009), a hard white wheat, both bred for the southeastern United States; Mace (2008), a hard, red winter wheat cultivar for the Midwest with resistance to Ug99 and wheat streak mosaic virus; and Anton (2008), a hard white wheat with low polyphenoxidase activity that yields superior flour for Asian noodles.

Graybosch, R.A., Peterson, C.J., Baenziger, P.S., Baltensperger, D.D., Nelson, L.A., Jin, Y., Kolmer, J.A., Seabourn, B.W., French, R.C., Hein, G.L. 2009. Registration of ‘Mace’ Hard Red Winter Wheat. Journal of Plant Registrations 3:51-56.

Graybosch, R.A., Peterson, C.J., Baenziger, P.S., Baltensperger, D.B., Nelson, L., Jin, Y., Kolmer, J.A., Seabourn, B.W., Beecher, B.S. 2011. Registration of Anton Hard White Winter Wheat. Journal of Plant Registrations. 5. DOI: 10.3198/jpr2010.08.0481crc.

Superior new woody landscape ornamentals. The ongoing introduction of superior new woody ornamental plants is a driving force behind the sustained growth of ornamental horticulture, providing growers and landscapers with a greater diversity of well-adapted landscape material from which to choose. Due to lack of cold-hardiness, camellias were limited to southern landscapes, but ARS researchers from Washington, D.C., selected ‘Anacostia’, a cold-tolerant variety hardy into USDA Zone 7 and possibly Zone 6, with abundant, large, semi-double medium-pink flowers, dark glossy evergreen foliage, rounded growth habit, and increased cold tolerance. They also released the viburnum variety ‘Nantucket,’ with superior compact growth habit, a profusion of large white lightly fragrant inflorescences, and outstanding dark green foliage. ARS researchers in Poplarville, Mississippi, released ‘Sahara Sunset,’ an African hibiscus cultivar with variegated foliage – the first stable variegated form of the purple leaf type – that can serve as color accent in shrub borders or as a contrast plant in mixed annual planters. They also released ‘USS Arizona,’ a new tropical hibiscus with orange flowers, ideal for landscape use that is adapted to hot summer heat.

Pooler, M.R. 2011. 'Anacostia' Camellia. HortScience. 46:139-140

Pooler, M.R. 2010. 'Cree' and 'Nantucket' Viburnum. HortScience. 45:1384-1385.

Pounders, C.T. 2009. Notice to Nurserymen etc. Germplasm Release. United States Department of Agriculture: Agriculture Research Service, Washington, D.C. 20250

Improved seed composition of crops that produce vegetable oils is needed to better meet the increasingly diverse needs of end users. Soybean oil is the world's most widely used edible oil, but peanut and sunflower oil also play prominent roles in the vegetable oil industry. ARS research addressed the top priorities for oilseed trait modifications, including reduced saturated fat, reduced linolenic acids, and increased oleic acids. ARS made significant advancements in both traditional breeding and biotechnology approaches, which were used to improve seed composition of the major oilseed crops. ARS research identified oil and meal traits, determined genes that influence them, and incorporated these genes into elite germplasm, without negatively impacting, or simultaneously increasing other agronomic traits, such as seed yield and tolerance to biotic and abiotic stress. Examples from soybeans and peanuts are listed below.

Disease-resistant peanut varieties. The peanut root-knot nematode and tomato spotted wilt virus in large yield losses for peanuts in the southeastern United States. Varieties were available with resistance to either, but there were no varieties resistant to both. ARS researchers and University of Georgia colleagues jointly released ‘Tifguard,’ a new variety with high yield and excellent resistance to the peanut root-knot nematode and tomato spotted wilt virus, and ‘Georganic,’ with good yield and multiple disease resistance. Georganic has a red testa, which is not acceptable for conventional peanut production, but it is being grown by several organic peanut producers.

Holbrook Jr, C.C., Timper, P., Culbreath, A.K., Kvien, C.K. 2008. Registration of 'Tifguard' Peanut. Journal of Plant Registrations. 2:92-94.

Holbrook Jr, C.C., Culbreath, A.K. 2008. Registration of 'Georganic' Peanut. Journal of Plant Registrations. 2:17.

Developing soybeans with desirable seed composition phenotypes. ARS scientists in Missouri developed several genetic technologies that are being commercialized with industry partnerships.

- Released germplasm containing modified stacchyose and raffinose levels along with corresponding molecular markers to rapidly identify the genes.
- Released improved soybean varieties containing more than 80 percent oleic acid and a method to identify soybeans with the trait.
- Released soybean lacking the 7S globulins that can be utilized as hypoallergenic protein source.
- Collaborated with university partners to released germplasm containing low linolenic acid along with a genomic strategy for identifying the trait. [Also contributes to Anticipated Product 4]

Emily C. Dierking and Kristin D. Bilyeu. 2008. Association of a soybean raffinose synthase gene with low raffinose and stachyose seed phenotype. Plant Genome 1:135–145.

Flores T, Karpova O, Su X, Zeng P, Bilyeu K, Sleper DA, Nguyen HT, Zhang ZJ (2008). Silencing of Gm FAD3 gene by siRNA leads to low α-linolenic acids (18:3) of fad3 -mutant phenotype in soybean [*Glycine max* (Merr.)] Transgenic Research *17*:839-850.

New cotton breeding lines with superior yield and fiber quality. About 75 percent of cotton produced in the United States is exported, and to be more competitive on a world market, U.S. cotton must be of higher quality. It has been a difficult challenge to combine high fiber quality traits with high yields in cotton. ARS researchers in Stoneville, Mississippi, have bred two cotton lines with superior yield, fiber quality, and disease resistance. These two lines are already contributing increased yields, better fiber quality, and insect resistance to several cotton breeding programs.

Superior new food legume varieties. ARS researchers in Pullman, Washington, have bred new food legume varieties with superior production and product quality, and resistance to diseases and pests, including 'Morton,' the first winter hardy lentil variety, and 'Specter,' a winter pea can be direct seeded into standing cereal stubble for a zero tillage system. The new smooth green pea variety, 'Stirling,' and the green dry pea, 'Medora,' are characterized by upright growth habit, adequate disease resistance, and superior seed quality. The new lentil cultivar 'Essex' is Spanish brown-type of lentil with greater yield and plant height than the industry standard. ARS researchers in Prosser, Washington, bred two high-yielding pinto bean lines with partial resistance to Sclerotinia, and researchers in Beltsville, Maryland, and University of Nebraska developed common bean lines with resistance to bean rust and other major diseases of dry beans in the United States. Finally, researchers in Charleston, South Carolina, bred two new cream-type southernpea cultivars that retain the superior green color after drying, and will contribute to the frozen food industry.

Fery, R.L. 2009. 'WhiteAcre-DG', a Small-seeded, Cream-type Southernpea with an Enhanced Persistent Green Seed Phenotype. HortScience. 44(5):1472-1473.

Fery, R.L. 2009. 'ZipperCream-GC', a Large-seeded, Cream-type Southernpea with a Green Cotyledon Phenotype. HortScience. 44(5):1474-1475

Miklas, P.N., Fourie, D., Wagner, J., Larsen, R.C., Mienie, C. 2009. Tagging and mapping Pse-1 gene for resistance to halo blight in common bean differential cultivar UI-3. Crop Science. 49:41-48.

Miklas, P.N., Seo, Y.S., Gilbertson, R.L. 2009. Quantitative Resistance to Bean dwarf mosaic virus in Common Bean is Associated with the Bct gene for Resistance to Beet curly top virus. Plant Disease. 93:645-648.

Mutlu, N., Urrea, C.A., Miklas, P.N., Steadman, J.R., Pastor Corrales, M.A., Lindgren, D.T., Reiser, J., Vidaver, A.K., Coyne, D.P. 2008. Registration of Common Bacterial Blight, Rust and Bean Common Mosaic Resistant Great Northern Bean Germplasm Line ABC - Weihing. Journal of Plant Registrations. 2:120-124.

Superior new potato varieties. ARS researchers in Beltsville, Maryland, and their university colleagues bred 'Peter Wilcox', a dark purple-skinned, yellow-fleshed fresh-market, specialty potato, with carotenoid content more than 15 percent greater than 'Yukon Gold', the current yellow-flesh standard variety in the United States. This new variety provides growers and consumers with a unique combination of skin and flesh colors for niche markets. In addition, ARS researchers at Aberdeen, Idaho, and their colleagues bred potato varieties with excellent processing quality, including low internal and external tuber-defects, high marketable yield, resistance to sugar ends, and retention of high quality following long-term storage.

Stark, J.C., R.G. Novy, J. L.Whitworth, N.R. Knowles, M.J. Pavek, S.L. Love ,M.I. Vales, S.R. James, D.C. Hane, C.R. Brown, B.A. Charlton, D.L. Corsini, J.J. Pavek, N. Olsen and T. Brandt. 2010. Classic Russet: A Potato Cultivar with Excellent Fresh Market Characteristics and High Yields of U.S. No. 1 Tubers Suitable for Early Harvest or Full-Season Production. Amer. J. Potato Res. 87:360-373.

Novy, R.G., Whitworth, J.L., Stark, J.C., Love, S.L., Corsini, D.L., Pavek, J.J., Vales, M.I., James, S.R., Hane, D.C., Shock, C.C., Charlton, B.A., Brown, C.R., Knowles, N.R., Pavek, M.J., Brandt, T.L., Gupta, S., Olsen, N. 2010. Clearwater Russet: A Dual-Purpose Potato Cultivar with Cold Sweetening Resistance, High Protein Content, and Low Incidence of External Defects and Sugar Ends. American Journal of Potato Research. 87:458-471.

Developing resistance to lettuce dieback and with long shelf life in fresh cut lettuce mixes. Resistance to lettuce dieback, caused by *Lettuce necrotic yellows tombusvirus*, has become essential for the Salinas Valley of California, the major U.S. lettuce growing area. Standard field screening methods for host-plant resistance are time-consuming, and frequently yield lines with very short shelf life in fresh cut salad mixes. ARS researchers in Salinas, California, developed a PCR-based molecular marker linked to the lettuce dieback resistance gene that eliminates the need for field-based testing. Two advanced lettuce breeding lines were released with complete resistance to dieback and significantly improved shelf life, and seeds have been provided to lettuce-breeding companies for further improvement.

Simko, I., Hayes, R.J., Subbarao, K.V., Sideman, R.G. 2010. SM09A and SM09B: Romaine Lettuce Breeding Lines Resistant to Dieback and with Improved Shelf-life. HortScience,Year 2010, volume 45, pages 670-672.

Root-knot nematode resistant new pepper varieties. ARS researchers in Charleston, South Carolina, transferred root-knot nematode resistance from Scotch Bonnet-type pepper into a yellow-fruited Habanero-type pepper, and released the yellow-fruited, root-knot nematode resistant, breeding line PA-560; a root-knot nematode resistant pimento-type pepper breeding line PA-566; and ‘Truhart Perfection’ cultivar. The latter is recommended for breeding root-knot nematode resistant cultivars for the organic, specialty crop, and home garden markets.

Fery, R.L., Thies, J.A. 2011. PA-566, A Root-knot Nematode Resistant, Pimento-type Pepper. HortScience. 46(4):668-669.

Fery, R.L., Thies, J.A. 2010. PA-559, a Root-knot Nematode Resistant, Red-fruited, Habanero-type Pepper. HortScience. 45(5):822-823.

New pecan cultivars for U.S. growers. The important U.S. pecan nut crop is primarily grown by small producers, so there is ongoing need for new pecan varieties to meet specific market niches to enhance marketability and profitability. ARS researchers in College Station, Texas, released to commercial growers and homeowners two new, improved pecan cultivars with early nut maturity and resistance to pecan scab disease that are suited for both the in-shell and shelling components markets.

Thompson, T.E., Grauke, L.J. 2010. 'Mandan' Pecan. HortScience. 45(9):1392-1393.

New pear varieties provide needed crop diversity. The lack of genetic diversity in current commercially grown pear cultivars limits opportunities to capitalize on specialty markets and consumer demand for new high quality fruit. ARS researchers in Kearneysville, West Virginia, and collaborators in California and Oregon, developed two cultivars, 'Sunrise' and a new selection yet to be named. These new releases were rated very high in consumer preference tests, and Sunrise is resistant to the devastating fire blight disease. The new, unnamed selection will provide an attractive new cultivar which can be stored for long periods and handled with minimal damage.

New improved peach rootstock and scions. 'Sharpe' rootstock, bred by ARS researchers in Byron, Georgia, is the first Armillaria (*A. tabescens*) resistant rootstock for peach in the United States, and is also more resistant to peach tree short life, the leading cause of peach mortality, and root-knot nematodes than is the industry standard. Two additional new varieties developed by ARS researchers bear fruit firmer and more attractive than industry standards. These new releases provide industry with long-needed, more reliable alternatives to older peach varieties.

Beckman, T.G., Chaparro, J.X. and Sherman, W.B.. 2008. 'Sharpe', a Clonal Plum Rootstock for Peach. HortScience 43(7):2236–2237. 2008.

Disease resistant citrus rootstocks. A complex disease and insect syndrome, the Phytophthora-Diaprepes root weevil disease complex, has destroyed thousands of acres of Florida citrus. ARS researchers in Fort Pierce, Florida, bred two promising new hybrid citrus rootstocks (US-802 and US-897) with field tolerance to the disease complex, combined with good fruit productivity. These new rootstocks confer dramatically different effects on fruit tree size, thereby providing a range of new management options for commercial citrus producers.

Evidence to tolerance to citrus greening disease in commercial citrus. Using titer of the *Ca. Liberibacter asiaticus* (*CL*as), the suspected causal agent of huanglongbing (HLB) or citrus greening disease, correlated with greening disease ratings, ARS scientists in Fort Pierce, Florida, demonstrated scion-specific differences regardless of location. The tangor variety 'Temple' showed the most consistently low incidence of HLB symptoms and CLas titer, whereas the tangor variety 'Murcott' and the tangelo variety 'Minneola' had the highest symptoms and CLas titer. The research indicates that there may be useful sources of tolerance to greening in current, commercial citrus cultivars.

Stover, E. and McCollum, G. 2011. Incidence and severity of Huanglongbing and Candidatus Liberibacter asiaticus Titer among Field-infected Citrus Cultivars. Hortscience 46(10):1–5.

New rootstock compatible with both stone fruits and almonds. A successful orchard requires trees having a rootstock that can resist infection of soilborne diseases and pests, as well as providing trees with water and nutrients necessary for a bountiful harvest. When an orchard is replanted with the same rootstock as was previously used, tree vigor can suffer and yields are generally reduced. ARS researchers released three new clonal rootstocks compatible with stone fruits and almonds. The new rootstocks are resistant to the prevalent root knot nematode, and produce trees of slightly smaller stature, which helps to reduce pruning costs, but fruit/nut bearing capability is not affected. Growers can expect enhanced growth of young trees when these rootstocks are utilized in previously planted orchard sites.

Ledbetter, C.A., Sisterson, M.S. 2007. Advanced Generation Peach-Almond Hybrids as Seedling Rootstocks for Almond: First Year Growth and Potential Pollenizers for Hybrid Seed Production. Euphytica. 160(2): 259-266.

New apple rootstocks released to industry. ARS researchers in Geneva, New York, released four new apple rootstocks that confer dwarfing traits tailored for high density production, fire blight resistance, and tolerance to *Phytophthora cactorum* and wooly apple aphid. These new rootstocks will replace less productive industry standard varieties.

Fazio, G., Robinson, T.L., Aldwinkle, H.S. 2010 Notice of release of G.210, apple rootstock. www.goodfruit.com/Good-Fruit-Grower/May-15th-2010/Geneva-releases-FOUR-new-rootstocks/

Superior new strawberry cultivar for the Pacific Northwest. A new strawberry cultivar, 'Valley Red,' that bears fruit with outstanding processing characteristics, including deep red internal and external color, good flavor, easy capping, and excellent quality after freezing. Those traits, combined with excellent yields of very uniform fruit on virus-tolerant plants, will give growers superior new germplasm for commercial production in the Pacific Northwest.

Finn, C.E., Moore, P.P., Kempler, C., Yorgey, B., Strik, B., Martin, R.R. 2009. 'Valley Red' Strawberry. HortScience. 44(5):1468-1471

New blackberry cultivars. ARS researchers in Corvallis, Oregon, bred a new blackberry cultivar, 'Newberry,' for production in California and the Pacific Northwest. This new variety has already earned the acceptance of major fresh market wholesale distributors because of its prolificacy and outstanding flavor. The researchers also bred ‘Wild Treasure,’ a thornless, small-fruited cultivar with superb flavor and appearance, which is best suited for mechanical harvesting and processing. It is grown mainly in Oregon and marketed for its small fruit size that fits better into bags of frozen berry mixes. Finally, they also released ‘Onyx,' now grown in the Pacific Northwest and California. Onyx is high-yielding with very high quality fruit in late season that is particularly suited to the wholesale fresh fruit market.

Finn, C.E., Strik, B.C., Yorgey, B., Martin, R.R., Stahler, M. 2010. 'Newberry' trailing blackberry. HortScience. 45(3):437-440.

Finn, C.E., Strik, B.C., Yorgey, B.M., Martin, R.R. 2011. 'Onyx' trailing blackberry. HortScience. 46:657-659.

Machine-harvestable pest resistant raspberry cultivars. ARS researchers in Corvallis, Oregon, along with colleagues at Agriculture and Agri-Foods Canada and Washington State University, developed the new red raspberry cultivar ‘Saanich’ that produces a very high yield of medium-sized, firm, sweet fruit, suited to machine harvesting, and which can be individually quick frozen. This cultivar is well adapted for the processing and fresh markets and resistant to the common strain of the North American raspberry aphid. The researchers also released ‘Cascade Bounty’ that is highly tolerant to root rot and produces high yields of mid- to late-season fruit on sites with severe root rot infestations. The fruit is recommended for machine harvesting, if intended for processing. More than 470,000 plants of Saanich and Cascade Bounty have been planted to the benefit of commercial red raspberry growers in the Pacific Northwest.

Kempler, C., Daubeny, H.A., Harding, B., Baumann, T., Finn, C.E., Moore, P.P., Sweeney, M., Walters, T. 2007. 'Saanich' red raspberry. HortScience. 42:176-178.

New southern highbush blueberry. ARS researchers in Stoneville, Mississippi, released ‘Pearl,’ a new highbush blueberry cultivar with good yield potential and large, attractive, light blue, firm berries with excellent flavor and small picking scars. Pearl has low-to-moderate chilling requirements and performs well in the Gulf coast region and in other areas where southern highbush blueberry cultivars are grown successfully. Growers will benefit from the early ripening that fits fresh market opportunities.

Stringer, S., Draper, A., Spiers, J. 2010. Pearl' Southern Highbush BlueberryC. 2002. International Journal of Fruit Science.

Anticipated Product 3: ***New alternative crops for diverse management and market systems for crop producers.***

"Energy cane" varieties. With an ever-increasing interest in sugarcane as an energy crop, sugarcane breeders around the world are developing "energy cane" cultivars as a biofuel feedstock, while continuing to genetically improve sugarcane primarily as a sucrose source. Collaborating with Louisiana State University colleagues, ARS researchers in Houma, Louisiana, released three high-fiber sugarcane varieties that excel in total solids accumulation. These new varieties provide various market system options: very high-fiber, low-sugar; high-fiber, moderate-sugar; and traditional moderate-fiber, high-sucrose sugarcane, that satisfy individual interests and regional constraints.

Hale, A.L. 2010. Notice of release of a high fiber sugarcane variety Ho 02-113. Sugar Bulletin. 88(10):28-29.

Natto and high protein soybeans for specialty markets. ARS researchers in Raleigh, North Carolina, have released a soybean cultivar, N8101, with 25 percent exotic germplasm in the pedigree that is suited to natto soybean export markets. This cultivar, with the smallest seeds of any cultivar ever released in the southern United States, adds diversity to the narrow genetic range of soybean breeding materials for this region. They also released N6202**,** a new high-yielding, high protein soybean breeding line for niche export markets with 50 percent exotic germplasm. This line has overcome the low yield trait that, up to now, has limited production of high protein soybean varieties adapted to the South.

Carter Jr, T.E., Burton, J.W., Rzewnicki, P.E., Villagarcia, M.R., Fountain, M.O., Taliercio, E.W.,

Bowman, D. 2009. Registration of 'N8101' small-seeded soybean. Journal of Plant Registrations, 3:22-27.

Carter Jr, T.E., Rzewnicki, P.E., Burton, J.W., Villagarcia, M.R., Bowman, D.T., Taliercio, E.W., Kwanyuen, P. 2010. Registration of N6202 soybean germplasm with high protein, good yield potential, large seed and diverse pedigree. J. of Plant Registrations. 4:77-79.

Feed and malting barley cultivars. Higher yielding feed barley cultivars were released by ARS researchers that are adapted to both dryland and irrigated regions. Also, a new malting barley cultivar was released with higher yields and that is adapted for the Intermountain west.

Obert, D.E., Wesenberg, D.M., Burrup, D.E., Windes, J.M., Erickson, C.A., Whitmore, J.C. 2008. Registration of 'Tetonia' barley. Journal of Plant Registrations. 2:10-11

Obert, D.E., Evans, C.P., Windes, J.M., Wesenberg, D.M., Ullrich, S., Budde, A.D., Chen, X., Jackson, E.W. 2009. Registration of Endeavor barley. Journal of Plant Registrations. 3:124-126.

Low phytate barley to reduce negative environmental impact of feed waste. Enhancing phosphorus utilization in livestock production is critically important to both animal productivity, and to managing the environmental impact of agricultural production. A feed-grain barley variety was developed with high available phosphorous and low phytate to enhance animal production efficiency, while reducing the phosphorus content of animal waste.

Jackson, C.A., Windes, J.M., Bregitzer, P.P., Obert, D.E., Price, W., Bradford, B. 2009. Low Phytic Acid Barley Responses to Phosphorus Rates. Crop Science. 49:1800-1806.

Bregitzer, P.P., Raboy, V., Obert, D.E., Windes, J., Whitmore, J. 2008. Registration of 'Clearwater' Low-Phytate Hulless Spring Barley. Journal of Plant Registrations. 2: 1-4.

Anticipated Product 4: *Flexible, crop-specific procedures for releasing and protecting plant material.*

Innovative and creative partnerships are required to effectively transfer new crop varieties and improved germplasm to an increasing diverse clientele. ARS has benefitted for many years from strong partnerships with land grant universities and their state-supported foundation seed programs. As budgets shrink, universities have started protecting and licensing their varieties to generate financial support for their breeding programs. The private sector has also expanded their research partnerships with ARS and universities. These joint research programs have become critical to ensuring successful transfer of varieties and improved germplasm bred by ARS researchers, so that U. S. agriculture can benefit from that breeding and genetic research. These partnerships can take the form of Cooperative Research and Development Agreements (CRADAs), Trust Agreements, joint intellectual property rights protection and licensing of inventions, and Cooperative Research Agreements. These agreements seek to provide incentives for partners to collaborate with ARS research programs, and to encourage commercial acceptance for ARS cultivars and plant products so that commodity interests and society as a whole benefits.

EXAMPLES OF RECENT NP 301 RESEARCH PARTNERSHIPS:

Northwest (Tri-State) Potato Variety Development Program. This cooperative program draws on plant genetics, agronomy, and other scientific disciplines to breed new, improved potatoes. The program pools resources and expertise from ARS, Oregon State University, Washington State University, and the University of Idaho. Each state's potato commission and industry representatives also participate. Old potato varieties are being replaced by new varieties from the Tri-State Program. In 2008, Northwest potato acreages planted with Tri-State varieties include 21 percent in Idaho; 29 percent in Oregon; and 42 percent in Washington. In 2009, three new varieties were released through this program, one of which offers 38 percent more protein than Russet Burbank and excellent fry color out of storage.

Trust agreement with New Varieties Development and Management Corp. for advanced citrus scion germplasm evaluation. The New Varieties Development and Management Corp. (NVDMC) has partnered with ARS researchers in Fort Pierce, Florida, to assist in the testing and evaluation of citrus scion materials for commercial markets. Growers will provide valuable data and input regarding the commercial acceptance of citrus clones, and thus will speed the evaluation process for ARS citrus breeders. This partnership will help the national citrus industry remain competitive in the face of new pests and diseases that threaten the survival and productivity of the Florida citrus industry.

The Germplasm Enhancement of Maize Project. The Germplasm Enhancement of Maize Project (GEM), a long-term partnership among ARS, land grant universities, private industry, and international and non-governmental organizations seeks to widen the germplasm base of commercial hybrid corn in the United States through the introduction and incorporation of novel and useful germplasm gathered from around the globe. In addition to the Federal support of the GEM Project, seed companies contribute more than $500,000 of annual in-kind support, and have granted access to proprietary germplasm worth billions of dollars. Despite the corporate consolidation in the seed industry, more than 30 companies and 25 public research programs (mainly universities) are currently GEM Project cooperators. During the last 5 years, ARS GEM researchers and their cooperators have identified more than 30 hybrids that equal or surpass the performance of commercial check lines, and that contain unique starch, protein, and oil properties; drought stress-tolerance; and outstanding silage productivity. More than 234 GEM lines with superior performance have been formally released and distributed freely to maize researchers worldwide.

Genetic Diversity Consortium for Saccharum and Perennial Grasses. Based on the organizational model of the GEM Project, this consortium will develop a pipeline of superior and genetically diverse sugarcane varieties and related bioenergy feedstocks. Despite the high quality of current ARS sugarcane varieties, new genetic diversity is needed to overcome biotic and abiotic constraints to production. A pilot program has been initiated, involving ARS, university, and private partners for sugarcane and energy cane germplasm enhancement. The resulting hybrid clones from crosses will be released as public germplasm and shared among members. ARS sugarcane clones have greatly enhanced the U.S. and international sugarcane industries, i.e., more than 95 percent of Florida sugarcane acreage is planted in ARS varieties. Additional opportunities are likely to exist beyond the initial Consortium efforts for further research agreements and commercialization.

National Grape and Wine Initiative. Through close collaboration with and support from the table grape, raisin, wine, and juice sectors of the grape industry, ARS researchers have released enhanced grape germplasm, rootstocks, and varieties aimed at meeting the needs for pest and disease resistance, new market niches, and more efficient (less labor and energy intensive) production systems. In addition, two varieties were released that were developed as tools for genetic studies. The raisin, rootstock, research, and Muscadine grape varieties developed since 2006 are public releases without patent protection. ARS table grape varieties that were released prior to the 2006 start date for this report have included both public releases and exclusive license of patented varieties. A patent application has recently been filed for a new table grape variety. The success of the ARS table grape breeding program can be seen in the current market share of California production. Seven of the top 10 highest volume table grape cultivars based on 2009-2010 box shipment numbers were developed by ARS researchers.

EXAMPLES OF NP 301 PRODUCTS RESULTING FROM PARTNERSHIPS:

Development of low allergen soybean seeds and commercialization with industry partners - United States Patent pending. ARS researchers from Columbia, Missouri, with their university colleagues, applied molecular markers for the P34 allele to develop low allergen soybean seeds. A mutation in the gene encoding the P34 protein in soybean which affects allergenicity was characterized, and assays were developed for this four-base-pair insertion and the mutant allele. This is being commercialized in the livestock industry.

Bilyeu, K.D., Ren, C., Nguyen, N.T., Herman, E. and Sleper, D.A. 2009. Association of a four basepair insertion in the P34 gene with the low allergen trait in soybean. The Plant Genome, 2(2):141-148.

Japan's deregulation of transgenic papaya. On September 1, 2011, Japan regulatory agencies announced the deregulation of the transgenic papaya in Japan. The *Papaya ringspot virus* (PRSV) resistant transgenic papaya, developed by ARS researchers and colleagues from Cornell University and the University of Hawaii helped save the small-farm-based Hawaiian papaya industry previously decimated by PRSV. After a 3-month waiting period, commercial shipment of transgenic papaya to Japan will be permitted, commencing in December 2011. PRSV is one of the most destructive diseases of papaya, occurs in nearly every region where papaya is grown. It is a major limiting factor for commercial papaya production in Hawaii, and regions of Asia and Latin America. This new variety became the first commercialized transgenic fruit crop and has been in commercial production in Hawaii since 1998. It now accounts for 80 percent of the papaya grown in Hawaii.

COMPONENT 4: Plant Biological and Molecular Processes

Under this component, ARS scientists conduct fundamental – often high-risk – research that generates an enhanced knowledge base and devises superior research tools that ultimately contribute to greater crop productivity and efficiency, better product quality and safety, improved protection against pests and diseases, enhanced tolerance to abiotic stress, and sustainable practices that maintain or enhance environmental quality.

Historically, crop breeding has successfully improved crops through empirical approaches, often unaided by fundamental knowledge of how the genes underlying key traits are controlled. The modern tools of biology offer new tools for improving plants either by exploiting existing variability in plant properties or by creating new variability when warranted. Knowledge of how plant biological mechanisms can be precisely modified will lead to new and potentially more effective approaches to crop genetic improvement. As the scientific principles that link molecular and genetic phenomena to phenotype are understood, the pace of crop breeding should be accelerated and its efficiency enhanced.

Translating research at the molecular level into information that can solve agricultural problems demands an integrated approach that exploits how variability in crop traits depends on gene expression and gene networks that enable the crop to respond to the environment. This Research Component must provide the means to coordinate and integrate the fundamental plant research with more applied approaches to develop focused strategies for solving specific problems. This area of research is often considered long-term, high-risk in nature, so it especially requires strong partnerships with other Federal, university, industry, and international partners who can contribute resources and expertise to substantially enhance the strong "public-good" impact of this component's outputs.

As mentioned in the introduction to this report (See page 3), the research within NP 301 Component 4 was formerly National Program 302, Plant Biological and Molecular Processes. In 2010, the NP 302 program was merged with NP 301. For the purpose of the current retrospective review, a sampling of NP 301 Component 4 accomplishments since 2010 is briefly described here to illustrate the synergy and complementarity that the former NP 302 projects have brought to NP 301. The examples were chosen to illustrate the unique research capacities that are now a part of and complement other research within NP 301.

Problem Statement 4A: *Functional Utilization of Plant Genomes – Translating Plant Genomics into Crop Improvement*

The focus of this problem statement is on applying the knowledge gained from analyses of model plants to crop genetics and genetic improvement. Translating the results of research at the molecular level into information that can catalyze solutions to constraints to crop productivity requires an integrated approach in which the experimental design may range from knowledge development at the level of gene ensembles, to yield testing in the field. As the following examples illustrate, the research conducted under this problem statement provides the means for such an integration of research.

Combinatorial code revealed for ethylene production. The key enzyme in the two-step biosynthetic pathway of ethylene, the plant signaling molecule, is 1-aminocyclopropane-1-carboxylate synthase (ACS). ARS scientists in Albany, California, with colleagues from the Salk Institute and the University of California, Riverside, analyzed the entire family of nine ACS isoforms encoded in the *Arabidopsis* genome. Phenotypic characterization of single and multiple mutants unmasked unique, but overlapping, functions in plant development for the various ACS isoforms – including multiple growth characteristics, flowering time, response to gravity, disease resistance, and ethylene production. A combinatorial code determined by the interaction of isoforms regulates plant development through ethylene production. The fundamental knowledge gained by this research has the potential of accelerating genetic gain for those traits in crop plants.

Tsuchisaka, A., Yu, G., Jin, H., Alonso, J.M., Ecker, J.R., Zhang, X., Gao, S., Theologis, A. 2009. A Combinatorial Interplay Among the 1-Aminocyclopropane-1-Carboxylate Isoforms Regulates Ethylene Biosynthesis in Arabidopsis thaliana. Genetics 183, 979-1003.

Genome-wide identification of microRNA networks in maize. ARS scientists in Columbia, Missouri Ithaca and Cold Spring Harbor, New York, and university collaborators, conducted a genome-wide survey of maize microRNA (miRNA) genes, identified 150 high-confidence genes within 26 miRNA families, and characterized their structure, expression, and evolution. Expression was verified for 25 families by deep-sequencing of small RNA libraries from an assortment of maize tissues. Comparison of mature miRNA sequences from diverse maize inbred lines and ancestral teosinte accessions identified those sequences which were subject to strong "purifying" selection, and also putative miRNA targets for coordinated regulation, many of which involving genes for regulatory and metabolic pathways.

Zhang, L., Chia, J.M., Kumari, S., Stein, J.C., Liu, Z., Narechania, A., Maher, C.A., Guill, K., McMullen, M.D., Ware, D. 2009. A genome-wide characterization of microRNA genes in maize. PLoS Genet 5, e1000716.

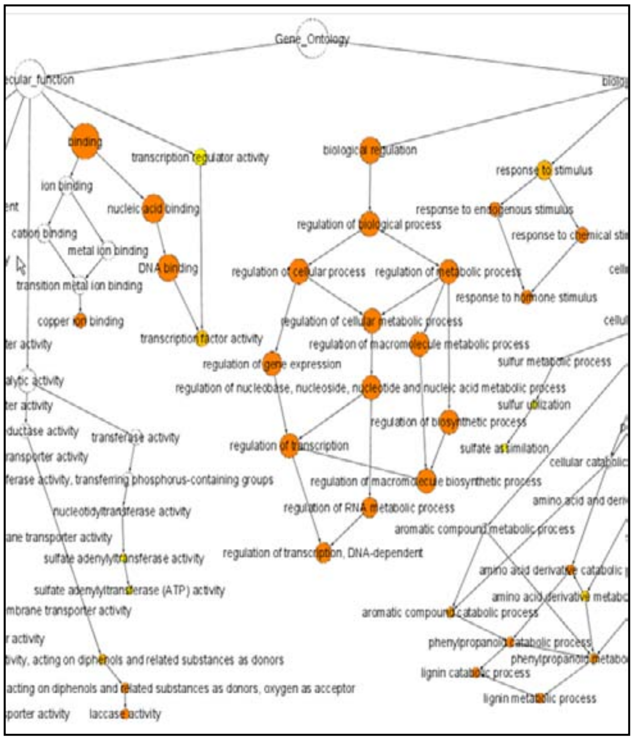

Figure 12. Schematic representation of the classification of maize microRNAs target gene functions as annotated using maize Gene Ontology terms. The size of a node is proportional to the number of targets in the GO category. The color represents enrichment significance; the deeper the color on a color scale, the higher the enrichment significance.

Genetic control of plant stem cell development. Plant architecture consists of repeated morphological units, called phytomers, which comprise a stem, leaf, and axillary bud. ARS researchers in Albany, California, and Cold Spring Harbor Laboratories, New York, showed that tasselsheath4, a maize microRNA-targeted transcription factor, plays a critical role in defining the boundaries in the phytomer that segregate cells into compartments. Subsequent cell fate decisions fix cell identities, and ultimately determine plant architecture, a critical aspect of crop adaptation and productivity.

Chuck, G., Whipple, C., Jackson, D., Hake, S. 2010. The maize SBP-box transcription factor encoded by tasselsheath4 regulates bract development and the establishment of meristem boundaries. Development 137, 1243-1250.

First plant interactome map. Plants have unique features that evolved in response to their environments and ecosystems. ARS scientists at Cold Spring Harbor, New York, as part of an international consortium of experts, constructed the first plant interactome map that contains 6,200 highly reliable interactions for 2,700 proteins from *Arabidopsis thaliana*. The interactome map serves as a protein network level model for how plant proteins physically interact and reflect how cells organize biochemical and biophysical processes and convey molecular signals. This, and future, plant interactome maps should facilitate development of systems approaches to better understand how crops function physiologically.

Arabidopsis Interactome Mapping Consortium. 2011. Evidence for Network Evolution in an Arabidopsis Interactome Map. Science 333, 601-607.

Lysine acetylation is a widespread modification for diverse proteins in *Arabidopsis*. ARS researchers in Urbana, Illinois, found that reversible lysine acetylation, a form of reversible protein post-translational modification previously known only for histone regulation in plants, might be an important and previously unknown regulatory mechanism for many non-histone proteins affecting a wide range of pathways and processes in all plants. Sixty-four lysine modification sites were identified for 57 proteins, which operate in a wide variety of pathways/processes and are located in various cellular compartments.

Wu, X., Oh, M.H., Schwarz, E. M., Larue, C.T., Sivaguru, M., Imai, B.S., Yau, P.M., Ort, D., Huber, S.C. 2011. Lysine Acetylation Is a Widespread Protein Modification for Diverse Proteins in Arabidopsis. Plant Physiology 155, 1769-1778.

Problem Statement 4B: Biological Processes that Improve Crop Productivity and Quality

Research in this problem statement is focused on three areas of plant biology and molecular processes: understanding plant growth; development and interactions with the environment; and developing high-value plant products. Because of the unifying impact of genomic and bioinformatics technology on crop genetics, physiology, and developmental biology, the research conducted under this problem statement has converged and has contributed strongly to the accomplishments reported for other NP 301 problem statements, most notably in 2B, 2C, 3A, and 3B. Consequently, the following accomplishment was chosen to illustrate unique NP 301 research achievements that have not been discussed elsewhere in this report.

Transcriptional control of metabolic shifts. The maturation of fleshy fruits, such as tomato, is tightly controlled genetically. ARS researchers at Ithaca, New York and colleagues from U. S. and German research institutions applied quantitative real-time polymerase chain reaction analysis of expression patterns for 1,000 tomato transcription factors, cDNA microarray analysis, and metabolite profiling of primary and secondary metabolites with GC-MS and LC-MS, respectively, to identify metabolites that are under the control of these transcription factors. These pioneering gene expression analyses provide new insights to fruit development which can be exploited for crop genetic improvement.

Rohrmann, J., Tohge, T., Alba, R., Osorio, S., Caldana, C., McQuinn, R., Arvidsson, S., van der Merwe, M.J., Riaño-Pachón, D.M., Mueller-Roeber, B., Fei, Z., Nesi, A.N., Giovannoni , J.J., Fernie, A.R. 2011. Combined transcription factor profiling, microarray analysis and metabolite profiling reveals the transcriptional control of metabolic shifts occurring during tomato fruit development. Plant Journal. Accepted manuscript online.

Problem Area 4C: Plant Biotechnology Risk Assessment

Research conducted for this problem statement is focused on improving and assessing the potential risks associated with genetic engineering technology; and the interactions of transgenic plants with their environment. This research area is unique not only within this National Program, but is also a distinct element within the ARS research portfolio. The research goals for Problem Statement 4C are strongly coordinated with the interagency (ARS and NIFA) Biotechnology Risk Assessment Grant (BRAG) Program.

Virus resistant biotech plum 'C5 HoneySweet' is advanced through deregulation. ARS scientists in Kearneysville, West Virginia, have developed, evaluated, and successfully guided a genetically engineered, plum pox virus-resistant plum tree cultivar 'C5 HoneySweet Plum' through the U. S. regulatory process. C5 HoneySweet Plum was deregulated by APHIS in 2007; by the Food and Drug Administration in January 2009, and registered by the EPA in May 2010. In August 2011, ARS submitted a request to the EPA that C5 HoneySweet Plum be exempted from regulation under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). HoneySweet is one of the few genetically engineered crop varieties to be developed by the public-sector. The EPA registration document is at: www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006354.pdf.

Manipulation of omega-5 gliadin genes in 'Butte 86', a commercial wheat variety. ARS scientists in Albany, California, designed an RNA interference strategy to silence the expression of genes encoding omega gliadins that trigger the food allergy termed wheat-dependent exercise-induced anaphylaxis (WDEIA). The U.S. bread wheat 'Butte 86' was chosen for transformation because a comprehensive proteome map existed for this variety in which flour proteins were distinguished by mass spectrometry and associated with specific gene sequences. Analysis of proteins in grain from transgenic plants demonstrated that the omega-5 gliadins were either absent or substantially reduced relative to non-transformed controls. The ability to genetically transform ‘Butte 86’ enabled flour composition to be changed in a targeted manner in a commercial U.S. wheat cultivar, and should accelerate future research on wheat flour quality and immunogenic potential.

Altenbach, S.B., and Allen, P.V. 2011. Transformation of the US bread wheat ‘Butte 86’ and silencing of omega-5 gliadin genes. GM Crops 2, 66-73.

Transgene containment technologies. Transgene escape, a major environmental and regulatory concern in transgenic crop cultivation, could be alleviated by removing transgenes from pollen, the most frequent vector for transgene flow. To do so, ARS scientists in Albany, California, tested a codon-optimized CinH recombinase and its recognition sites RS2 in tobacco in combination with a pollen specific promoter. For three transgene events, less than 1 percent of the test pollen contained the target gene, compared to 75 percent of controls. In addition, three additional recombinase systems were optimized for use in plants – phiC31, Bxb1, and ParA. These novel recombination systems will provide the biotechnology industry convenient options for removal of unwanted DNA and more precise genomic engineering of transgenic crop plants prior to commercialization.

Moon, H.S., Abercrombie, L.L., Eda,S., Blanvillain, R., Thomson, J.G., Ow, D.W. and Stewart, C.N. 2011. Transgene excision in pollen using a codon optimized serine resolvase CinH-RS2 site-specific recombination system. Plant Molecular Biology, 75, 621-631.

Thomson, J.G., Chan, R., Thilmony, R., Yau, Y.-Y., and Ow, D.W. 2010. PhiC31 recombination system demonstrates heritable germinal transmission of site-specific excision from the Arabidopsis genome. BMC Biotechnology 10, 17.

Pollinator mediated geneflow. Validated geneflow management strategies are important for ensuring successful coexistence between the production of genetically-engineered crops and conventional crops. ARS researchers in Madison, Wisconsin, examined the impact of pollinators on gene flow in alfalfa, a crop that can be visited by different bee species. Honeybees had the greater potential risk for carrying pollen from transgenic alfalfa to non-transgenic alfalfa, relative to three wild bumble bee species. The potential for gene flow mediated by honeybees was not affected by plant density although, for the three wild bumble bee species, it was highest in high-density fields.

Brunet, J. and Stewart, C.M. 2010. Impact of Bee Species and Plant Density on Alfalfa Pollination and Potential for Gene Flow. Psyche 2010, Article ID 201858, 7 pages.

Durable benefits of Bt maize. ARS scientists in Ames, Iowa, and university, State agency, and industry colleagues, documented that area-wide suppression of the European corn borer with Bt maize generated economic benefits for non-Bt maize growers. Cumulative benefits during 14 years were estimated at $3.2 billion in Illinois, Minnesota, and Wisconsin, with more than $2.4 billion accruing to non-Bt maize growers. Comparable estimates for Iowa and Nebraska are $3.6 billion in total, with $1.9 billion for non-Bt maize growers. These results affirm theoretical predictions of pest population suppression and highlight economic incentives for growers to maintain non-Bt maize refugia for sustainable insect resistance management.

Hutchison, W. D.,Burkness, E. C.,Mitchell, P. D.,Moon, R. D.,Leslie, T. W.,Fleischer, S. J.,Abrahamson, M.,Hamilton, K. L.,Steffey, K. L.,Gray, M. E.,Hellmich, R. L.,Kaster, L. V.,Hunt, T. E.,Wright, R.,J.,Pecinovsky, K.,Rabaey, T. L.,Flood, B. R.,and Raun, E. S. 2010. Areawide Suppression of European Corn Borer with Bt Maize Reaps Savings to Non-Bt Maize Growers. Science 330, 222-225.