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National Program 302: Plant Biological and Molecular Processes

Accomplishment Report 2004-2009

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Cover Photo: Barley GeneChip probe assay data from a NP 302 research project (Ames, Iowa). Parallel profiling reveals a link between basal and gene-specific resistance. "Cutting Edge TranscriptomeAnalysis: It's all about design". **Plant Cell** 16:2249-2251 (2004)

NATIONAL PROGRAM 302
Plant Biological and Molecular Processes

BACKGROUND AND GENERAL INFORMATION

The vision for the USDA-ARS National Program (NP) 302 Plant Biological and Molecular Processes is to conduct scientific research that leads to tomorrow's advances in crop production, quality, and safety. The NP 302 mission is to conduct fundamental research on plants that forms the basis for greater crop productivity and efficiency, better product quality and safety, improved protection against pests and diseases, and sustainable practices that maintain environmental quality. This National Program's mission follows the USDA, Agricultural Research Service Strategic Plan (see http://www.ars.usda.gov/SP2UserFiles/Place/00000000/ARSStrategicPlan2006-2011.pdf),which in turn, is directed towards achieving goals mandated by the USDA Research, Education, and Extension Mission Area Strategic Plan and the USDA Strategic Plan for 2006-2011 (see http://www.ocfo.usda.gov/usdasp/sp2005/sp2005.pdf).

The products of research conducted in this national program contribute toward broader goals (termed "Actionable Strategies" or targets) associated with the following specific Performance Measures from the ARS Strategic Plan for 2006-2011 and the previous ARS Strategic Plan for 2003-2007. These include:

ARS Strategic Plan 2006-2011 Objective 2.2: Increase the Efficiency of Domestic Agricultural Production and Marketing Systems

Performance Measure 2.2.3: Expand, maintain, and protect our genetic resource base, increase our knowledge of genes, genomes, and biological processes, and provide economically and environmentally sound technologies that will improve the production, efficiency, health, and value of the Nation's crops.

Examples of Actionable Strategies:

- Describe the structure, function, and regulation of agriculturally important genes and their protein products in model plants and crop plants.
- Identify new genetic methods and tools to identify specific genes that mediate end product traits desired by consumers, such as nutritional content, oil, grain quality, disease resistance, and stress tolerance in agricultural crops.
- Improve plant genetic transformation systems to expand their usefulness, and improve exploitation of genome sequence information to identify valuable genes in raw germplasm collections.

ARS Strategic Plan 2003-2007 Objective 1.2: Contributions to the Efficiency of Agricultural Production Systems

Performance Measure 1.2.5: Provide producers with scientific information and technology that increases production efficiency, safeguards the environment, and reduces production risks and product losses. **Target:** Technology will be developed that optimizes management practices for sustainable production with available soil microbial, mineral nutrient (including carbon and nitrogen), and water resources. Production systems and technologies that harness genetic potential will be developed to maximize profits and provide secure supply and market competitiveness.

Performance Measure 1.2.6: Improve understanding of the biological mechanisms that influence plant growth, product quality, and marketability to enhance the competitive advantage of agricultural commodities. **Target:** Information will be available to guide manipulation of regulatory metabolic processes that influence plant growth, product composition, product quality, and profitability.

Performance Measure 1.2.7: Identify genes responsible for plant product quality and resistance to disease, pests, and weather losses. **Target:** A more complete understanding will be developed of the structure and function of genes responsible for quality, growth, and health of crops and how those individual genes are regulated in the context of gene systems or networks.

Actionable Strategies are not included from the ARS Strategic Plan 2003-2007.

The rationale for NP 302 is based on the fact that plant growth and development is the result of the coordinated expression of genes and gene networks within a given environment which gives rise to a multiplicity of phenotypes. Progress in crop improvement requires that agricultural scientists understand the genomic, genetic, biochemical, and physiological principles governing phenotype. As such, NP 302 was intentionally constituted differently from most other National Programs by its emphasis on fundamental knowledge of biological and molecular mechanisms underpinning long-term advances in crop production, protection, product value, and food safety.

PLANNING AND COORDINATION FOR NP 302

USDA-ARS National Programs follow a five-year program cycle, initiated by a Customer and Stakeholder Workshop. This is the second 5-year cycle of National Program 302. The cycle began with a Customer and Stakeholder Workshop in April, 2004 in St. Louis, Missouri. ARS scientists and administrators met with customers, stakeholders, and partners and identified major crop agricultural issues and research priorities. At the Workshop, three major research components for the NP 302 were identified that serve as the basis for the NP 302 Action Plan (see http://www.ars.usda.gov/NP302/ActionPlan). A list of relevant research planning, coordination, and stakeholder workshops is provided in Appendix 1.

The NP 302 Action Plan was drafted by a writing team comprised of ARS scientists and national program leaders in the USDA-ARS Office of National Programs. The writing teams combined input from workshops, their own knowledge of the science subject matter, and input from other ARS scientists and cooperators to identify the key, priority needs that could be addressed by ARS research. These needs were aggregated into Problem Areas for each NP Research Component. After a public comment period, the draft Action Plan was revised and completed in December 2004.

After the Action Plan was completed, ARS project teams each developed a Project Plan. All Project Plans included statements of the agricultural problem being addressed, the anticipated products or information to be generated by the Project, how the planned research contributed to solving the larger National Program problem areas, and time lines and milestones for measuring progress toward achieving the Project objectives. All Project Plans associated with NP 302 were then evaluated for scientific quality by external peer panels. The project peer reviews were handled by the ARS Office of Scientific Quality Review. Project Plans were revised in response to review panel recommendations, and then implemented. Project Plans were approved for the period of 2006-2011.

Day-to-day coordination of this ARS National Program in Plant Biological and Molecular Processes is the task of the National Program Leaders who comprise the NP 302 Leadership Team. The National Program Leaders also coordinate NP 302 with other ARS National Programs and with other agencies and departments. Much of the interagency coordination is conducted through the Interagency Working Group on Plant Genomes, which includes leaders from USDA-CSREES (Cooperative State Research, Education, and Extension Service), 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).

Ongoing coordination and planning during this NP 302 Program Cycle, is led by USDA-ARS Office of National Programs, Area Offices, and NP 302 scientists. Multiple scientific and stakeholder workshops, meetings, and other interaction help NP 302 researchers update and reprioritize their research as needed. These activities enable NP 302 scientists to address emerging agricultural problems, exploit new scientific discoveries, and partner with collaborators. A partial list of NP 302 related workshops conducted during this 5-year cycle is in Appendix 1.

It has been five years since the NP 302 Customer-Stakeholder Workshop in 2004, and progress achieved in attaining the Action Plan goals is being assessed by an external assessment panel.

ARS CROP PRODUCTION AND PROTECTION RESEARCH

NP 302 research contributes to the ARS Crop Production and Protection Program. This includes national programs that safeguard the national seed collections (NP 301) to more applied programs that enhance crop production and protection. The dynamic interaction of NP 302 with other Crop Production and Protection National Programs is shown in Fig. 1. The arrows in the figure highlight the benefits of NP 302 research that often contributes new hypothesis for testing and new mechanisms (screening, mutant analysis, candidate genes) for assessment. These NP 302 contributions advance both basic and applied crop research projects and national programs.

NP 302 also contributes to ARS national program areas, particularly, NP 306 Quality and Utilization of Agricultural Products; NP 307 Bioenergy and Energy Alternatives; and NP 215 Rangeland, Pasture, and Forage Systems.

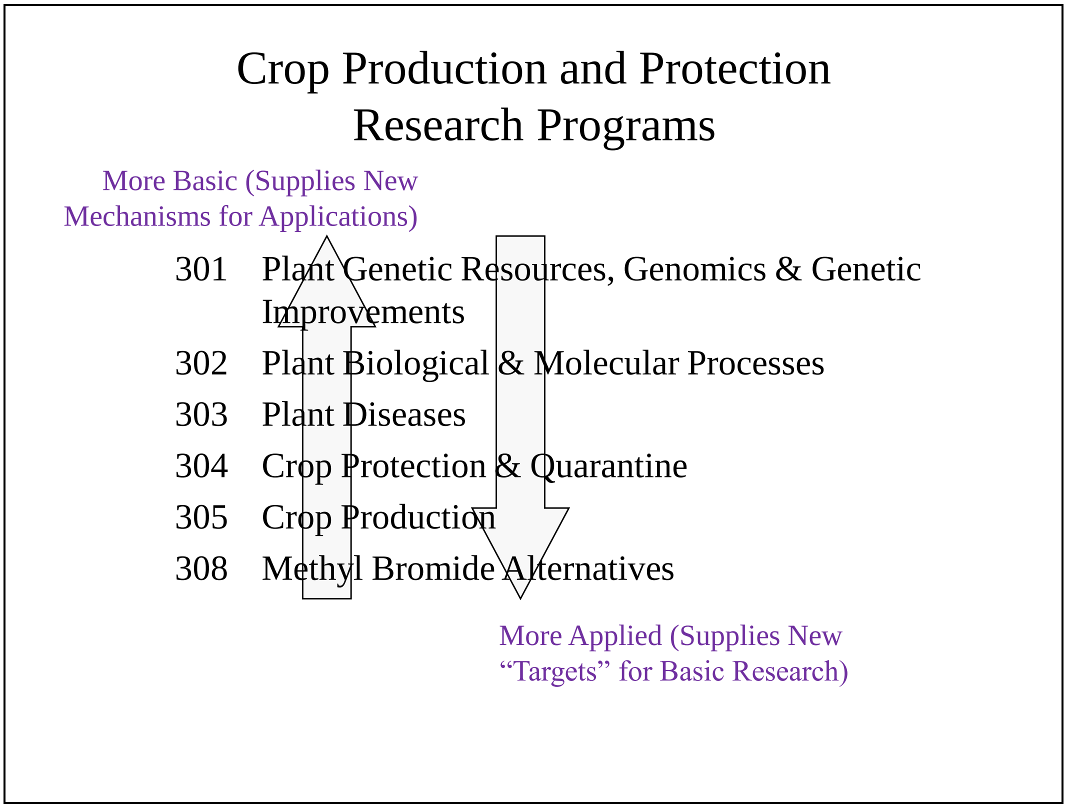

Figure 1. Dynamic interaction of NP 302 with other national programs in Crop Production and Protection

NP 302 Partners with NP 301: Plant Genetic Resources, Genomics and Genetic Improvement

National Program 302 focuses on gene discovery and developing new technologies to support crop production and protection, but cannot achieve its vision of scientific discovery without close partnership with the plant genetics and breeding research that occurs in National Program 301. NP 301 includes (1) Plant Genetic Resource Management including the National Plant Germplasm System, (2) Crop Informatics, (Structural) Genomics and Genetic Analysis, and (3) Genetic Improvement of Crops. NP 302 research often contributes to NP 301 in research that expands, maintains, and protects the genetic resource base. NP 302 supports NP 301 objectives that increase knowledge of genes and genomes, develop novel tools and approaches, and in managing and delivering vast amounts of genetic and phenotypic information. NP 302 is targeted to functional genomics, while NP 301 is targeted to structural genomics. However, NP 302 and NP 301 researchers often collaborate in research that advances crop production and protection through new advances in plant genomics and genetics.

Genomics-Enabled Genetic Improvement: During the past five years National Programs 302 and 301 have developed research synergies that have advanced “Genomics-Enabled Genetic Imrovement” (Fig. 2.) Specifically, NP 302 research in the three Component Areas of the Action Plan has advanced genomic, plant biology, and biotechnology strategies that have contributed to crop production and protection. Those accomplishments have contributed to the ARS release of over 100 new crop varieties and more than 500 improved germplasm lines (2004-2009). Many more varieties have been released by research partners from university and industry breeding programs.

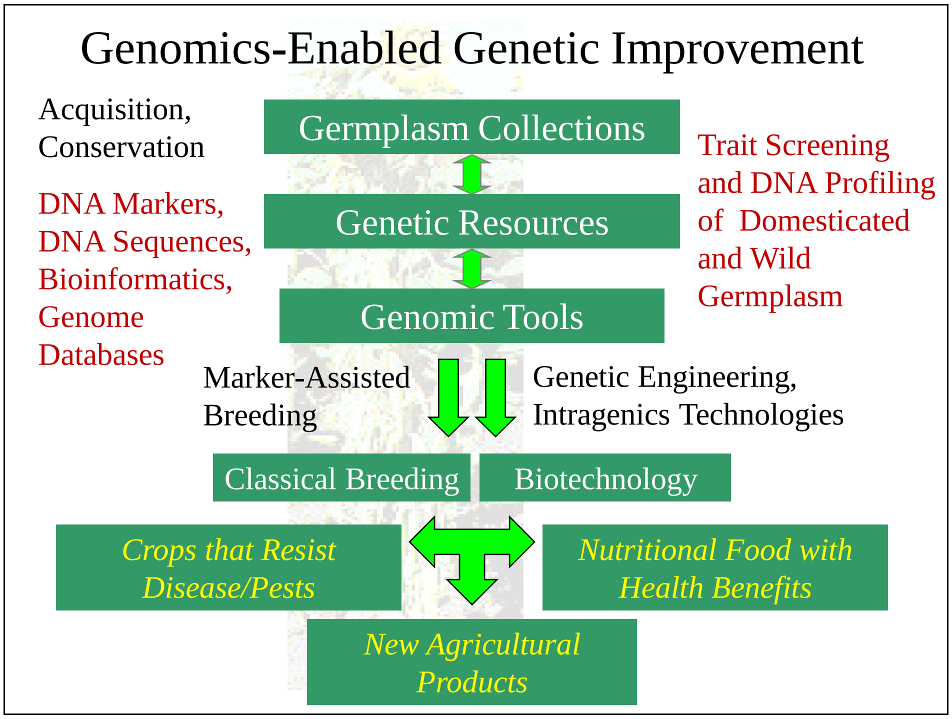

Figure 2. Genomics-Enabled Genetic Improvement. This figure illustrates synergies between National Programs 301 and 302 in capitalizing on untapped genetic diversity in the national seed collections, developing more efficient genetic selection methods, and using both conventional breeding and genetic engineering to develop improved crop varieties and new agricultural products.

During the past 5 years, NP 302 projects have been significantly strengthened by collaborative and coordinated research supported by other governmental agencies, various non-governmental organizations, universities, private industry, and international partners. National Plant Genome Initiative awards from NSF, USDA-CSREES, and DOE have enabled NP 302 projects to expand research objectives and contributions to the plant biology research community. Many accomplishments of NP 302 projects have also achieved in close cooperation with public and private sector collaborators. These are often supported through in-kind contributions, trust agreements or Cooperative Research and Development Agreements (CRADAs).

Many NP 302 projects are integrated into commodity and other targeted research communities. Examples of integrated ARS soybean and wheat improvement research initiatives that include NP 302 projects are presented in Figures 3 and 4. During the past 5 years, NP 302 national program leaders and scientists have often participated in strategic research planning and coordination with these commodity communities to optimally integrate NP 302 research. These partnerships enable NP 302 to effectively leverage and transfer research results to address critical agricultural needs and problems.

Figure 3. NP 302 Wheat Research is Integrated into Overall ARS Wheat Improvement Research.

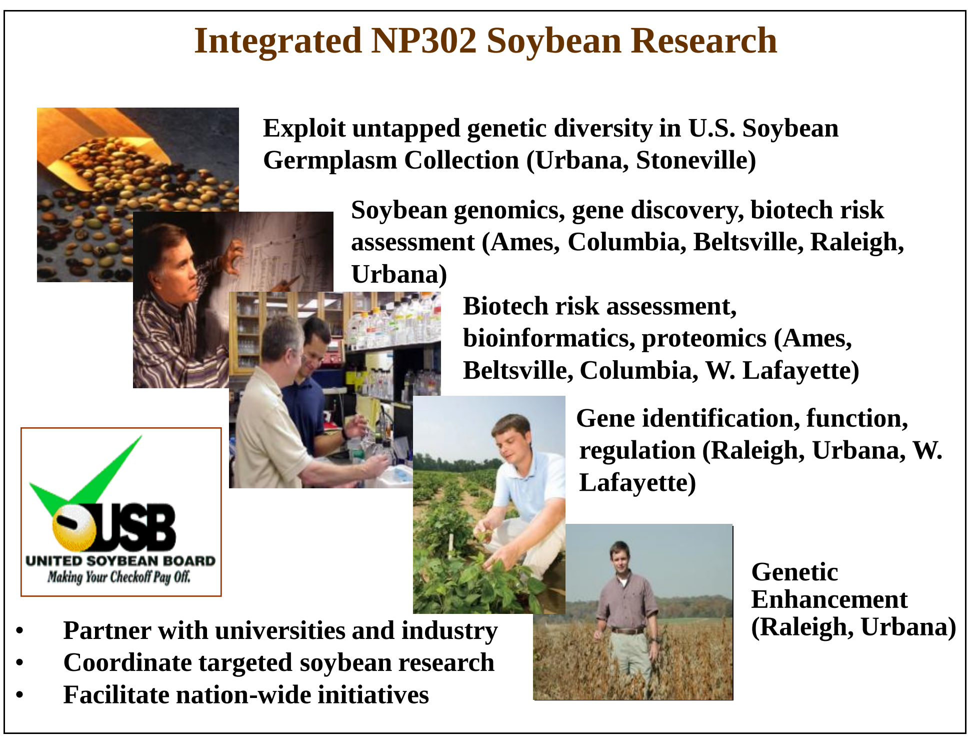

Figure 4. NP 302 Soybean Research is Integrated into Overall ARS Soybean Improvement Research.

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

In this Report, information about NP 302 achievements and their impact is organized according to National Program Research Components and their constituent Problem Areas, as described in the National Program Action Plan. The report first outlines the three NP 302 Research Components. This is followed by a section for each of the components. The actual language from the current Action Plan is given to outline the Research Needs and Expected Outputs for each of the Problem Areas within each Component. These are followed by a summary of NP 302 accomplishment and impact for each Problem Area. Then, selected accomplishments are listed that were achieved under the current Action Plan aimed at meeting the high priority needs identified by customer/stakeholders in the NP 302 Action Plan.

For the most part, the content of this report is derived from responses to a recent data call of the NP 302 scientists who were asked to summarize their project's major accomplishments in terms of impact, and key references documenting those accomplishments. NP 302 encompasses over 40 appropriated research projects. The titles of the individual projects, objectives, funding levels, and scientific staffing are listed in Appendix 2 Listing of Individual NP 302 Projects. NP 302 Publications are in Appendix 3.

This report does **not** include **all** accomplishments achieved in the national program but, rather, only those **selected** by the NP 302 Scientific Writing Team and National Program Leaders who authored this report. As a result, the scope of this report encompasses a subset of the total spectrum of NP 302 accomplishments, chosen to illustrate and exemplify the total progress and achievements *at the national level*. Individual scientists or projects are not identified since the purpose of the review is to assess the overall National Program, not individual scientists or projects.

Finally, a word about how NP 302 achievements and accomplishments were documented. Just as only selected accomplishments are reported, the details of those accomplishments are documented selectively so as to illustrate the overall variety of products and knowledge generated by this National Program.

NP 302 – PLANT BIOLOGICAL & MOLECULAR PROCESSES ACTION PLAN 2006-2011

Goal: National Program (NP) 302, *Plant Biological and Molecular Processes*, supports fundamental research that creates a knowledge base and research tools that will 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. The rationale for NP 302 is that all plant traits (phenotypes) depend upon genes and gene networks that respond to their environment.

Plant breeding has a long history of improving crop plants for agriculture, usually without fundamental knowledge of how the underlying genes are controlled. However, modern tools of biology offer plant scientists ways to improve plants either by using existing variability in plant properties or by creating new variability when warranted. Knowledge of how biological mechanisms can be modified will provide new and potentially more effective approaches to improve crop plants. Thus, crop improvement will be accelerated and strengthened, as agricultural scientists understand the scientific principles that link molecular and genetic phenomena to phenotype.

Translating research at the molecular level into useful information for problem-solving demands 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. This NP provides the means for the integration of research. NP 302 scientists will draw upon relevant expertise within NP 302 to coordinate and integrate the use of resources to develop focused strategies for solving specific problems. NP 302 scientists may also attract federal, university, industry and international partners. Objectives of NP 302 collaborative projects will be consistent with constituent ARS base projects and are expected to substantially enhance the impact of research outputs.

RESEARCH COMPONENT 1: Functional Utilization of Plant Genomes

In the past five years, DNA sequencing of model plant genomes such as Arabidopsis (eudicot) and rice (monocot) has contributed to an exponential gain in information about plant genes and gene expression. These efforts have produced vast EST resources and extensive amounts of microarray-based transcript expression data. It is now possible not only to observe whole-genome patterns of gene expression in response to treatment variables, but also to differentiate genetic variation in those responses. New technologies for quantifying gene function through changes in mRNA, protein, and metabolites (transcriptomics, proteomics, metabolomics) create additional opportunities to understand how plants convert their genetic potential into form. Confirming the function of specific gene sequences enables advanced strategies for crop improvement that involve the modification of specific gene targets. However, to support useful applications, the information garnered through plant genomics must be reduced to practice in a timely manner.

Five years ago, USDA/ARS committed to conducting an extensive program of plant biology and functional genomics, focusing particularly on crop traits that can be exploited for genetic improvement. Only one project focused entirely on model crops, while many projects leveraged model plant genomic information to address genomic objectives for crop plants. Many NP 302 projects contribute to both Component 1, Problem Area 1B, and also Component 2, Problem Areas 2A, 2B, 2C. It is feasible to report only a sampling of the complete spectrum of accomplishments for Components 1 and 2.

Engaged ARS Locations: Albany, California; Ames, Iowa; Beltsville, Maryland; Columbia, Missouri; Ithaca, New York; Kearneysville, West Virginia; St. Paul, Minnesota; Urbana, Illinois; West Lafayette, Indiana.

Problem Area 1A: Advancing From Model Plants to Crop Plants

(NP 302 Action Plan text related to Problem Areas are shaded in gray)

Problem Statement: Most genomic information is derived from model plants, such as Arabidopsis. However, the extent to which knowledge of model systems can be generalized to the complex genomes of crop plants depends on the degree of synteny or how closely plant species are related on an evolutionary scale. Thus, regulation of many important processes in crop plants often differs from those in model systems. This inadequacy limits the utility of model systems to resolve complex problems in agricultural crops. To remedy this dilemma, ARS research will explore gene-rich regions in important agricultural crops to advance genomics and alleviate the inherent limitations of model plant genomes.

Research Needs: 1) Knowledge of how important genes function in model plants, for subsequent extension to crop plants. **2)** Methods useful in model species, such as gene disruption to identify function, will be modified or extended to systematically define the biological function of crop plant genes.

Outputs: 1) Description of the function of agriculturally important genes in model plants and agronomic crops. 2) Verified knowledge of gene families or genetic networks that mediate or are associated with important traits in agronomic crops.

Problem Area 1A: Accomplishment Summary

This section summarizes the overall achievements in Problem Area 1A with a focus on scientific impact and potential benefits. Selected accomplishments follow this summary.

Fundamental discoveries about important genes were made in model plants that will serve as the basis for new advances in crop plants. The *Arabidopsis CLV*3 gene, was identified as the first small polypeptide ligand known to regulate plant development and these results led to discovery of a family of related molecules that are conserved among higher plants. From this research a model was formulated describing how *CLV*3 acts as a diffusible signaling molecule that communicates cell fate information to neighboring cells. This model has served as a new paradigm for ligand-receptor signal transduction in plant stem cell maintenance that has been widely adopted by the plant biology community. Discoveries of three genetic pathways in this model plant that confer different levels of stem cell regulation were made, which can serve as the basis for genetic manipulation of these regulatory pathways to modify shoot and flower structure in crop species.

The roles of pollen- and sperm-expressed genes in Arabidopsis were elucidated, which expands knowledge of the biological and molecular processes that ensure seed set. Novel signaling pathways involved in pollen tube growth were characterized and these findings can be exploited to expand interspecific hybrids for crop improvement.

Major advance in understanding of the molecular basis of phytochrome-regulated gene expression were made. This opens up new opportunities for manipulating this system for agronomic improvement of crop species.

Promoters, other genomic resources, methods, and tools from this model plant research has been provided to multiple researchers and widely used by the research community.

Genome resources were developed from the model legume plant, *M. truncatula*, and a model grass for desiccation-tolerance, *Sporobolus stapfianus*. Genechips were developed for the model legume, so that whole genome transcript expression could be assessed in legumes. Transcription factors were identified and catalogued in *Sporobolus stapfianus*. These new genomic resources can be exploited to leverage model plant information to better understand biological and molecular processes in crop plants. Results also can be applied to speed up and improve the sensitivity of molecular markers for legume and cereal crop genetic improvement.

Selected Accomplishments:

Output 1: ***Description of the function of agriculturally important genes in model plants and agronomic crops.***
Output 2: ***Verified knowledge of gene families or genetic networks that mediate or are associated with important traits in agronomic crops.***

Discovery of genetic pathways that regulate plant stem cell activity. Because of their fundamental importance in providing the source cells for new growth, plant stem cell activity is under strict genetic control throughout development. ARS researchers characterized three genetic pathways in the model plant Arabidopsis that confer different levels of stem cell regulation: a cell proliferation control pathway, a chromatin remodeling pathway, and a small RNA-mediated pathway. These can be manipulated to modify shoot and flower structure in crop species.

Carles, C.C., Choffnes-Inada, D., Reville, K., Lertpiriyapong, K. and Fletcher, J.C. 2005. ULTRAPETALA1encodes a SAND domain putative transcriptional regulator that controls shoot and floral meristem activity in Arabidopsis. **Development** 132: 897-911.

Williams, L., Grigg, S.P., Xie, M., Christensen, S. and Fletcher, J.C. (2005). Regulation of Arabidopsis shoot apical meristem and lateral organ formation by microRNA miR166g and its AtHD-ZIP target genes. **Development** 132: 3657-3668.

Ha, C.M., Jun, J.H., Nam, H.G. and Fletcher, J.C. (2007). BLADE-ON-PETIOLE1 and 2 control Arabidopsis lateral organ fate through regulation of LOB-domain and adaxial-abaxial polarity genes. **Plant Cell** 19: 1809-1825.

Characterization of novel signaling pathways during pollen tube growth. Pollen grows by tip growth. Tip growth was known to be regulated by a GTPase protein called Rop, which is active when in the GTP-bound form and inactive when in the GDP-bound form. How the pollen tube regulated the activation state of Rops and thereby established polar growth was unknown, and how pollen tubes perceived signals from the female was unknown. ARS researchers identified pollen receptor kinases and the extracellular signals (ligands) they interact with, showing that yeast two hybrid screening can be used to identify proteins that interact extracellularly. They showed that one of the ligands promoted pollen tube growth. They also identified a protein that interacted with the cytoplasmic domain of pollen receptor kinases, and it turned out to be a Guanine Exchange Factor (GEF) for Rop. GEFs in plants and animals are completely different in their amino acid sequences, but ARS researchers showed that the plant and animal proteins have domains that perform similar functions. For example, the back part of the protein inhibits the ability of the GEF to switch Rop from the GDP to GTP form. They showed that this inhibition is released when the RopGEF interacts with pollen receptor kinases. The interactions between pollen receptor kinases and RopGEFs supplied the missing link from perception of extracellular signals to regulated tip growth.

Kaothien, P., Ok, S. H., Shuai, B., Wengier, D., Cotter, R., Kelley, D., Kiriakopolos, S, Muschietti, M. and McCormick, S. 2005. Kinase Partner Protein interacts with the LePRK1 and LePRK2 receptor kinases and plays a role in polarized pollen tube growth. **Plant J** 42: 492-503.

Zhang, Y. and McCormick, S. 2007. A distinct mechanism regulating a pollen-specific guanine nucleotide exchange factor for the small GTPase Rop in *Arabidopsis thaliana.* **Proc. Natl. Acad. Sci. USA** 104: 18830-18835.

Zhang, D., Wengier, D., Shuai, B., Gui, C.P., Muschietti, J., McCormick, S. and Tang, W.H. 2008.The pollen receptor kinase LePRK2 mediates growth-promoting signals and positively regulates pollen germination and tube growth. **Plant Physiol** 148: 1368-1379.

Tools for analyzing gene function in pollen and sperm. Pollen tube growth is critical for completion of the plant life cycle. Understanding the roles pollen- and sperm-expressed genes and the proteins they encode play in this process is important for ensuring seed set. Pollen biologists were stymied in studying pollen tube growth in the model plant *Arabidopsis thaliana*. To overcome these barriers, ARS researchers developed methods for collecting large amounts of pollen, and more importantly established techniques for robust *in vitro* pollen germination, with the critical component being incubation temperature. Additionally they identified and used sperm-specific promoters to facilitate determining the total transcriptome (i.e. all expressed genes) of sperm cells.

Engel, M., Holmes-Davis, R. and McCormick, S. 2005. Green Sperm. Identification of male gamete promoters in *Arabidopsis thaliana*. **Plant Physiol** 138: 2124-2133.

Boavida, L.C. and McCormick, S. 2007. Temperature as a determinant factor for increased and reproducible in vitro pollen germination in *Arabidopsis thaliana*. **Plant J** 52: 570-582.

Borges, F., Gomes, G., Gardner,R., Moreno, N., McCormick, S., Feijo, J.A., Becker, J.D. 2008. Comparative transcriptomics of *Arabidopsis thaliana* sperm cells. **Plant Physiol** 148: 1168-1181.

New discovery of how plants perceive and respond to light. All aspects of plant biology hinge on the plant's ability to perceive light. Because the phytochrome (phy) family (phyA to phyE) are the major light receptors for plants, understanding how they function in regulating gene expression, is crucial for crop improvement. ARS and University of California, Berkeley, researchers have found that red light induces very rapid phosphorylation and subsequent degradation of a transcription factor, PIF5, via the proteasome system, in a manner similar to that previously shown for PIF3. Collectively, the data support the proposal that the rapid phy-induced phosphorylation of PIF3 and PIF5 represent the biochemical mechanism of primary signal transfer from photoactivated photoreceptor to binding partner, and that phyA and phyB may signal to multiple shared partners, utilizing this common mechanism.

Khanna, R., Shen,Y., Toledo-Ortiz,G. Kikis,E., Johanneson, H., Hwang,Y.S. and Quail, P.H. 2006. Functional profiling reveals that only a small number of phytochrome-regulated early-response genes in Arabidopsis are necessary for optimal deetiolotation. **Plant Cell** 18:2157-2171. [Julin Maloof: Faculty of 1000 Biology, 29 Sep 2006 http://www.f1000biology.com/article/id/1047207/evaluation

Al-Sady,B., Kikis,E.A.,Monte,E., and Quail,P.H. 2008. Mechanistic duality of transcription factor function in phytochrome signaling. **Proc. Natl. Acad. Sci. USA** 105: 2232-2237.
[Julin Maloof: Faculty of 1000 Biology, 19 Feb 2008
http://www.f1000biology.com/article/id/1101111/evaluation]

Development of novel strategies for improvement of drought tolerance. Improving drought tolerance is hampered by its complexity and by the lack of gene targets for improvement strategies. ARS researchers developed genomic resources (expressed sequence tags) to evaluate components that contribute to dehydration tolerance in the desiccation-tolerant grass *Sporobolus stapfianus*, a model plant related to maize. Previous work established that complex traits can be genetically defined by gene networks under the control of a few, but specific, transcription factor genes. ARS researchers catalogued 750 maize transcription factors. Detailed expression analyses of each transcription factor in non-stressed and water-stressed maize seedlings under both mild and severe water deficits showed that unique subsets of transcription factors regulate the drought response in a tissue-specific manner in maize.

Cho, I-J., Joshi, T., Srivastrava, G., Xu, D., Hearne, L., and Oliver MJ. Large scale Q-PCR reveals maize transcription factors that are regulated under water deficit in a tissue-specific manner. Preliminary results presented at Gordon Research Conference, Salt and Water Stress in Plants, Big Sky Montana, Sept. 2008.

Genomic tools developed for model plants can be used in crop plants. Advances in understanding alfalfa genes and genetics have been limited because alfalfa has a very large genome and requires outcrossing (i.e., it is difficult to self-pollinate). The model legume *M. truncatula* is a close relative of alfalfa, has a small genome that is being sequenced, and is self-fertile. Moreover, a genechip having more than 50,000 probe sets has been developed for *M. truncatula* and has been used to study whole genome transcript expression. ARS scientists showed that the *M. truncatula* genechip can be used for whole genome transcript expression in alfalfa, to identify genetic variation in alfalfa and to develop molecular markers for alfalfa. Other ARS researchers annotated the MapMan visualization software, originally developed for Arabidopsis, by creating a visualization platform for soybean Affymetrix arrays and cDNA arrays.

Tesfaye, M., K.A. Silverstein, B. Bucciarelli, D.A. Samac, and C.P. Vance. 2006. The Affymetrix Medicago GeneChip Array is applicable for transcript analysis of alfalfa (*Medicago sativa*). **Funct. Plant Biol.** 33:783-788.

Problem Area 1B: Applying Genomics to Crop Improvement

Problem Statement: Gene families or gene networks in crop plants often control complex traits, such as seed quality and vigor, flower color and scent, mineral nutrient use efficiency, and forage digestibility. Knowledge of how these genes interact to influence gene expression in crops is essential to understand inheritance and regulation of traits. Without this knowledge, inheritance of such traits is hard to predict, and assembling the required genes in an improved genotype is difficult. ARS research will analyze, interpret and use the voluminous increase in crop genomic resources to characterize gene networks governing fundamental processes in crops and thereby identify opportunities for crop improvement.

Research Needs: 1) Transcript and protein expression profiles associated with genetic variation in complex traits will be determined to identify genes that control phenotypic expression. **2)** DNA sequence differences (polymorphisms) associated with variants of complex traits will be identified to enable mapping, inheritance studies, and eventual manipulation of valuable traits. **3)** Multiple sets of information within and across species and environments will be "mined" to relate gene action to plant phenotype and productivity. **4)** Quantifying gene action and crop biological processes that contribute to productivity will require application of new algorithms and computational tools for data analysis and integration.

Expected Outputs: 1) Identification of specific genes that mediate end-product traits desired by consumers, such as nutritional content, oil and grain quality, and disease resistance and abiotic stress tolerance in agricultural crops. **2)** Expansion of genomic information on the function and regulation of gene systems that govern expression of important traits in agricultural crops. **3)** Expanded macro-array and micro-array capabilities to visualize functional changes in gene expression during development of agronomic crop species. **4)** Proteomic technologies to extend genomic understanding to the level of gene products.

Problem Area 1B: Accomplishment Summary

This section summarizes the overall achievements in Problem Area 1B with a focus on scientific impact and potential benefits. Selected accomplishments follow this summary.

The overarching goal of research conducted as part of NP 302 Problem Area 1B is to discover new genomic knowledge that enables "genome-enabled plant breeding" under ARS National Program 301 (focused strongly on germplasm enhancement and varietal breeding), and by other public and private-sector crop improvement programs. Genomic information can improve the effectiveness of screening diverse unimproved germplasm and breeding lines for their agronomic and horticultural potential, of choosing optimal breeding materials and progeny earlier and more accurately, and can contribute to formulating more effective decision-making strategies for crop breeding.

Several of the most important achievements of NP 302 Problem Area 1B during the last five years involve identifying "candidate genes" for key agronomic traits, applying that knowledge to developing "functional genetic markers," and developing ways to accomplish the preceding tasks far more effectively than ever before. NP 302 maize researchers developed a ground-breaking population genetics approach for systematically screening and identifying "candidate genes" for traits of any organism—crop, animal, human--far more effectively than did any prior methods. Deploying this and other novel, genomically-assisted methods for germplasm screening, they uncovered major genetic variants from maize's wild relative which have already altered maize breeding strategies. Other researchers identified and cloned agriculturally-important genes and genetic factors (transcription elements, microRNAs) from maize that serve as more effective functional markers for enhancing maize biomass quality for biofuel applications and for expanding crop markets.

And, these achievements have not been limited to maize. A new functional marker-- a novel aluminum tolerance gene--was identified for breeding sorghum (and perhaps other cereals) that yields more on acidic soils. The discovery of a novel pathogen host-plant resistance mechanism in barley, involving small peptides, has furnished additional breeding targets and functional markers for cereal genetic improvement. Candidate genes for cold-resistance in blueberries valuable for breeding broader adaptation were identified through microarray analyses of EST libraries. In strawberries, candidate genes were identified to extend the growing season during the warm summer months.

Research under this Problem Area has also elucidated the biosynthetic and metabolic pathways underlying key traits so that they can be more effectively manipulated. NP 302 researchers uncovered seed-expressed genes apparently regulated by a circadian clock. Understanding this previously unknown property of the metabolic pathways determining seed fill has yielded a new avenue for more effectively manipulating soybean seed yield and composition. Comparing microarray profiles of total barley RNA with those from polyribosomal RNA that actually produce proteins yielded an analytical approach for more effectively identifying genes key for seed germination and GA signaling—traits which are important for improving seed quality and function (e.g., in malting). This program producedthe first publicly-accessible microarray chip for peanut, which enabled more rapid identification of critical gene networks that breeders can exploit to enhance resistance to biotic and abiotic stresses.

NP 302 researchers have been particularly productive in developing more effective methods for improving critical seed protein quality and quantity traits. They devised the means for accurately determining how expression of individual plant proteins varies according to specific plant phenotypes or environmental parameters, so that the proteins that determine agronomically-important traits can be more rapidly identified. New proteome analytical approaches were developed for more rapidly identifying selectable seed protein markers for key peanut sensory and nutritional traits. Similarly, determining which wheat proteins are keys for gluten (bread dough) polymeric structure, and how the environment affects gluten protein composition, have yielded new analytical approaches and functional markers for wheat product quality.

Finally, the many genetic/genomic tools (e.g., genetic and physical maps, genomic and insertion libraries, specialized genetic stocks, and a plethora of genomic databases) generated by research under this Problem Area during the last five years collectively constitute a strong, sustainable infrastructure for extending, enhancing, and effectively conducting "genomically-directed crop breeding" in the future. For example, maize/teosinte "introgression lines" enable breeders to insert agronomically-superior teosinte genetic variants into maize more accurately. The new population genetic approach developed for identifying maize candidate genes will boost gene discovery efforts for all crops. Similarly, TEnest, a bioinformatic tool for detecting transposable element insertion, is accelerating the isolation of agronomically-important traits throughout the cereals. NP 302 researchers have contributed much of the data for the full tomato genome sequence, plus specialized tomato microarray and gene expression databases for intensifying gene discovery throughout the family Solanaceae. Likewise, a genome database for the Cucurbitaceae constructed by NP 302 geneticists will continue to generate markers and key insights for bolstering genetic research and breeding in that economically-important plant family.

Selected Accomplishments:

Accomplishments are reported according to the four expected outcomes listed in the NP 302 Action Plan.

Output 1: ***Identification of specific genes that mediate end-product traits desired by consumers, such as nutrition, oil and grain quality, and disease resistance and abiotic stress tolerance in agricultural crops.***

Genes important for biomass and seed yield identified in maize. Genes that regulate leaf and inflorescence development were cloned from maize, including transcription factors that regulate leaf expansion and differentiation of the vascular system. A single mutation in the *Corngrass* gene makes a corn plant unrecognizable as *Zea mays*. ARS researchers discovered that *Corngrass* encodes a microRNA that targets seven transcription factors, thus explaining its pleiotrophic phenotype. *Corngrass* mutants have altered cell walls with less lignin and more sugar, properties that would be advantageous for biomass applications. Another microRNA gene, *tasselseed4*, regulates the *indeterminate spikelet* gene, and thereby influences seed number and fertility, important components of grain yield.

> Chuck, G, Cigan, M., Saeteurn, K. Hake, S. (2007) The heterochronic maize mutant *Corngrass1* results from overexpression of a tandem microRNA. **Nature Genet**, 39:544-549.
> [Vivian Irish: Faculty of 1000 Biology, 25 Apr 2007
> http://www.f1000biology.com/article/id/1080894/evaluation]
>
> Chuck, G. Meeley, R. Irish, E., Sakai, H. Hake, S. (2007) The *tasselseed4* microRNA of maize controls meristem cell fate and sex determination by targeting the *indeterminate spikelet1/Tasselseed6* gene. **Nature Genet** 12:1517-1521.

Analysis of wild ancestors gives corn breeders new tools for crop improvement. In maize (corn), artificial selection during domestication 7500 years ago and/or during modern plant breeding over the last century diminished genetic variation in key genes responsible for traits that define differences between the crop and its wild ancestor teosinte. ARS scientists evaluated seed characteristics (kernel size and germ to endosperm ratios), kernel composition (starch, protein, oil), amino acid profiles, and seed storage protein (zein) profiles for a diverse set of modern inbred lines, teosintes, and landraces (the evolutionary intermediate between inbred lines and teosinte). Teosinte differs significantly from inbred lines and landraces for most traits examined, including those instrumental in determining maize yield and product quality. For example, teosinte seeds have twice the protein and higher levels of the essential amino acids lysine, methionine, and tryptophan, which are deficient in modern corn. In addition, ARS scientists developed ten sets of introgression lines, each derived by backcrossing a different teosinte accession into an elite maize inbred line. These introgression lines represent precision tools for introducing valuable genetic variation into maize breeding material. Eight such introgression sets, comprising 640 maize lines, were characterized with molecular markers to define the chromosomal regions from the teosinte parent; on average each line contains 3 different chromosomal segments encompassing ~4% of the teosinte genome.

> Briggs, W.H., McMullen, M.D., Gaut, B.S., and Doebley, J. 2007. Linkage mapping of domestication loci in a large maize-teosinte backcross resource. **Genetics** 177:1915-1928.

Using population genetics approaches to identifying agronomically important genes for maize. A major constraint for applying biotechnology tor crop improvement is our limited knowledge of which genes control agronomic traits. ARS scientists, in collaboration with colleagues at University of Wisconsin and University of California-Irvine, developed and validated a novel approach to identifying candidate genes for agronomic traits by contrasting the sequence diversity in maize inbred lines of maize to that in wild teosintes and maize landraces. Genes selected as the wild progenitor teosinte was transformed into modern maize should have greatly reduced levels of genetic diversity among varieties, relative to unselected genes. Paradoxically, genes that have undergone the greatest selection have the least genetic variation remaining and therefore cannot be further improved by standard plant breeding. This population genetics approach has identified, efficiently and systematically, numerous genes of agronomic importance for traits influencing growth, and productivity, and kernel nutritional value. It is being emulated by other research groups in other crops.

Hufford, K.M., Canaran, P., Ware, D.H., McMullen, M.D., and Gaut, B.S. 2007. Patterns of selection and tissue-specific expression among maize domestication and crop improvement loci. **Plant Physiol** 144:1642-1653.

Yamasaki, M., Schroeder, S., Sanchez-Villeda, H., Gaut, B., and McMullen, M.D.2008. Empirical analysis of selection screens for domestication and improvement loci in maize by extended DNA sequencing. **Plant Genome** 1:33-43.

Novel regulator of cereal disease defense characterized from barley. Pathogenic fungi, viruses, bacteria, insects, and nematodes impact agronomic and horticultural crops. ARS researchers discovered a previously unrecognized role for small peptides as negative regulators of plant defense. They isolated a novel regulator of disease defense, a monocot-specific gene encoding a family of small cysteine-rich peptides designated blufensins. *BLUFENSIN1* (*BLN1*) is highly induced during infection by pathogenic fungi.

Meng, Y., Moscou, M.J., and Wise, R.P. 2009. *Blufensin1* negatively impacts basal defense in response to barley powdery mildew. **Plant Physiol** 149:271-285.

Novel aluminum tolerance gene characterized from sorghum. In acid soils, toxic forms of aluminum (Al) solubilized from clay minerals damage root systems, greatly reducing crop yields. ARS researchers have isolated a novel aluminum tolerance gene that can improve crop yield on acid soils. The gene encodes a citrate efflux transporter that mediates release of citric acid into the soil where it binds and detoxifies aluminum, so it does not damage the growing root tip.

Hoekenga, O.A., Maron, L.G., Pineros, M.A., Cancado, G.M.A., Shaff, J.E., Kobayashi, Y., Ryan, P.R., Dong, B. et al, and Kochian, L.V. 2006. *AtALMT1* is a novel, essential factor for aluminum tolerance in Arabidopsis thaliana and encodes an aluminum-activated malate transporter. **Proc. Natl. Acad. Sci USA** 103:9738-9743. [Nicolaus Von Wiren: Faculty of 1000 Biology, 24 Aug 2006 http://www.f1000biology.com/article/id/1033879/evaluation]

Mongalhaes, J. M. et al., and Kochian, L.V. 2007. A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. **Nature Genetics** 39:1156-1161. [Enrico Martinoia: Faculty of 1000 Biology, 4 Sep 2007 http://www.f1000biology.com/article/id/1089611/evaluation]

Global transcript analyses identifies cold-responsive genes in blueberry and genes that respond to abiotic stresses in strawberry. Lack of winter hardiness is a major genetic limitation for current blueberry cultivars so introduction of cold tolerance could expand the geographical range of blueberry cultivation. ARS scientists analyzed expressed genes called ESTs (Expressed Sequence Tags) and microarrays to identify cold-responsive genes in blueberry, under both cold room and field conditions. Gene expression differences in the two environments indicate that cold room studies are not a reliable proxy for field studies. Similarly, the growing season of current strawberry cultivars is restricted by lack of hot and cold temperature tolerance. ARS researchers greatly expanded the publicly available ESTs for strawberry by generating cDNA libraries from abiotically stressed *F. vesca* plants and seedlings; they generated ~45,000 new ESTs representing genes expressed in response to cold, heat, drought, and increased salinity. These ESTs represent >90% of the available *Fragaria* ESTs, and are invaluable for annotation of the newly sequenced *F. vesca* genome. The new strawberry ESTs will provide useful molecular marker development for breeders, and have already revealed gene candidates for developing day-neutral strawberries capable of sustained production during the hot summer months.

Dhanaraj, A.L., Alkharouf, N.W., Beard, H.S., Chouikha, I.B., Matthews, B.F., Wei, H., Arora, Rowland, L.J. 2007. Major differences observed in transcript profiles of blueberry during cold acclimation under field and cold room conditions. **Planta** 225:735-751.

Circadian clock control of gene expression in soybean seeds. Many plant biosynthetic pathways are controlled or influenced by day/night cycles and the circadian clock. Previous research in model plants examined the timing of gene expression in leaves or whole seedlings in diurnal or circadian cycles, but the importance of circadian regulation for gene expression within seeds was unknown. If occurring, such regulation would be critical for controlling seed yield and composition. ARS researchers found significant differences between circadian gene expression patterns in seeds and leaves, and identified several seed-expressed genes that could be targets for improving seed composition. This work represents one of the first examinations of how metabolite importation into filling seeds is controlled, and adds to a growing body of data indicating that circadian clock function in sink tissues is modulated by metabolites such as sucrose.

Output 2: ***Voluminous expansion of genomic information on the function and regulation of gene systems that govern expression of important traits in agricultural crops.***

Development of tools for genomic and genetic research in strawberry, blueberry, blackberry, and apple. Inbred lines of the diploid strawberry, *F. vesca*, were developed for genome sequencing and *F. vesca* was developed as a reference plant for commercial octoploid strawberry. ARS and Towson University scientists developed the first database for blueberry genomic data, which will elucidate mechanisms of cold acclimation and freezing tolerance in perennials. ARS and U. Maine scientists showed that DNA markers developed from highbush blueberry DNA sequences are effective for distinguishing individuals and degrees of relatedness among lowbush blueberry plants. ARS and Clemson University scientists sequenced about 3000 blackberry genes, from which nearly 700 molecular markers were developed. ARS and U. Arkansas scientists identified molecular markers linked to thorn production and repeat fruiting in blackberry. *Malus sieversii,* the progenitor of the modern, domesticated apple, is a valuable genetic resource for many novel genes for fruit quality (aroma, taste, texture, color, size, etc.) and both disease and environmental stress resistance. A detailed genetic framework map, constructed of the GMAL4593 mapping population (*Malus sieversii* x Royal Gala), will facilitate incorporation of novel genes into new commercial cultivars by marker-assisted-breeding. Transcriptome analyses in fruit crops were previously limited to SSH and EST studies, but University of Illinois and ARS researchers constructed a 38,000 unigene apple microarray, that can contribute to gene expression analyses, in other Rosaceae species, focused on the genetic control of flower and fruit development, disease resistance, stress resistance, and plant architecture in temperate tree fruit crops.

Bell, D.J., Rowland, L.J., Polashock, J.J., Drummond, F.A. 2008. Suitability of EST PCR markers developed in highbush blueberry for genetic fingerprinting and relationship studies in lowbush blueberry and related species. **J. Amer Soc Hort Sci** 133:701-707.

Davis, T.M., DiMeglio, L.M., Yang, R., Styan, S.M.N., Lewers, K.S. 2006. Assessment of SSR marker transfer from the cultivated strawberry to diploid strawberry species: functionality, linkage group assignment, and use in diversity analysis. **J Amer Soc Hort Sci** 131:506-512.

Tomato genome sequencing and tomato and melon genomics resources. Tomato serves as a model system for many biological inquiries including those related to fruit development, quality and pathogen response. ARS researchers lead the US contribution (supported by NSF and ARS) to the International Tomato Genome Sequencing Project. ARS researchers have generated clone resources, coordinated mapping and localization activities, and will complete the sequencing of two chromosomes. ARS researchers created two public tomato microarrays, TOM1 (cDNA) and TOM2 (long oligo) and distribute publicly available tomato cDNA and genomic clone resources world-wide. They consolidated existing data into a tomato gene expression database (www.ted.bti.cornell.edu), widely used for large-scale gene expression analyses in tomato and related crops (such as pepper and eggplant). ARS researchers also created a cucurbit genome database (http://www.icugi.org/) to house melon EST data and facilitate communication among an international network of cucurbit researchers.

Fei, Z., Tang, X., Alba, R. and Giovannoni, J. (2006) Tomato Expression Database (TED): A suite of data presentation and analysis tools. **Nucl. Acids Res**. 34: D766-D770.

Wetcher, P., Levi, A., Harris, K., Davis, A., Fei, Z., Katzir, N., Giovannoni, J., Salman-Minkov, A., Hernandez, A., Thimmapuram, J., Tadmor, Y., Portnoy, V., Trebitsh, T. (2008) Gene expression in developing watermelon fruit. **BMC Genomics** 9:275.

Mueller, L. et al. (2009) A snapshot of the emerging tomato genome sequence. **Plant Genome** (in press).

New bioinformatic tool to accelerate genome assembly in grasses. An organism's genome may contain many repetitive sequences that either no longer function or could have been modified by evolution. ARS scientists developed TEnest, a bioinformatic tool for accelerating the process of genome assembly and isolation of important agronomic genes. Transposable elements (TEs) are mobile segments of DNA that produce the repetitive segments. TEnest enables researchers to identify repeat insertions in the genome, calculate the age since integration into the DNA, and model their evolution. Initially, TEnest has been applied to four major cereals—maize, barley, wheat, and rice—to accelerate international efforts to assemble these crops' genome sequences.

Kronmiller, B.A., and Wise, R.P**.** 2008. TEnest: Automated chronological annotation and visualization of nested plant transposable elements. **Plant Physiol** 146:45-59.

Output 3: ***Expanded macro-array and micro-array capabilities to visualize functional changes in gene expression in the development of agronomic crop species.***

High-throughput gene expression studies provide clues for how cereal crops grow. Access to complete genomic sequences and rapidly accumulating information about RNA and protein expression patterns can generate insights regarding how genes control complex phenotypes. Using the Barley1

GeneChip, ARS scientists worked with an international team of cereal scientists to evaluate in parallel the expression of 22,000 genes in fifteen tissues or stages of barley development. The resulting "Barley Gene Atlas" provides detailed information on individual genes in a unified developmental framework available to the worldwide research community, and is also a useful comparative dataset for investigating genes or regulatory networks in globally-important cereal crops.

Druka, A., Muehlbauer, G., Druka, I., Caldo, R., Baumann, U., Rostoks, N., Schreiber, A., Wise, R., Close, T., Kleinhofs, A., Graner, A., Schulman, A., Langridge, P., Sato, K., Hayes, P., McNicol, J., Marshall, D., and Waugh, R. 2006. An atlas of gene expression from seed to seed through barley development. **Funct Integrat Genomics** 6:202-211.

Potokina, E., Druka, A., Luo, Z., Wise, R., Waugh, R., and Kearsey, M. 2008a. Gene expression quantitative trait locus analysis of 16,000 barley genes reveals a complex pattern of genome-wide transcriptional regulation. **Plant J** 53:90-101.

Improved protocol for transcriptome analysis. Researchers usually examine total RNA when analyzing microarrays for gene expression. In the only comparison to date of microarray analytical results from total RNA with those from polyribosomal RNA (the form actually used to produce protein), ARS researchers showed that half of the genes differ by two-fold in expression level, simply due to the RNA source This approach will improve global gene expression analysis, and specifically identify genes potentially related to germination and the GA signaling pathway. Identifying the preceding genes may enable their use in marker-assisted breeding programs for improving malting quality.

Skadsen, R.W., Jing, P. Transcriptome profile of barley aleurone differs between total and polysomal RNAs: implications for proteome modeling. **Molecular Breeding** 21: 261-269. 2007

Gene expression in peanuts. ARS scientists, in collaboration with Agilent Technologies, have developed the first publicly available microarray for gene expression profiling in peanut, as a tool for elucidating molecular and physiological responses to water-deficiency and heat stress. The array represents approximately 10,000 unique genes from leaf, root, stem, and pod assayed under a variety of growth conditions, including drought, pathogen infection, and heat stress, in addition to genes from wild species. Two expression-profiling studies have already demonstrated the utility of the array with both vegetative and reproductive tissues.

Output 4: *Proteomic technologies to extend genomic understanding to the level of gene products.*

New tools to optimize proteomics experiments. Plant protein extracts typically contain 10^4-10^5 proteins but high performance 2-D gels only resolve 10^3 protein spots, so virtually all protein spots contain multiple proteins. Reducing the frequency of false positive protein identifications would enable researchers to more rapidly identify proteins whose expression really correlates with the phenotype or stress response under study, thereby accelerating the pace and reducing the cost of research. ARS scientists have developed a protein isoelectric focusing method to assign fractional spot volumes to each protein component of a gel spot, making it possible to determine how expression of each protein changed with respect to a particular phenotype or stress stimulus.

Yang, Y., Thannhauser, T., Li, Li, Zhang, S., (2007) Development of an Integrated Approach for Evaluation of 2-D Gel Image Analysis: Impact of Multiple Proteins in Single Spots on Comparative Proteomics in Conventional 2-D Gel/MALDI Workflow **Electrophoresis**, 28, 2080-2094.

Optimization of electrophoresis-based proteomics for peanut seeds. In peanut, seed proteins might serve as genetic markers for cultivar identification and for underlying sensory and nutritional traits of peanut cultivars. ARS scientists, in collaboration with colleagues at New Mexico State and Texas A&M Universities, have optimized a 2-dimensional gel electrophoresis-based proteomics approach for analyzing peanut seed as a first step for establishing comprehensive seed proteomics capacity in peanut.

Kottapalli, K., Payton, P., Rakwal, R., Agrawal, G., Shibato, J., Burow, M., Puppala, N. 2008. Proteomics analysis in mature seed of four peanut cultivars using two-dimensional gel electrophoresis reveals distinct differential expression of storage, anti-nutritive, and allergenic proteins. **Plant Science.** 175:321-329.

Improved methods for assessing wheat flour protein composition. Determining the effects of environment on gluten proteins is essential for minimizing variation in wheat flour quality, which is so important for millers and bakers. ARS researchers developed a sequential separation method that extracted up to 95% of flour protein, including the difficult-to-extract polymers, and significantly improved mass spectrometry approaches to identify individual gluten proteins. The improved methodology resulted in a three-fold increase in the number of gluten proteins identified. More than 50 individual gluten proteins were distinguished with high levels of confidence, including some that may function as terminators of the polymer chains. Consequently, for the first time analysts can readily identify changes in gluten protein composition resulting from different environmental conditions and determine the roles of specific constituent proteins in the gluten polymer.

DuPont, F.M., Chan, R., Lopez, R., and Vensel, W.H. 2005. Sequential extraction and quantitative recovery of gliadins, glutenins, and other proteins from small samples of wheat flour. **J. Ag. Food Chem**. 53:1575-1584.

Hurkman, W. J., Tanaka, C. K. 2006. Extraction of wheat endosperm proteins for proteome analysis. J. **Chromatography** B 849: 344–350.

RESEARCH COMPONENT 2: Biological Processes that Improve Crop Productivity and Quality

Crop productivity is determined by a plant's capacity to convert energy, nutrients, and water into harvestable yield of high quality and high value. Thus, ample supply of harvestable products is a function of a plant's genetic potential to capture energy, and use available water and nutrients. The molecular and biochemical factors that actuate regulatory mechanisms are poorly understood for fundamental biological processes that underpin crop plant productivity and the production of high-value end-products. A highly coordinated integration of research is needed to develop knowledge of how biological processes may be regulated to overcome factors that limit crop yield and quality in a manner that reduces costly inputs, expands area suitable for production, and protects the environment.

Engaged ARS Locations: Albany, California; Beltsville, Maryland; Columbia, Missouri; Corvallis, Oregon; Dawson, Georgia; Fargo, North Dakota; Gainesville, Florida; Hilo, Hawaii; Ithaca, New York; Kearneysville, West Virginia; Lubbock, Texas; Madison, Wisconsin; New Orleans, Louisiana; Phoenix, Arizona; Raleigh, North Carolina; St. Paul, Minnesota; Oxford University, Mississippi; Urbana, Illinois; Washington, DC; and West Lafayette, Indiana.

Problem Area 2A: Understanding Growth and Development

Problem Statement: Biological processes that regulate crop plant growth and development include photosynthesis (conversion of sunlight energy to chemical energy); initiation and growth of reproductive tissues; root and shoot growth; transport of metabolic assimilates from leaves to and among plant organs; seed development and maturation. These processes often are inefficient or poorly adapted to agricultural growing conditions that may seriously restrict productivity and crop quality. ARS research will develop a better understanding of the fundamental principles governing these processes and findings will be applied to improve crop quality, increase product value, and achieve more sustainable production systems.

Knowledge of the processes that regulate growth and development is necessary to develop effective strategies for enhanced crop productivity under variable growing conditions. Hormonal signals that trigger important developmental changes and the genes that control the signaling pathways will be identified. The potential of gene modification to beneficially influence the performance of biological processes will be assessed for ability to enhance crop quality and production efficiency.

Research Needs: 1) Improved knowledge of how genes and gene networks that mediate crop development and productivity are regulated. **2)** Improved knowledge of how metabolic processes that mediate crop quality and productivity of agricultural commodities are regulated. **3)** Technology to enhance flavor, nutritional value, or other quality traits of plant products.

Expected Outcomes: 1) Ability to regulate fundamental biological mechanisms will enable technologies that beneficially alter processes such as photosynthesis, cellulose and lignin accumulation, carbohydrate partitioning to harvested organs, and fruit ripening. **2)** Improved efficiency of nutrient assimilation and metabolism will support higher crop productivity and nutritional value.

Problem Area 2A: Accomplishment Summary

This section summarizes the overall achievements in Problem Area 2A with a focus on scientific impact and potential benefits. Selected accomplishments follow this summary.

Output 1: *Improved knowledge of genes that mediate development and productivity*

Research in this problem area advanced understanding of how genes mediate development and crop productivity. Major advances were made in identifying important genes and elucidating metabolic processes that mediate productivity and crop quality. In particular, the *RIPENING-INHIBITOR* (*RIN*) transcription factor was identified as a master regulator of tomato fruit ripening. The RIN gene sequence is now widely used as a breeding tool in selecting for long shelf-life lines in fresh market breeding programs for tomatoes and other fresh-fruit crops. Some breeders also use the *rin* allele (and the associated marker) for developing higher solids fruit in processing lines.

Gene underlying critical processes of nitrogen fixation in root nodules of legumes and in nutrient uptake in developing corn kernels were identified. Determining these genes serves as the first step in modifying these important processes.

Output 2: ***Improved knowledge of metabolic processes that mediate quality and productivity.***

Sugars are central to all aspects of plant growth and development, and recent results in corn indicate that failure to metabolize sucrose, as in the *miniature-1* mutant, not only limits carbohydrate accumulation but also affects other key growth-related hormones that collectively control sink size and seed mass.

The sucrose-metabolizing enzyme, sucrose synthase, was also identified as a potential cause of postharvest sucrose loss in sugarbeet root. These results provide a foundation for manipulation of import function in corn kernels and will advance development of sugarbeet lines with improved sucrose storage during storage.

A new metabolic component of sugar sensing was identified with the discovery that this soluble, globular protein can bind directly to membranes and to actin cytoskeleton. The perception of sucrose synthase has shifted from an enzyme largely devoid of interesting regulatory properties to one that is controlled post-translationally by multisite phosphorylation, is dynamically located in specific subdomains of the cytoplasm, and may be part of a novel sucrose-sensing mechanism. Controlling the intracellular localization of sucrose synthase opens new approaches to manipulate how imported assimilates are utilized in growing plant organs. An interesting spin-off of this work is a synthetic peptide that may have utility as a human therapeutic agent (patent application).

Polyamines have also been identified as anabolic growth regulators that control tomato fruit ripening, and thus manipulation of their content may provide another approach to regulate fruit ripening.

Advances in the signaling area involved the receptor-like protein kinases that sense extracellular ligands or signals and transmit that information to the inside of the cell. The signal transduction process involves the cytoplasmic protein kinase domain of the receptor, and at least in one case, is now known to involve phosphorylation of tyrosine residues in addition to serine and threonine residues. This has started a paradigm shift in plant signal transduction and importantly, new targets have emerged for manipulation to control receptor kinase function. The results open a new level of potential regulation of plant receptor kinases and understanding of growth-promoting signal transduction mechanisms that control brassinosteroid signaling with beneficial impact on agriculture.

In wheat endosperm, new proteomic results have established the compartmentation and potential redox regulation of key enzymes and suggest that the amyloplast plays an important role in coordinating the synthesis of starch and protein in cereal crops.

Collectively these results provide the foundation for future efforts to manipulate crop quality and productivity. New candidate genes, mutant screens, and molecular tools have been provided to test new hypotheses for the genetic basis of crop quality and productivity.

Output 3: ***Technology to enhance quality traits of plant products***

The impact of environmental conditions during crop growth was determined for important quality traits. The basis for deleterious effects of high temperature on wheat flour quality was elucidated. That information will be used by wheat breeders in germplasm screens and genetic selection methods to develop new cultivars that provide millers and bakers a more consistent grain product. The number of people who suffer from wheat food product sensitivities and allergens is increasing, so new results identifying specific wheat proteins that cause allergies is useful. This finding is being used to develop transgenic plants with reduced levels of specific proteins for evaluation and is advancing methods for detecting wheat allergens in food products. Research on wheat allergens responds to a need expressed by the Food and Drug Administration to expand research on identifying and reducing allergens in wheat food products.

Research results were obtained confirming that soybean seed protein and oil concentrations are similar in normal and low phytic acid lines. That information is enabling soybean breeders to confidently develop new low-phytate lines with good end-product quality. Low phytate lines are desired to reduce excretion of phosphorous in animal waste and thus lower the environmental impact. Low phytate lines are being evaluated by ARS aquaculture researchers and industry partners for fish and livestock feed. Other research results documents that changes in the temperature during soybean seed development can alter the fatty acid composition of soybean oil. Findings indicate that transcriptional regulation of seed-specific desaturases account, at least in part, for an elevated oleic acid phenotype observed at higher temperatures. That critical information is being used by soybean geneticists in cultivar development and screening for fatty acid composition.

Selected Accomplishments:

Output 1: *Improved knowledge of genes that mediate development and productivity*

Characterization of transcription factors regulating ripening. While a number of ripening-associated genes have been isolated from tomato and other species, few "master regulator" genes have been identified to date. One such gene previously identified by ARS researchers is the *RIPENING-INHIBITOR* (*RIN*) transcription factor. In an effort to expand our understanding of fruit ripening control and to develop new gene targets for breeding and genetic engineering, the scientists identified several new regulatory genes, which are necessary for coordinating ripening and impact downstream ripening traits including accumulation of nutritionally important carotenoids and important quality attributes such as softening and ascorbate (vitamin C) accumulation. These genes include a NAC transcription factor encoded by the *nor* (*non-ripening*) locus which regulates *RIN*, another MADS-box gene *TAGL1*, and two members of the ERF family. With collaborators, the researchers showed that RIN homologs control ripening in other fruit species (strawberry and melon) and are currently determining the range of species these additional regulators act in for ripening control. In summary, understanding of ripening from ethylene and ethylene response genes has been advanced and transcription factors, which regulate ripening in species such as strawberry, have been identified that do not require ethylene.

> Barry, C. and Giovannoni, J. (2006) Ripening inhibition in the tomato Green-ripe mutant results from ectopic expression of a novel protein which disrupts ethylene signal transduction. **PNAS USA** 103: 7923-7928.

Manning, K., Tor, M., Poole, M., Hong, Y., Thompson, A., King. G., Giovannoni, J. and Seymour, G. (2006) A naturally occurring epigenetic mutation in a gene encoding an SPB-box transcription factor inhibits tomato fruit ripening. **Nature Genetics** 38:949-952.

Ripening inhibition in the tomato *Green-ripe* mutant results from ectopic expression of a novel protein which disrupts ethylene signal transduction. Co-Inventors: Barry, C and Giovannoni, J. Filed May 26, 2006. Serial number 11/442,028. Patent pending.

Genes identified within legume nodules that fix nitrogen from the air. All plants require nitrogen for growth. While many plants obtain nitrogen from fertilizer, legumes have bacteria in their root system that can make use of nitrogen from the air. Symbiotic nitrogen fixation within the root nodules is critical for legume crop growth but little is known about the genes affecting this process. ARS researchers at St. Paul, Minnesota, have assessed the expression of more than 500 genes in the nodules of a model legume, *Medicago truncatula*. Results revealed that more than 80 genes had enhanced expression in root nodules including one putative plant disease resistance gene (R).

Tesfaye, M., D.A. Samac, and C.P. Vance. 2006. Insights into symbiotic nitrogen fixation in Medicago truncatula. **Mol. Plant-Microbe Interact**.19:330-341.

Genes of sugar-hormone crosstalk in seed development in maize are identified.
Sugar metabolism and its control are critical to all parts of the plants, including developing seeds - a major site of sugar utilization for normal seed development. Hormones such as auxin and cytokinins also have an important role in seed development presumably through regulation and control of numerous cellular processes as well as sugar metabolism. Molecular details of these interactions are however unknown. ARS researchers have conducted molecular studies on a single gene mutant, *miniature-1* (*mn1*) seed, which is primarily impaired in sucrose utilization and the loss of ~ 70% seed weight, and has been determined to also show major alterations in the levels of both auxin and cytokinins in the mutant seed. Significant changes were seen in the expression levels of a few but not all genes related to biosynthesis of the two hormones.

LeClere, S., Schmelz, E. A. and Chourey, P. S. (2008) Cell wall invertase-deficient *miniature 1* kernels have altered phytohormone levels. **Phytochemistry** 69:692-699.

Critical gene for nutrient uptake identified in developing endosperm of maize. Endosperm of cereal seeds provide 60% or more of calories of human diet; thus, it is critical to understand the basic cellular structures and processes that are critical to its development. Specifically, developing endosperm harbor highly specialized cells in the basal endosperm transfer layer (BETL) which is presumed to perform nutrient uptake from the mother plant and may also act as a barrier to pathogenic entry. However, no molecular or cellular evidence is thus far available in support of either function. Using state-of-the-art imaging technologies, laser micro-dissection and high throughput gene expression tools, ARS researchers in collaboration with the University of Florida scientists provide new major insights on the role of the *miniature1* (*Mn1*) gene in the appropriate development and differentiation of plasma membrane in these cells that is essential to their import function of the photoassimilates. Additionally, many new BETL-specific genes with nutrient import and metabolism functions are identified.

Isolation and characterization of genes involved in sugarbeet root postharvest loss. It is not uncommon for sugarbeet roots to lose 10 to 15% of their sucrose during postharvest storage, costing sugarbeet companies $100 to 150 million annually. Research established sucrose synthase as the principal sucrose-degrading enzyme during sugarbeet root storage and provided evidence for its likely role in postharvest sucrose loss. While one sucrose synthase gene was previously identified, ARS research identified a second sucrose synthase gene and characterized the expression of both genes during storage and in response to typical postharvest stresses.

Campbell. L.G., Klotz, K.L. 2007. Characterizing sugarbeet varieties for postharvest storage losses is complicated by environmental effects and genotype X environment interactions. **Can. J. Plant Sci**. 87:121-127.

Output 2: ***Improved knowledge of metabolic processes that mediate quality and productivity.***

Regulation of tomato fruit ripening by polyamines. Until recently, fruit ripening was considered a senescence program that had reached a point of no return because of enhanced degradation of cell integrity. However, ripening is delayed in tomato mutants that accumulate polyamines, such as putrescine, spermidine and spermine. To gain an insight into the role of polyamines in fruit biology, ARS scientists genetically altered the levels of spermidine and spermine by over-expressing a gene for polyamine biosynthesis. Such a molecular engineering technology enabled development of nutritionally enhanced tomato lines that have become a unique genetic resource to study the function of polyamines in plants. Employing the techniques of DNA arrays for analysis of global gene expression, immunoblots for protein expression profiling, and nuclear magnetic resonance-based metabolite profiling of these transgenic fruits by ARS scientists in collaboration with researchers at the Purdue University and at CNR, Rome, Italy have revealed previously unknown facets of polyamine role in living cells which include: initiation of anabolic pathways, nitrogen-carbon interactions, and positive impact on nutrient composition including effects on aspartate family of amino acids, antioxidant lycopene, and the micronutrient 'vital amine' choline. Aspects particularly related to fruit metabolism and signaling include: presence of sensing mechanisms for polyamines, revival of metabolic memory, and cross talk with other plant hormones.

Srivastava, A., Chung, S.H., Fatima, T., Datsenka, T., Handa, A.K., and Mattoo, A.K. Polyamines as anabolic growth regulators revealed by transcriptome analysis and metabolite profiles of tomato fruits engineered to accumulate spermidine and spermine. **Plant Biotechnol.** 24: 57-70. 2007.

New metabolic component of sugar sensing identified. Sucrose synthase (SUS) is an important enzyme of sucrose metabolism in growing plant organs, and the activity of SUS is thought to be a marker or determinant of growth. However, details of the biochemical regulation of SUS proteins are not well understood. In a series of studies, ARS scientists determined that sucrose synthase was regulated by multisite protein phosphorylation and that the soluble, globular protein could bind directly to membranes and to the actin cytoskeleton. The ability to bind to membranes and F-actin was promoted by sucrose, which may facilitate channeling of carbon into biosynthetic pathways (e.g., cell wall glucan) and may function as a metabolic component of sugar sensing.

Duncan, K.A. and Huber, S.C. Sucrose synthase oligomerization and F-actin association are regulated by sucrose concentration and phosphorylation. **Plant Cell Physiol.** 48: 1612-1623. 2007.

Patent application (ser. no. 10/576,757) "Synthetic peptides that cause F-actin bundling and block actin depolymerization." Inventors: H. Winter, S.C. Huber and C. Larabell. U.S. National case filed Apr 20, 2006 (PCT/US2004/34996) and published as US-2008-0280358-A1 in the US Patent and Trademark Office on Nov 13, 2008. (http://www.uspto.gov/patft/index.html).

Unexpected role for tyrosine phosphorylation in plant receptor kinase signaling. Brassinosteroids are essential plant hormones that promote growth and increase tolerance to many stress conditions. Plant cells sense brassinosteroids when they bind to a receptor protein known as BRASSINOSTEROID INSENSITIVE 1 (BRI1), which in the presence of its ligand undergoes autophosphorylation on multiple serine and threonine residues in its intracellular protein kinase domain. ARS scientists demonstrated for the first time that BRI1 can also autophosphorylates on tyrosine residues and identified tyrosine-831 in BRI1 as one of the major sites of autophosphorylation that plays an important role in brassinosteroid signaling in vivo. The occurrence of tyrosine phosphorylation in BRI1 was completely unexpected and resulted in a major change in thinking about plant receptor kinases.

Oh, M.-H., Wang, X., Kota, U., Goshe, M.B., Clouse, S.D. and Huber, S.C. 2009. Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component of brassinosteroid signaling in Arabidopsis. Proc. **Natl. Acad. Sci. USA** 106: 658-663.
Kay Schneitz: Faculty of 1000 Biology, 16 Jan 2009
http://www.f1000biology.com/article/id/1146867/evaluation

Amyloplasts play a role in balancing starch and protein biosynthesis in the wheat grain. Amyloplasts are specialized organelles whose primary function is the synthesis and storage of starch, a major component of wheat endosperm. Relatively little is known about other biochemical processes taking place in this organelle. ARS researchers identified nearly 300 soluble and membrane-associated proteins in amyloplasts isolated from developing endosperm. These proteins function in a range of processes, including carbohydrate metabolism, cytoskeleton/plastid division, energetics, nitrogen and sulfur metabolism, nucleic acid-related reactions, synthesis of various building blocks, protein-related reactions, transport, signaling, and stress/defense. In addition, 42 potential thioredoxin target proteins were identified, including 13 novel targets. These findings provide new evidence for a much broader spectrum of biosynthetic activities in amyloplasts than previously known. The presence of carbohydrate and amino acid biosynthesis pathways and their potential regulation by thioredoxin provide the first evidence that amyloplasts play a role in balancing starch and protein biosynthesis in the endosperm.

Dupont, F. M. 2008.Metabolic pathways of the wheat (Triticum aestivum) endosperm amyloplast revealed by proteomics. **Biomed Central (BMC) Plant Biology**. 8:39 (http://www.biomedcentral.com/1471-2229/8/39)

Unexpected growth patterns in soybean leaves. The paradigm of dicotyledonous leaf expansion describes high relative growth rates at the leaf base, dampening towards the leaf tip. Soybean leaflet expansion was measured under different growth conditions with both conventional and time-lapse video techniques. In contrast to findings from other species, maximum growth rates occurred at ~2 a.m. and the basipetal growth pattern was absent, suggesting the factors controlling soybean expansion are distinct from other species. It is essential to define the inherent spatial and temporal patterns of growth and to recognize the differences from model organisms to correctly interpret subsequent experimentation on the genetic and environmental controls of leaf growth.

Ainsworth, E.A., Walter, A. and Schurr, U. *Glycine max* leaves lack a base-tip gradient in growth rate. **J. Plant Res.** 118:343-346. 2005.

Output 3: ***Technology to enhance quality traits of plant products***

High temperature during grain fill alters albumin and globulin levels in the developing wheat grain. High temperature during grain fill adversely affects wheat yield and quality. ARS researchers utilized a proteomics approach that combined two-dimensional gel electrophoresis with mass spectrometry to discover changes in accumulation of albumins and globulins in the endosperm from developing grain produced under different temperature regimens. Comparison of over 250 protein profiles during grain fill demonstrated that accumulation of proteins shifted from those active in biosynthesis and metabolism to those functioning in storage and stress/defense. Although high temperatures shortened the duration of grain fill, the developmental timing of protein accumulation was not substantially altered. However, a number of proteins increased in the endosperm in response to high temperature, including proteins hypothesized to have roles in bubble formation in dough as well as proteins that are structurally similar to known food allergens.

> DuPont, F. M., Hurkman, W. J., Vensel, W. H., Chan, R., Lopez, R., Tanaka, C. K., Altenbach, SB. 2006. Differential accumulation of sulfur-rich and sulfur-poor wheat flour proteins is affected by temperature and mineral nutrition during grain development. **J. Cereal Chem.** 44: 101-112.

Expression of genes encoding potential food allergens is enhanced by high temperatures or post-anthesis fertilizer during wheat grain development. Wheat is one of the top eight foods responsible for IgE-mediated food allergies, but few genes encoding wheat allergens have been characterized in detail. DNA sequences encoding potential allergenic proteins from the spring wheat Butte 86 were assembled from wheat ESTs and levels of gene expression were evaluated. ARS researchers found that a number of genes encoding potential food allergens were up-regulated in response to environmental conditions during grain fill. Transcript levels of genes encoding omega gliadins, known to cause an anaphylactic response in sensitive individuals, were higher when plants were supplied with post-anthesis fertilizer. In comparison, transcript levels of genes encoding two lipid transfer proteins, and two globulin-2 proteins increased when plants were subjected to high temperatures.

> Altenbach, S.B., Tanaka, C.K., Hurkman, W.J., Vensel, W.H. 2009. Expression of globulin-2, a member of the cupin superfamily of proteins with similarity to known food allergens, is increased under high temperature regimens during wheat grain development. **J. Cereal Sci.** 49: 47-54.

Low seed phytic acid trait in soybean. Commercialization of soybean varieties with low seed phytic acid depends on the stability of the trait when grown in soils with a wide range of phosphorus availabilities, and on the impact of altered seed phosphorus composition on seed protein and oil concentrations. When external phosphorus supply was increased from deficient to excessive levels, the seed phytic acid concentration in the low phytic acid line was stable at less than 2 grams phytic acid-P kg^{-1} seed dry wt. As the phosphorus supply increased, seed inorganic phosphorus concentrations increased from 0.8 to 4.0 grams kg^{-1} seed dry wt, compared to an increase of 0.2 to 0.6 grams kg^{-1} dry wt for the normal phytic acid lines. Seed protein and oil concentrations did not differ significantly between normal and low phytic acid lines. The stability of the low phytic acid trait to high levels of P supply supports continued development of varieties with low seed phytic acid and high yields. Soy proteins, whether from normal or low phytic acid soybean genotypes, exhibited normal to enhanced functionality.

Israel, D.W., P. Kwanyuen, J.W. Burton**,** and Walker, D.R. 2007. Response of low seed phytic acid soybeans to increases in external phosphorus supply. **Crop Sci.** 47: 2036-2046.

Mechanism of the canola "green seed problem" identified. Canola, the world's third most important oil seed crop, is in general a cold hardy plant but a freezing episode early in seed development causes green seed at harvest which is the highest priority seed quality issue within the canola industry. ARS researches showed that the induction of the enzyme pheophorbide a oxygenase (PaO), a key control point in the overall regulation of chlorophyll degradation, was inhibited in the seeds that remained green. Moreover, they discovered that the induction of PaO activity in canola seed was largely post-translationally controlled and that freezing interference with PaO dephosphorylation is the basis of the canola "green seed problem."

Chung, D.W., Pruzinska, A., Hortensteiner, S., and Ort, D.R. The role of pheophoribe a oxygenase (PAO) expression and activity in the canola green seed problem. **Plant Physiol.** 142:88-97. 2006

Environmental temperature during seed development can alter the fatty acid composition of soybean oil. Growth chamber experiments demonstrated that a relatively warm temperature during seed development results in oil with higher oleic and lower linoleic acid and linolenic acid content, and at a cooler temperature, vice versa. To assess the contribution of gene transcriptional regulation to this phenomena, the expression of the seed-specific omega-6 fatty acid desaturase genes Gm*FAD2-1A* and *FAD2-1B* and the omega-3 fatty acid desaturase genes Gm*FAD3A*, *FAD3B* and *FAD3C* were measured in the seeds of three soybean varieties grown at cool (22/18°C), normal (26/22°C), or warm (30/26°C) temperatures during pod fill. At the cool temperature, gene expression of both the Gm*FAD2-1s* and Gm*FAD3s* was significantly upregulated, but at the warm temperature their expression was significantly downregulated. Decreased Gm*FAD2-1* and Gm*FAD3* expression at the warm temperature was positively associated with increased oleic and decreased linoleic acid and linolenic acid in seeds.

Byfield, G.E. and R.G. Upchurch. 2007. Effect of temperature on microsomal omega-3 linoleate desaturase gene expression and linolenic acid content in developing soybean seeds. **Crop Sci.** 47:2445-2452.

Problem Area 2B: Understanding Plant Interactions with Their Environment

Abiotic and biotic stresses during a growing season can significantly limit agronomic crop yield and alter the population dynamics of plant species. Natural plant defense mechanisms often provide only limited protection, and the process of how crops adapt to unfavorable growth environments is poorly understood. ARS research will establish knowledge of how gene networks for fundamental biological processes perceive and translate 'signals' in response to environmental stimuli. Innovations governing fundamental processes that influence crop performance will be incorporated into useful crop protection strategies.

Genes important to crop adaptation, tolerance and defense will be identified. The capacity these genes confer on crops to withstand stress or attack will be determined. How crop plants and the organisms that affect them "recognize" and respond to each other will be assessed. Critical sites for intervention will be distinguished by disrupting interactions between organisms.

Expected Outputs: 1) DNA markers for genes that confer resistance or tolerance to abiotic and biotic stresses. 2) Discovery of genes that govern processes involved in plant adaptation and response to environmental stimuli. 3) Knowledge of biological mechanisms and regulatory processes that condition crop response to abiotic and biotic stresses. 4) Discovery of genes that condition biological processes associated with crop plant interactions with weeds, fungi, and bacteria.

Problem Area 2B: Accomplishment Summary

This section summarizes the overall achievements in Problem Area 2B with a focus on scientific impact and potential benefits. Selected accomplishments follow this summary.

Every crop has an optimal growth environment—the right mix of temperature, moisture, atmosphere, nutrients, beneficial neighboring plants, symbiotic microbes, beneficial insects, nematodes, etc.—for realizing the maximum potential crop yield and/or product quality. But, these optimal conditions rarely if ever occur (or co-occur) and may shift relatively rapidly. Consequently, a central challenge of agriculture is to deploy crop plants which can somehow yield acceptably high product quality and quantity under suboptimal growth conditions. For a broad spectrum of crops, NP 302 researchers working in Problem Area 2B have 1) discovered those optimal growth conditions; 2) revealed how crops sense sub-optimal conditions and mobilize their genetic and physiological systems to adapt to biotic and abiotic stresses; and 3) discovered the genes and gene networks controlling the preceding systems, thereby producing genetic markers and genomic tools valuable for breeding varieties with superior adaptation, yield stability and product quality. This National Program has also developed durable research capacity that can be mobilized for reacting to future challenges, such as more rapid climate change, emerging pests and pathogens, etc. Examples of these major NP 302 achievements are presented below according to the key crop biotic and abiotic stresses that were addressed. What unifies this diverse set of achievements is that they resulted from NP 302 researchers developing and applying leading-edge genomic, genetic, and molecular biological tools and insights to first understand, and then manipulate, the crop's inherent genetic and biological potentials.

Extreme Cold – NP 302 researchers identified specific genes and transcription factors that enhance cold tolerance, thereby providing new targets and genetic markers for crop improvement. For example, transcription factors were identified from apple that are cold-induced and that confer improved cold hardiness. A transcription factor and other genes that enhance freezing tolerance in blueberries were identified. The preceding efforts also generated valuable tools (e.g., clones) and approaches for future research. NP 302 small fruit researchers and their industry and university collaborators (e.g., the "Generating Tools for Blueberry Improvement", CSREES Specialty Crop Research Initiative team Driscoll Strawberry Associates, Berry Blue LLC, and the Wild Blueberry Commission of Maine) have applied genetic tools and information to speed up genetic selection for cold hardiness in new blueberry cultivars.

Extreme Heat - Susceptibility to high temperatures can also negatively impact vegetative and reproductive plant growth. At the physiological level, NP 302 researchers achieved a major conceptual breakthrough in crop physiology by discovering that heat stress reduces plant growth through photosynthetic inhibition resulting from thermal inactivation of Rubisco activase, a key enzyme controlling CO_2 fixation. This critical insight immediately yielded highly effective new methods for screening crop germplasm for heat tolerance, and for more effective breeding and genetic engineering of more heat-tolerant activases and other photosynthetic compounds. In cooperation with public-and private-sector collaborators, NP 302 researchers are striving to alter these activase to increase temperature tolerance in crop plants. Other NP 302 researchers established that the content of the "signaling lipid," phosphatidic acid, was correlated with heat tolerance of maize and, consequently, that variation in phospholipase D might be exploited for enhancing heat tolerance.

Critical genes in sorghum associated with reduced seed set and development at high temperatures were identified, providing a suite of new candidate genes for breeders to manipulate. A novel meta-analysis of gene expression profiles across several plant species under stress conditions identified 17 genes that may govern basal heat tolerance in plants. Finally, NP 302 scientists directly applied some of the preceding novel insights regarding heat tolerance mechanisms to develop and release new upland cotton germplasm lines with improved heat tolerance and fiber quality, and to develop new irrigation technology that maximize resource conservation. These new cotton lines are starting to improve cotton production over much of the cotton belt and are providing a new source of germplasm for future improvement.

Changes in atmospheric carbon dioxide concentrations - Increasing carbon dioxide [CO_2] concentration has been implicated as a major cause of more rapid climate change. Notably, research results generated by NP 302 scientists have overturned the long-held view that elevated [CO_2] inhibits plant respiration. Contrary to expectation, elevated [CO_2] increased gene expression that upregulates the plant's respiratory machinery and thereby stimulates respiratory CO_2 loss, reducing the net benefit of elevated [CO_2] on photosynthesis. These findings demonstrated that the projected increases in crop yield from "CO_2 fertilization," resulting from elevated [CO2], may have been significantly overestimated.

Weeds - A detailed understanding of plant-plant interactions is a vital prerequisite for devising biological control measure for weeds. NP 302 researchers discovered that sorgoleone, a benzoquinone produced in root hairs, is the major allelopathic compound of sorghum. They characterized patterns of gene expression in sorghum root hairs, and have identified nearly all the genes that govern sorgoleone biosynthesis. This new information represents a key step for developing a new broad-spectrum "natural herbicide."

Insect pests - NP 302 researchers characterized a new insect elicitor peptide that triggers general plant defense reactions against insects. This discovery might help uncover new candidate genes for genetically-engineering crops with increased host-plant resistance to insects. Other NP 302 researchers uncovered 500 ESTs (putative genes) associated with root maggot feeding on sugar beets, and a particular gene that enhances resistance to maggot feeding. These results have led to a patent, and extensions of this research with industry partners have begun. Russian wheat aphid, one of the most serious insect pests of wheat and barley, is most effective controlled by intrinsic host-plant resistance. NP 302 barley researchers have discovered and mapped quantitative trait loci (QTLs) and molecular markers strongly linked to host-plant resistance against Russian wheat aphids. The genetic markers and knowledge of the QTLs genomic location have already been applied to develop resistant new barley germplasm and cultivars for the western U.S. Other ARS researchers applied similar markers and methods to incorporate Russian wheat aphid resistance into new wheat cultivars for every wheat market class in the U.S.

Fungal pathogens –Asian soybean rust and dry bean rust are threatening major legume crops world-wide. NP 302 researchers have identified 1000 genes from soybean rust uredia and 3400 genes from infective structures of dry bean rust. This research, jointly supported by USDA/ARS and the United Soybean Board, led to valuable insights about the pathogen-infection process, resulting in novel genetic (SNP) markers for soybean rust resistance.

For cereal crops, other NP 302 researchers discovered a hitherto unknown class of regulators for cereal disease defense in barley that encodes a family of small cysteine-rich peptides, designated blufensins (referenced in Problem Area 1B). Also, lemma-specific promoters were identified and applied to characterizing candidate genes for Fusarium head blight (scab) resistance in genetically-engineered barley.

Parasitic nematodes –NP 302 researchers have identified more than 7500 soybean cyst nematode genes that are expressed in the feeding structures formed by the nematodes during contact with resistant and susceptible soybeans. These findings represent invaluable new research tools for developing genetic resistance to an economically-damaging pest. Sequence information for the preceding genes and molecular resources has been shared with the research community, leading to CRADA and grant support that enhanced NP 302 research on resistance to soybean cyst nematodes, the major pest of soybeans in the U. S.

Selected Accomplishments:

Outputs 1-3:
DNA markers that condition plant responses to (a)biotic stresses.
Genes that confer resistance or tolerance to (a)biotic stresses
Genes that govern plant adaptation and response to environmental stimuli.

Cold

Transcription factors for improved cold hardiness of trees. Much of the information known about cold acclimation and injury in plants has been obtained from model plants. ARS scientists extended this knowledge to temperate tree fruits and have identified numerous cold regulated genes and proteins using global proteomic and transcriptomic approaches. They isolated several cold induced transcription factors from apple and have demonstrated that overexpression of these transcription factors improves cold hardiness and sensitizes the plant to dormancy signals.

> Bassett, C.L., Wisniewski, M.E., Artlip, T.S., Norelli, J.L., Renaut, J and Farrell, Jr., R.E. 2006. Global analysis of genes regulated by low temperature and photoperiod in peach bark.. **J. Amer. Soc. Hort. Sci.** 131:551-563.

Eskimo 1 protects against the big chill. ARS scientists have identified a gene mutation that enhances freezing tolerance in plants. The *Eskimo1* (*esk1*) mutation of Arabidopsis resulted in a 5.5º C improvement in freezing tolerance in the absence of cold acclimation. The results indicate that ESK1 is a novel negative regulator of cold acclimation. These findings provide a new strategy for enhancing freezing tolerance in crops.

Xin, Z., Chen, J., Mandaokar, A., Last, R., Browse, J. 2007. Arabidopsis ESK1 encodes a novel regulator of freezing tolerance. **Plant J.** 49:786-799.

Isolation of an important transcription factor in the cold response pathway from blueberry, the CBF gene. ARS scientists have isolated and sequenced a full-length clone from blueberry that encodes an important transcription factor in the cold response pathway of plants, termed CBF. CBF 'turns on' a cascade of genes involved in cold tolerance. In collaboration with scientists at Iowa State University, blueberry CBF constructs were introduced into Arabidopsis for testing gene function. Introduction of the blueberry CBF was shown to increase freeze tolerance in non-acclimated plants. This work has resulted in a current collaboration with scientists at Michigan State University to overexpress the CBF gene in cold-sensitive blueberry genotypes to test its effect on cold tolerance.

Naik, D., Dhanaraj, A.L., Arora, R., Rowland, L.J. 2007. Identification of genes associated with cold acclimation in blueberry (*Vaccinium corymbosum* L.) using a subtractive hybridization approach. **Plant Science** 173:213-222.

Heat

Identifying the basis for inhibition of photosynthesis by heat stress. Moderate heat stress inhibits photosynthesis, reducing the productivity and agronomic yield of crops. Using both intact plants and isolated enzymes from cotton and other plant species, ARS researchers have provided definitive evidence that the inhibition of photosynthesis under moderate heat stress is caused by the inhibition of the regulatory protein, Rubisco activase. The discovery that the thermal sensitivity of Rubisco activase limits the thermotolerance of the entire photosynthetic process represents a major conceptual breakthrough and provides a new strategy for improving the heat tolerance of crop plants.

Salvucci, M.E., Deridder, B.P., Portis Jr, A.R. 2006. Effect of activase level and isoform on the thermotolerance of photosynthesis in Arabidopsis. **J. Exp. Bot.** 57: 3793-3799.

Phosphatidic acid and corn heat tolerance. A positive correlation was identified between the amounts of phosphatidic acid (PA) and heat tolerance in corn. The findings were verified in two maize inbred lines by lipid profiling. The likely role of PA in heat tolerance in maize was further suggested by the significant induction of PA in leaf tissues of maize plants upon high temperature treatments. Several recombinant inbred lines (RILs) of distinctive heat tolerance were identified from the cross of these two inbred lines. The role of PA in heat tolerance is being further examined by lipid metabolic analysis of these RILs. Phosphatidic acid emerges as a new signaling lipid that may play a critical role in tolerance to high temperature stress and as a result, phospholipase D, which produces PA, becomes a possible target.

Welti, R., Shah, J., Li, W., Li, M., Chen, J., Burke, J.J., Fauconnier, M., Chapman, K., Chye, M., Wang, X. 2007. Plant lipidomics: Discerning biological function by profiling plant complex lipids using mass spectrometry. **Frontiers in Bioscience.** 12:2494-2506.

Low cost infrared thermometry system. ARS scientists in conjunction with a private sector cooperator have incorporated off-the-shelf low cost infrared thermometers (IRTs) into a custom designed wireless IRT system. Comparisons of performance between the lower cost IRT ($20/unit) and the IRTs previously used in the laboratory ($250/unit) indicate that the lower cost IRTs are suitable replacements for the higher cost units in agricultural settings. This technology is used in conjunction with the ARS BIOTIC (Biologically Identified Optimum Temperature Interactive Console) protocol is the basis for the SmartCrop™ irrigation scheduling technology. Sensors in the field keep track of how much heat stress has accumulated in the crop and sends out an "IRRIGATE" signal only when an adjustable time threshold has been reached. This allows farmers to irrigate when and where water is really needed and thereby contributes to conservation and sustainable practices.

> Mahan, J.R., Burke, J.J., Wanjura, D.F. 2005. Determination of temperature and time thresholds for BIOTIC irrigation of peanuts on the Texas Southern High Plains. **Irrigation Science.** 23:145-152.

Stress-induced genes identified. ARS scientists have developed novel techniques to monitor stress effects and have identified 17 genes contributing to basal heat tolerance in plants, and used a meta-analysis of heat and water-deficit stress expression profiles in cotton, peanut, and Arabidopsis to identify candidate genes for ectopic expression studies. Fifty-eight full-length clones are currently being prepared for ectopic expression studies in cotton and peanut. This represents one of the first public, large-scale examinations of genetically engineered crop plants under relevant field conditions and will allow scientists to develop more heat-tolerant crops.

Genes identified with heat susceptibility in sorghum. Exposure to higher than optimal temperature, heat-stress, is becoming increasingly common to all crop plants worldwide. Heat stress in developing pollen, especially during the pollen maturation phase, marked by starch biosynthesis, is often associated with starch-deficiency and male sterility and, ultimately, greatly reduced crop yields. ARS researchers in collaboration with the University of Florida scientists, and in part funded by the BARD grant, have demonstrated that sorghum plants grown in high temperature environment show changes in the expression of several critical genes of starch biosynthesis, reduced starch deposition and reduced pollen fertility, which led to reduced seed set (reduced crop yields). Further characterization, selection or manipulation of such 'heat-sensitive' genes will provide superior germplasm that is expected to be better adapted to growth conditions associated with global warming.

> Jain, M.**,** Vara Prasad P. V., Boote K. J., Allen, H. L. and. Chourey, P.S. (2007) Effects of season-long high temperature growth conditions on sugar-to-starch metabolism in developing microspores of grain sorghum (*Sorghum bicolor* L. Moench). **Planta** 227:67-79.

Release of new heat tolerant cotton germplasm with excellent fiber quality. ARS released three improved germplasm lines of upland cotton to the public (jointly with Cotton Incorporated) that possess superior fiber length and strength characteristics and improved yield performance under heat stress environments. Future profitability for US cotton growers requires improvements in fiber quality and plant productivity under abiotic stress, including high heat conditions. The lines provide public and private breeders with resources for concurrent improvement of fiber quality and heat tolerance in upland cottons for the mid-south and southeastern United States. The lines also serve as genetic resources for improving heat tolerance in Acala cottons of the southwestern and western United States. The new germplasm has the potential to provide superior germplasm that will impact cotton production over a larger portion of the cotton belt in the US.

> Percy, R.G., May, O.L., Ulloa, M., Cantrell, R.G. 2006. Registration of AGC85, AGC208, and AGC375 upland cotton germplasm lines. **Crop Sci.** 46: 1828-1829.

Other Abiotic Stresses

Increasing atmospheric CO_2 stimulates respiration in crop plants. It has been well documented that photosynthesis increases in C_3 plants grown at elevated carbon dioxide concentrations; however, it is unknown how respiration is affected by elevated carbon dioxide concentrations. Soybean was grown at elevated carbon dioxide concentration under open air conditions for two consecutive growing seasons, and the molecular, biochemical and physiological adjustment of respiratory capacity was measured. Greater respiration rates in soybean were driven by increased expression of genes encoding many enzymes in the respiratory pathway. Current models of ecosystem responses to elevated carbon dioxide may not account for the significant increase in plant respiration. If all C_3 plants respond to elevated carbon dioxide concentration with a similar increase in respiration, the modeled stimulation of net primary productivity would be reduced in magnitude by 7.4-11.1 petagrams of carbon per year, a flux similar to anthropogenically produced carbon dioxide.

Leakey, A.D.B., Xu, F., Gillespie, K.M., McGrath, J.M., Ainsworth, E.A. and Ort, D.R. The genomic basis for stimulated respiration by plants growing under elevated carbon dioxide. **Proc Natl Acad Sci USA**, (in press). 2009.

Long, S.P., Ainsworth, E.A., Leakey, A.D.B., Nösberger, J., and Ort, D.R. Food for thought: Open-air field experiments suggest lower than expected crop yield stimulation with rising [CO_2]. Science. 312:1918-1921. 2006. [Evan DeLucia: Faculty of 1000 Biology, 5 Jul 2006 http://www.f1000biology.com/article/id/1033016/evaluation]

Increasing atmospheric CO_2 improves water use efficiency in crop plants. With very few exceptions, decreased stomatal conductance (g_s) is one of the most conserved responses of leaves to growth at elevated [CO_2]. Since many changes at the soil, plant, and canopy microclimate levels feed back on evapotranspiration (ET), it is not certain how a decrease in g_s will impact ET and water use efficiency in rain-fed crops. ARS researchers discovered that elevated [CO_2] caused ET to decrease up to 16% for soybean. Ecosystem ET was linked with g_s of the upper canopy leaves when averaged across the growing seasons, such that a 10% decrease in g_s resulted in an 8.6% decrease in ET. The findings demonstrate that despite system feedbacks, decreased g_s of upper canopy leaves at elevated [CO_2] results in decreased transfer of water vapor to the atmosphere. It was further shown that the reduction in ET at elevated [CO_2] conserved soil moisture, which translated into greater photosynthesis and yield during periods of drought.

Leakey, A.D.B., Bernacchi, C.J., Ort, D.R., Long, S.P. Growth of soybean under free-air [CO_2] enrichment (FACE) does not cause stomatal acclimation. **Plant Cell Environ.** 29:1794-1800. 2006.

Impact of mulch type on growth of transgenic tomato. Genetic modification of crop plants is increasingly seen as a promising technology for sustainable agriculture and boosting food production in the world. Independently, cultural practices that utilize alternative agriculture strategies including organic cultivation subscribe to sustainable agriculture by limiting chemical usage and reduced tillage. How the two together affect fruit metabolism or plant growth in the field or whether they are compatible has not been tested thus far. ARS scientists compared the field performance of non-transgenic and transgenic tomato plants (that accumulate polyamines in a fruit-specific manner) in leguminous hairy vetch (*Vicia villosa* Roth) (HV) mulch and conventional black polyethylene (BP) mulch. Significant genotype x mulch dependent interactions on fruit phenotype were revealed by gene expression analysis and differential profiles of fruit metabolites. These studies revealed that polyamines work in concert with nitrogen and cytokinins to orchestrate the carbon-nitrogen metabolism in tomato fruit. Thus, a coordination exists between the environmental (mulch type) cues and differential gene expression and metabolism, revealing environment-dependent and transgene-dependent changes in fruit metabolism but without any apparent qualitative deviation from normal fruit metabolites. This observation bodes well for the incorporation of genetically engineered (transgenic) crops into alternative agriculture practices.

Neelam, A., Cassol, T., Mehta, R.A., Abdul-Baki, A.A., Sobolev, A., Goyal, R.K., Abbott, J., Segre, A.L., Handa, A.K., and Mattoo, A.K. A field-grown transgenic tomato line expressing higher levels of polyamines reveals legume cover crop mulch-specific perturbations in fruit phenotype at the levels of metabolite profiles, gene expression and agronomic characteristics. **J. Exp. Bot.** 59: 2337-2346. 2008.

Unique adaptations in root architecture enhance phosphorus availability and uptake. All plants require phosphorus (P) for growth and seed production and many soils are deficient in available P. Many legume plants are adapted to growth under P-deficient conditions but little is known about these adaptations. ARS scientists identified novel modifications in legume root architecture that improved P acquisition. Lupin plants form complex, compound roots known as cluster roots in response to P deficiency. Cluster roots increase surface area available for P-uptake by 50-100 fold. Over 100 genes related to cluster root formation have highly enhanced expression under P-deficiency, including a gene that regulates root meristematic centers. Identification of genes regulating root architecture will be useful in improving crop plant growth in nutrient poor soil.

Tesfaye, M., J. Liu, D.L. Allan, and C.P. Vance. 2007. Genomic and genetic control of phosphate stress in legumes**. Plant Physiol.** 144:594-603.

Output 4: ***Discovery of genes that condition biological processes associated with crop plant interactions with weeds, fungi, and other organisms***

Weeds/Herbicides

Allelopathic interactions have been proposed to have profound effects on the evolution of plant communities, and also play a significant role in the ability of grain crop species such as barley, rye, and sorghum to suppress weeds and have potentially broad application as natural herbicides.

Transcriptome of allelochemical-producing root hair cells described. The compound sorgoleone, a benzoquinone produced in root hair cells, accounts for much of the allelopathy of sorghum. ARS scientists executed a strategy for identifying genes involved in the biosynthesis of sorgoleone by developing a database of over 5,000 expressed sequence tags from purified sorghum root hair cells in collaboration with the University of Georgia. Detailed bioinformatic analyses of the resulting data set led to many novel insights concerning the root hair cell transcriptome; additionally, all of the enzyme classes hypothesized to be required for sorgoleone biosynthesis were identified. These efforts represented an important first step towards identifying the genes involved in the biosynthesis of this important allelochemical, and provide a foundation for the investigation of its biosynthesis using functional genomics. The broad-spectrum phytotoxicity, chemical stability, and apparently complex mode-of-action of sorgoleone have made it a promising candidate for development as a natural product alternative to synthetic herbicides.

Baerson, S.B., F.E. Dayan, A.M. Rimando, N.P.D. Nanayakkara, C.-J. Liu, J. Schröder, M. Fishbein, Z. Pan, I.A. Kagan, L.H. Pratt, M.-M. Cordonnier-Pratt, and S.O. Duke. 2008. A functional genomics investigation of allelochemical bioysynthesis in *Sorghum bicolor* root hairs. **J. Biol. Chem.** 283:3231-3247.

***In planta* mode of action of sorgoleone.** Sorgoleone is a strong inhibitor of photosynthesis and it may also act on other target sites. However, the actual mode of action of this compound in allelopathic interactions with plants is unknown. Experiments designed to test the *in planta* mode of action of sorgoleone demonstrated that it has no effect on the photosynthesis of older plants, but inhibits photosynthesis in germinating seedlings. Sorgoleone is not translocated acropetally in older plants, but can be absorbed through the hypocotyl and cotyledonary tissues and inhibit photosynthetic electron transport. The mode of action of sorgoleone may be the result of inhibition of photosynthesis in young seedlings in concert with inhibition of its other molecular target sites in older plants.

Kagan, I.A., A. Michel, B.E. Scheffler, A. Prause, S.R. Baerson, and S.O. Duke. 2006. Global gene expression approaches to mode-of-action studies with natural product-based pesticides. **Amer. Chem. Soc. Symp.Ser.** 927: 255-264.

Insects

Plant diseases and pest problems are responsible for decreases in crop yields. To improve disease resistance, a better understanding of the molecular mechanisms controlling plant defense responses is needed.

Barley resistance to Russian wheat aphids identified. Damage from Russian wheat aphid (RWA) has been an ongoing problem limiting barley production in the western USA. ARS scientists have released resistant germplasm and cultivars to address RWA infestation. In collaboration with those scientists, this project mapped quantitative trait loci (QTL) for RWA resistance in the first two germplasm releases, STARS9301B and STARS9577B. Three resistance loci were identified in STARS9301B and two of the same loci were present in STARS9577B. ARS scientists have introgressed Russian wheat aphid resistance into new wheat and barley varieties for every market class in the U.S. These results provide linked molecular markers to accelerate breeding of new RWA resistant barley cultivars and show that STARS9301B is a better choice for breeding RWA resistance for new aphid biotypes.

Mittal, S., L. S. Dahleen and D. Mornhinweg. Locations of quantitative trait loci (QTL) conferring Russian wheat aphid resistance in barley germplasm STARS-9301B. **Crop Science** 48:1452-1458. 2008

Genes involved in plant defense mechanisms identified in sugar beet roots. Utilizing the sugar beet root and root maggot (SBRM) interaction as a model system, innovative approaches were conceived and implemented to decipher the molecular aspects of root defense and response mechanisms. SBRM is one of the most devastating insect pests of sugar beet that is found in two-thirds of all U. S. sugar beet fields and accounts for 10-100% reduction in sugar yield valued at more than $1.2 billion. ARS scientists identified and provided global access to nearly 500 root genes (ESTs, GenBank) whose expression is modulated by insect feeding. Results showed that about 100 genes were specifically associated with the plant response to insect attack. Identifying the genes involved in defense responses is the first step for developing effective, environmentally safe, biotechnologically based strategies to improve insect and disease resistance in crop plants.

> Puthoff, D.P. and A. Smigocki. *Beta vulgaris* root ESTs modulated by sugar beet root maggot feeding and their regulation by wounding and defense signaling molecules. **Plant Cell Rep.** 26, 71-84, 2007.

Sugar beet gene identified, isolated and demonstrated to have a role in mediating insect resistance to several major plant pests. Disease and pest problems are responsible for decreases in yield of sugar from sugar beet, an industry valued at $1.2 billion. ARS scientists launched a comprehensive approach to attack an emerging problem incited by the most devastating pest of sugar beet, the sugar beet root maggot. The first *in vitro* root maggot bioassay using transformed *in vitro* propagated hairy roots was established, biochemistry of the root maggot midgut was determined, and the newly gained knowledge was resourcefully exploited to devise control strategies targeting the insect's digestive system. From a plant library enriched for root maggot responsive genes, one unique gene targeting the midgut was identified and utilized in a functional genomic study based on over expression of the gene in a transgenic model root system and model plants. Results showed that this gene increases insect resistance of the genetically modified plants to several pests. This research will provide methodology to enhance the development of improved elite germplasm lines with enhanced disease and pest resistance that will lead to increases in yields and the quality and nutritional value of cultivated crops.

> Smigocki, A.C., D.P. Puthoff, S.D. Ivic-Haymes and S. Zuzga. A *Beta vulgaris* serine proteinase inhibitor gene (*BvSTI*) regulated by sugar beet root maggot feeding on moderately resistant F1016 roots. **Am. Soc. Sugar Beet Tech. Proc.** 34:143-150, 2007.

Identification of a new insect elicitor that triggers plant defense reactions. Induced plant defenses to pests and pathogens are often initiated by perception of elicitors derived from the invading organisms. ARS scientists identified a new peptide elicitor from the oral secretions of *Spodoptora frugiperda*, an herbivorous insect that feeds on several species including cowpea. The elicitor is a peptide derived from the plant's plastid ATP synthase that has been proteolysed in the insect gut and released in oral secretions. The elicitor peptide, termed inceptin, is active at the fmol level to initiate specific plant responses. These novel findings enlarge the spectrum of insect-derived elicitors to a potentially large class of peptides and proteins produced by the insect during digestion of the plant. Further, new strategies to engineer plants with recombinant proteins containing multiple copies of the elicitor sequence can be considered as approaches to increase pest resistance.

> Schmelz EA, Carroll MJ, LeClere S, Phipps SM, Meredith J, Chourey PS, Alborn HT and Teal PEA (2006) Fragments of ATP synthase mediate plant perception of insect attack. **Proc. Natl. Acad. Sci. USA** 103: 8894-8899.

> Faculty of 1000 Biology: evaluations for Schmelz EA et al *Proc Natl Acad Sci U S A* 2006 Jun 6 103 (23) :8894-9 http://www.f1000biology.com/article/id/1000294/evaluation

Fungi

Identifying genes and proteins of fungi is a first step to develop resistance against pathogenic fungi and to foster interactions with beneficial fungi using biotechnological approaches.

Discovery of new genes involved rust interactions with plants. ARS scientists have sequenced almost 1,000 genes from soybean rust uredia, the asexual development structures, and 3,400 genes (19,480 ESTs) from infective structures (hyphae and haustoria) of dry bean rust. More than 600 proteins were detected from spores and germinating spores of dry bean rust using mass spectrometry and many changed in abundance as spores germinated and infected plants. These data provide the first detailed knowledge about rusts during pathogen invasion and may be useful in a target-based approach for fungicide discovery. This research, supported by the United Soybean Board and USDA-CSRESS, may also provide new opportunities for developing resistance in soybean and dry bean.

> Choi, J.J., Alkharouf, N.W., Schneider, K.T., Matthews, B.F. and R.D. Frederick. 2008. An enriched cDNA library and microarray analysis reveals expression patterns in soybean resistant to soybean rust. **Functional and Integrative Genomics** 8: 341-59.

Resistance against Fusarium head blight in cereals. Fusarium head blight is the main pathogen affecting barley and wheat seed quality and food safety. Barley has no appreciable resistance to F. graminearum. A program was started to transform barley with barley genes in a manner that would lead to resistance against Fusarium. A gene promoter (Lem2) that would specifically overexpress attached genes in the lemma and epicarp was cloned. A gene encoding a thionin protein (LemThio1) toxic to Fusarium was cloned, attached to Lem2, and used to transform barley. Many transformant lines were produced, and preliminary tests produced evidence of resistance. These and other promoters and thionin genes have been provided to other labs combating fungal diseases. A promoter has been developed that enables antifungal proteins to be targeted to the lemma and epicarp of grains.

> Carlson, A., Skadsen, R.W., Kaeppler, H.F. Barley hordothionin accumulates in transgenic oat seeds and purified protein retains anti-fungal properties in vitro. **In Vitro Cellular and Developmental Biology - Plant** 42: 318-323. 2006.

Mycorrhizal fungi cause hormone-induced increases in root formation. Root formation is the rate limiting step in vegetative propagation of horticultural and forest tree crops. Knowledge of hormonal regulation of root formation is needed to improve the success of root induction, thus improving production efficiency and profitability. ARS researchers, with collaborators from the Universities of Helsinki, and the Finnish Forest Research Institute, demonstrated mycorrhizal fungi increase the success of propagation from cuttings of several different woody perennial crops, inoculation with mycorrhizal fungi increases polyamines (PAs) in plants before mycorrhizae formation, and fungal-induced PA accumulation is linked to induction of lateral roots. Increased PAs in plants is a result of both PAs produced by the fungus and PA-production by the plant induced by the establishment of the symbiosis. Additionally an *in vitro* method was developed to induce adventitious roots in Scots pine cuttings by inoculating them with specific mycorrhizal fungi. Knowledge from this research is significant since results have expanded knowledge on the mechanisms involved in the regulation of root growth by determining changes in hormones associated with increased growth can be altered by mycorrhizal fungi and also has demonstrated practical application to propagation of plants from tissue culture and cuttings.

> Niemi *K*, Sutela S, Häggman H, Scagel CF, Vuosku J, Jokela A, Sarjala T. 2006. Changes in polyamine content and localization of *ADC* and *ODC* mRNA transcripts during mycorrhiza formation by *Suillus variegatus* on *Pinus sylvestris* in an in vitro cultivation system. **J. Exp. Bot**. 57: 2795-2804.

Scagel CF. 2005. Isolate-specific rooting responses of *Leucothoe fontaesiana* cuttings to inoculation with ericoid mycorrhizal fungi. **J. Hort. Sci. Biotechnol**. 80:254-262.

Nematodes

Soybean cyst nematode is the major pest of soybean in the US. While some resistant varieties are available additional technologies are needed to produce genotypes that are broadly resistant to different races of the nematode.

Identification of genes expressed at the nematode feeding site. Soybean cyst nematode is the major pest of soybean in the US. ARS researchers used microarrays to examine the expression of over 35,000 soybean genes and 7,500 soybean cyst nematode genes in syncytia (feeding structures) formed during a resistant interaction and a susceptible interaction. Syncytia were collected using laser capture microdissection. RNA was extracted from the syncytia, labeled and hybridized to Affymetrix microarray chips. In addition to broadly defining changes in gene expression linked to SCN colonization, cell wall modifying genes were targeted for detailed analysis and potential use in bioengineering projects because of early microscopy reports demonstrating marked changes in cell wall structures during syncytium formation. Affymetrix and RTPCR results demonstrate a very large and infection-specific increase in the transcripts for several cell wall modifying proteins. All pertinent data was placed on-line. A pool of candidate genes that are expressed specifically at the nematode feeding site was identified for further testing and analysis.

Klink, V.P., Overall, C.C., Alkharouf, N.W., MacDonald, M.H. and B.F. Matthews. 2007. Laser capture microdissection (LCM) and comparative microarray expression analysis of syncytial cells isolated from incompatible and compatible soybean roots infected by soybean cyst nematode (*Heterodera glycines*). **Planta** 226:1389-1409

Problem Area 2C: Developing High-Value Products

Problem Statement: Plants are increasingly being recognized as sources of compounds that have important roles in nutrition, medicine, or industrial products. ARS research on natural products will help develop new high-value feedstocks for biobased products and pharmaceuticals. Natural products will embody valuable biological, nutritional, pharmacological, or other beneficial materials including domestic source of feedstocks for biobased products.

Research Needs: 1) New sources of novel phytochemicals will be identified. Constituent compounds will be isolated and characterized for appropriate chemical and biological activities. 2) This information will be related to the biosynthetic pathways, genes that control those pathways and the development of probes to enable rapid screening of plants for new sources of high-value compounds.

Expected Outputs: 1) Tools and methods to identify specific genes that mediate secondary end-product traits desired by consumers, such as nutritional and pharmaceutical compounds. 2) Identification and characterization of functional compounds and components in agricultural commodities and their byproducts that provide the basis for enhanced crop value; and 3) New sources of valuable bioactive compounds and potential sources for commercialization identified.

Problem Area 2C: Accomplishment Summary

This section summarizes the overall achievements in Problem Area 2C with a focus on scientific impact and potential benefits. Selected accomplishments follow this summary.

Output 1: *Tools and methods to identify specific genes that mediate end-product traits desired by consumers*

Tools and methods have been identified that mediate product traits desired by producers and consumers. A strength of NP 302 accomplishments in this problem area is that genes and associated methods have been determined that regulate useful agricultural traits and food value. For example, rotating sorghum with other crops has always been a problem due to allelopathic effects of what we now know as the compound surgoleone in the sorghum plant. NP 302 scientists and collaborators identified the genes involved in surgoleone biosynthesis and then determined that suppressing two specific genes produced surgoleone-null sorghum lines. The impacts are two-fold: isolation of a compound that can be developed as a natural-product herbicide and offering the ability to use sorghum in rotation with other crops without detrimental effects to the proceeding crop.

Genes that affect the end-product quality and nutritional value of food crops have been identified. Research aimed at increasing the nutritional value of wheat has resulted in the isolation and characterization of a wheat gene associated with grain protein, zinc and iron content. A version of this gene found in a wild ancestor of durum wheat was isolated and sequenced. Then researchers used genetic engineering to definitively demonstrate that the allele from wild durum wheat can increase protein, zinc, and iron content in bread wheat. Notably, wheat biotechnology methods developed by NP 302 researchers have provided the tools and methods needed to determine the functional role of specific genes for end-product quality as well as disease resistance. Results from this NP 302 molecular research are providing wheat breeders new candidate genes and genetic targets to increase wheat protein and nutrition levels in new cultivars for malnourished people, as well as protect the grain supply.

Other research has provided new methods to produce effective vaccines in seeds. NP 302 scientists have constructed a gene for a model vaccine protein in soybean and demonstrated its expression in seeds. The soybean-produced protein provided protection against antigen challenge in mice, demonstrating that the methods can be useful as a low-cost vaccination option.

Providing direct benefits to science and industry, ARS scientists developed an assay useful for screening natural products for the reversal of fluconazole resistance, identified the target pathway of a plant-derived alkaloid that possesses antifungal or fungicidal activity, and discovered and characterized new cyclopentenedione antifungal agents. Antifungal properties of acetyleneic acid were identified in yet another high value research effort in this area, thus offering potential for developing new antifungal agents.

NP 302 teams have been productive in transferring technology to the appropriate customers.Technology transfer contributions included distribution of molecular resources and tools, collaborative projects among crop breeders, CRADAs for end-product quality, and new uses of wheat. Numerous crop germplasm lines have been transferred to major private companies, and have been used in hybridizations to develop commercial cultivars. In many cases advanced breeding lines developed by ARS scientists were transferred via MTA documents, and were assessed by private companies to select ones appropriate for their specific programs. Specialty cultivars or genetic traits have also been developed and transferred to private companies for direct commercialization with appropriate agreements that facilitate wide distribution. Technology transfer from ARS NP 302 scientists in this problem area also included a natural immunostimulant product preparation for support of the immune system “Immune XT”, which was developed by the NCNPR, and recently released in U.S. markets. This product had been clinically studied and marketed in Europe for two years before being licensed and released in the U.S. in 2008. Several patent applications from NP302 scientists included: 1) a product from among the classes of compounds developed from natural compounds that showed potent phytotoxic, antifungal, antibacterial, and/or antimalarial activities 2) O-methyltransferase - a gene that can be used to enhance sorgoleone production in soghum or to impart it in other species, and can also be used to enhance pterostilbene production in blueberries, grapes, and other crops. This technology has been discussed with companies interested in pterostilbene. 3) A unique lipid desaturase - This gene encodes a unique lipid desaturase that desaturates the bond between the terminal two carbons of a lipid, and is the first such enzyme found in a plant. Cooperative Research and Development and other agreements include aquaculture-optimized soybeans from ARS-developed genetic materials, a swine model to evaluate compositional improvements in soybean meal digestibility, and construction of a peanut proteome map.

Output 2: ***Identification and characterization of functional compounds and components in agricultural commodities and their byproducts that provide the basis for enhanced crop value. New sources of valuable bioactive compounds and potential sources for commercialization identified.***

Functional compounds have been identified that provide a basis for enhanced crop value and end-product benefits for consumers. NP 302 soybean researchers addressed soybean customer/stakeholder needs initially identified in the United Soybean Board 2002 strategic plan to improve soybean seed quality through the “Better Bean Initiative” (http://www.agbioforum.missouri.edu/v6n12/v6n12a07-durham.pdf). Collaborative work with university scientists, coordinated by ARS, resulted in major impacts from the development of new soybean genetic resources and germplasm with enhanced functionality. ARS scientists developed soybeans with lowered levels of a highly allergenic protein, lower trans fatty acid-generating linolenic acid, increased oleic acid levels, and increased levels of vitamin E and sulfur-containing amino acids. In addition, ARS scientists developed a technique that soybean breeders seeking to alter the chemistry of the seed storage reserves can use to monitor the impact of breeding or biotech manipulations on fatty acid reserves. These improved genetic resources have provided needed incentives for breeders to design soybeans for specific human food and animal feeds.

Selection for low linolenic acid soybean and the ability to recombine different sources of mutant alleles have facilitated incorporation of the low-linolenic traits into high-yielding soybean varieties. This NP 302 research has contributed to the increased U.S. production of low linolenic soybeans (1.8-1.9 million acres in 2007 and 3.0-3.5 million acres in 2008) http://www.qualisoy.com/brochures/files/34140%20Qualisoy%20Insert.pdf), indicating the importance of this trait. The perfect molecular markers developed by ARS scientists have been transferred to private industry, and are being used in further development of low linolenic soybeans. As the relative importance of oil and protein in the marketplace has fluctuated, soybean breeders have sought new ways of manipulating the relative proportions of these two negatively correlated traits. In discovering how oil bodies are assembled by the endoplasmic reticulum, ARS scientists developed innovative ways to design new oil traits. These efforts have provided research techniques, tools, genetic materials, and methods for the soybean research community. Research results and resources have attracted and enabled soybean breeders across the country to focus on soybean seed improvement to specifically address customer/stakeholder needs to increase soybean competitiveness by improving the seed quality. These efforts also are impacting animal industries, such as aquaculture, to assess and adopt the improved and low allergen seeds, and poultry which will benefit from increased methionine levels.

Important advances have also been made in identifying functional compounds in other crop plants in addition to soybeans. Notably, key genes that regulate the vitamin, mineral, and other nutritional compounds in food crops have been identified. An example is that genes have been identified and characterized that control elevated levels of carotenoids in cauliflower. This new information enables plant scientists to develop cauliflower and other specialty crops with altered levels of specific carotenoids, that can be provided to nutrition and clinical scientists for hypothesis testing of human assimilation and utilization of carotenoid compounds. Another example is identification of a gene that modulates selenium levels. Identifying the gene that controls selenium accumulation provides new methods and molecular resources for use in improving nutritional benefits, as well as provides options to address environmental concerns involving high selenium.

Genes with key roles in food product functionality have been identified. A benefit of this research is that specific genes that affect function have been determined that could not be previously identified through genetics and breeding strategies. For example, millers and bakers need improved wheat cultivars that produce grains with reliable, consistent end-product qualities. Wheat scientists developed transgenic lines of bread and pasta wheats with elevated levels of specific high-molecular-weight glutenin proteins. As a result, transgenic lines were identified that produce flour with improved dough elasticity and bread loaf volume. These transgenic lines have been provided to wheat breeders in U.S. and international breeding programs for introgression into their breeding material and are currently being evaluated for end-product quality. Development of strong-gluten wheat by ARS scientists without adverse effects on seed yield and other agronomic traits impacts both producers and the US milling and baking industries for both bread and pasta wheats.

New sources of valuable bioactive compounds were identified. These include successful screening of plants for new sources of high-value compounds. Examples include antifungal drugs and fungicidal agents.

Selected Accomplishments:

Output 1: ***Tools and methods to identify specific genes that mediate end-product traits desired by consumers***

Identification of genes for sorgoleone biosynthesis. Sorghum root hair cells produce a compound called sorgoleone that suppresses weeds. The broad-spectrum phytotoxicity, chemical stability, and apparently complex mode-of-action of this compound have made it a promising candidate for development as a natural product alternative to synthetic herbicides. ARS scientists in collaboration with the University of Georgia identified genes involved in the biosynthesis of sorgoleone by developing an

EST database from sorghum root hair cells and testing candidate genes through functional assays with recombinant proteins and RNA interference studies. Suppression of two of the genes resulted in the generation of the world's first sorgoleone-deficient sorghum line. The sorgoleone-deficient sorghum line has potential utility in the elimination of replant problems with crops planted in rotation with sorghum. The identification of sorgoleone biosynthesis genes in sorghum represents a crucial step toward engineering its production in other important crops.

Baerson, S.B., Dayan, F.E., Rimando, A.M., Nanayakkara, N.P.D., Liu, C.-J., Schröder, J., Fishbein, M., Pan, Z., Kagan, I.A., Pratt, L.H., Cordonnier-Pratt, M.-M., Duke, S.O. 2008. A functional genomics investigation of allelochemical bioysynthesis in Sorghum bicolor root hairs. **Journal of Biological Chemistry**. 283:3231-324

Functional genomics for important wheat traits. The combination of wheat genomics and molecular mapping has yielded many genetic markers for traits important for wheat productivity and utilization. In the absence of a complete genome sequence, candidate sequences for individual wheat genes can be isolated by map-based cloning and other approaches, but verification of their identities requires that their functional properties be demonstrated by transforming them back into wheat. ARS researchers have used wheat genetic transformation in collaborations with university researchers to verify the functionality of several candidate genes isolated from wheat. DNA sequences were identified that comprise three major vernalization genes, a wild emmer wheat gene that supports increased nutritional value in domesticated wheat grains, and a kernel hardness gene that determines important milling properties. The sequences have been used to design molecular markers with which breeders can follow inheritance of particularly useful alleles in breeding programs.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., Dubcovsky, J. 2006. A *NAC* gene regulating senescence improves grain protein, zinc, and iron content in wheat. **Science** 314: 1298-1301.

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S., Dubcovsky, J. 2006. The wheat and barley vernalization gene VRN3 is an orthologue of FT. **Proceedings of the National Academy of Science USA** 103: 19581-19586.

Fu, D., Uauy, C., Distelfeld, A., Blechl, A.E., Epstein, L., Chen, X., Sela, H., Fahima, T., Dubcovsky, J. 2009. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. **Science**. 323:1357-1360.

Production of a model vaccine protein in soybean. Vaccines for human and animal immunizations are expensive and difficult to produce in sufficient quantities to be fully effective. Plant based synthesis of proteins has the potential for a low cost, high quantity, production approach to generate otherwise expensive protein based medicines. ARS scientists have constructed a gene for a model vaccine, transferred it into soybean and demonstrated its expression in seeds. The soybean-produced protein is identical to the native protein and accumulates to significant levels. The soybeans produced were used in a crude form to orally immunize mice. The soybean oral immunization provided protection against subsequent antigen challenge. Soybean seeds can provide a safe way to produce authentic vaccine proteins and an inexpensive pathway to produce oral immunizations. Low cost vaccination approaches using plant-based vaccine production has potential use in applications where cost is of primary concern, such as in vaccination of production animals and wild populations.

Moravec, T., Schmidt, M.A., Herman, E.M., Woodford-Thomas, T. 2006. Production of Escherichia coli heat labile toxin (LT) B subunit in soybean seed and its analysis of its immunogenicity as an oral vaccine. **Vaccine**. 25:1647-1657.

NIR for soybean fatty acid profiling. No suitable method was previously available for analysis of seed storage reserves during development, germination, and postgerminative growth to verify changes that might take place during transgenic events. A research method was developed to allow for the rapid analysis of soybean seed quality using near infrared reflectance (NIR) to profile the fatty acids of soybean cotyledons. The new method requires one-tenth of the usual sample size and allows for a rapid analysis of seed reserve fatty acids during seedling growth. Soybean breeders seeking to alter the chemistry of the seed storage reserves to make soybeans more nutritious for humans and animals, and can monitor the impact of breeding or biotech manipulations on fatty acid reserves.

Roberts, C.A., Ren, C., Beuselinck, P.R., Benedict, H.R., Bilyeu, K.D. 2007. Fatty acid profiling of soybean cotyledons by near-infrared spectroscopy. **Applied Spectroscopy**. 60:1328-1333

Development of an assay to identify natural products that enhance the anti-fungal activity of fluconazole in resistant organisms. The rapid development of drug resistance in pathogenic fungi, particularly for azole antifungals such as flucanazole, requires research into new strategies for effective antifungal therapies. Scientists at NCNPR developed an assay to screen natural product extracts for reversal of fluconazole resistance. Two compounds, capisterone A and B, were identified that significantly enhanced fluconazole activity and were not toxic to human cells. These compounds did not have antifungal activity when directly tested against several target pathogens. They may be useful in combination therapies of fungal infections caused by clinically relevant fluconazole-resistant strains. The assay that was developed can be used to screen for agents that overcome resistance to other antifungal drugs or fungicidal agents.

Li, X.C., Jacob, M.R., Ding, Y., Agarwal, A.K., Smillie, T.J., Khan, S.I., Nagle, D.G., Ferreira, D., Clark, A.M. 2006. Capisterones A and B, which enhance fluconazole activity in Saccharomyces cerevisiae, from the marine green alga Penicillus capitatus. **Journal of Natural Products.** 69:542-546.

Output 2: ***Identification and characterization of functional compounds and components in agricultural commodities and their byproducts that provide the basis for enhanced crop value. New sources of valuable bioactive compounds and potential sources for commercialization identified.***

Soybeans

Soybeans with enhanced vitamin E content. Plant derived oils suffer from instability when exposed to the air resulting in off flavors or a deterioration in lubricant properties dependent upon the specific usage. The vitamin E component of soybean seeds is important for conferring oxidative stability to the extracted vegetable oil in food processing and bio-based lubricant applications. Soybeans were engineered to co-express genes for two key enzymes in the vitamin E biosynthetic pathway. Seeds obtained from the genetically enhanced plants had up to 10-fold higher levels of vitamin E than non-transgenic counterparts. The transgenic soybeans also accumulated novel forms of vitamin E, some of which have greater antioxidant capacity than those typically found in soybeans.

Hunter, S.C., Cahoon, E.B. 2007. Enhancing vitamin E in oilseeds: unraveling tocopherol and tocotrienol biosynthesis. **Lipids** 42:97-108.

Identification and testing of a low allergenicity soybean. The use of soybean meal for both human and animal consumption is limited by high levels of certain proteins that act as major allergens. The immunodominant human seed allergen was shown to be a member of the papain super-family of cysteine proteases, P34. The first systematic and successful survey of an entire germplasm collection was conducted to isolate two lines that are essentially null allergen varieties of soybean that lack the P34 protein. These soybean lines formed the basis of a low allergenic soybean commercial line. In addition the P34 protein was used as a key challenge-antigen to develop a clinically relevant animal model, swine, to assess soybean biotechnology products. The combination of the nulls and the animal model compose a complex program to alleviate the problem of soy allergenicity. Meal from the low allergen soybeans is being tested for use as a feed supplement. The results of this research will lead to novel uses of soybean in both human and animal feed and increase the competitiveness of the U.S. soybean industry.

Josephs, L.M., Hymowitz, T., Schmidt, M.A., Herman, E.M. 2005. Evaluation of Glycine germplasm for nulls of the immunodominant allergen P34/Gly m Bd 30k. **Crop Science**. 46:1755-1763.

Soybean meal with enhanced sulfur-containing amino acid content. Although soybeans are a rich source of proteins, protein fractions from soybean seed-meal are deficient in the essential sulfur-containing amino acids, methionine and cysteine. A maize seed storage protein exceptionally rich in methionine residues was cloned and expressed in soybean plants. A two-pronged approach involving metabolic engineering of the sulfur assimilatory pathway, plus high level expression of methionine-rich proteins was shown to be the most promising way of enhancing the overall sulfur amino acid content of seeds. The information obtained from these basic studies will help in the development of genetic or biotechnology-based strategies for manipulation of the sulfur-assimilatory enzymes, leading to an improvement in overall quality of seed proteins. Superior quality soy proteins can be utilized in a multitude of food and feed applications.

Krishnan, H.B. 2005. Engineering soybean for enhanced sulfur amino acid content. Crop **Science.** 45:454-461.

Mahmoud, A.A., Natarajan, S.S., Bennett, J.O., Mawhinney, T.P., Wiebold, W.J., Krishnan, H.B. 2006. Effect of six decades of selective breeding on soybean protein composition and quality: A biochemical and molecular analysis. **Journal of Agriculture and Food Chemistry**. 54:3916-3922.

Low linolenic acid soybeans generated. Linolenic acid (18:3) is an oxidatively unstable component of cooking oil. Removal by hydrogenation creates unhealthy *trans* fatty acids. The molecular genetic basis for reduced accumulation of linolenic acid in soybean seed oil was identified. It was shown that mutations in multiple fatty acid desaturase genes could be combined to produce low 18:3 oil without *trans* fatty acids. The discovered mutations were translated into molecular markers that allowed plant breeders to efficiently capture the desired genes and rapidly develop elite cultivars with the low 18:3 trait. Low linolenic (18:3) soybeans are grown on an increasing numbers of acres each year to meet the needs of the food industry and consumer demands for the high value oil.

Chappell, A.S., Bilyeu, K.D. 2007. The low linolenic acid soybean line PI 361088B contains a novel GmFAD3A mutation. Crop Science. 47:1705-1710.

Flores, T., Karpova, O., Su, X., Zeng, P., Bilyeu, K., Sleper, D.A., Nguyen, H.T., Zhang, Z.J. 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.

Suppression of soybean oil body protein restructures oil bodies. Alteration of the oil content and oil to protein ratio in soybeans has been a major objective of soybean breeders for many years in an effort to improve the value of U.S. soybeans. These efforts have been limited by our understanding of how oil bodies are formed and if manipulation of their formation could alter the ratio of oil to protein. ARS scientists have constructed a total knockdown of the 24 kDa oleosin protein, the major soybean oil body structural protein. The results revealed a critical role for oleosin in the assembly of oil bodies by the endoplasmic reticulum and as a macromolecular surfactant. Understanding the role of the endoplasmic reticulum in forming oil bodies provides a foundation for engineering new oil traits.

Schmidt, M.A., Herman, E.M. 2008. Suppression of soybean oleosin produces micro-oil bodies that aggregate into oil body/ER complexes. **Molecular Plant.** 1:910-924.

Output 2: ***Identification and characterization of functional compounds and components in a variety of crop plants that provide the basis for enhanced value (continued)***

Other Crops

Novel gene controls accumulation of carotenoids. Biofortification of staple crops with increased levels of carotenoids is considered to be an effective and sustainable approach to improve human nutrition and health. Current approaches rely primarily on overexpression of the biosynthetic genes, which have not resulted in desired levels of carotenoids. ARS researchers have isolated a novel gene from orange cauliflower and found that it controls a high level of provitamin A accumulation by providing a sink for storage. Now that the gene has been identified plant scientists can block or silence the gene as a new strategy to ensure that the nutritional and health benefits value of vegetables are maintained and provided to consumers.

Lu, S., Van Eck, J., Zhou, X., Lopez, A.B., O'Halloran, D.M., Cosman, K.M., Conlin, B., Paolillo, D.J., Garvin, D.F., Vrebalov, J., Kochian, L.V., Kupper, H., Earle, E.D., Cao, J., Li, L. 2006. The cauliflower Or gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high-levels of ß-carotene accumulation. **Plant Cell** 18:3594-3605.

Li, L., Lu, S., Garvin, D.F., Vrebalov, J., O'Halloran, D.M. 2005. The Or gene and its use in manipulating carotenoid content and composition in plants and other organisms**.** Patent Application Filed on 12/6/2005.

Target gene identified for control of selenium levels. Selenium is an essential micronutrient for animals and humans, but is toxic at high levels. The difference between the beneficial and toxic levels of selenium is narrow, making both selenium deficiency and selenium pollution common problems. A methyltransferase was identified that mediates selenium volatilization in both bacteria and plants. This gene can be used to modify plants that accumulate selenium for enhanced phytoremediation performance in selenium-contaminated environments. It may be useful as a target to suppress conversion of selenium to the volatile forms, especially in cases where accumulation of bioactive forms of selenium are required.

> Lyi, S.M., Heller, L.I., Rutzke, M., Welch, R.M., Kochian, L.V., Li, L. 2005. Molecular and biochemical characterization of the selenocysteine *Se*-methyltransferase gene and *Se*-methylselenocysteine synthesis in broccoli. **Plant Physiology**. 138:409-420.

Strong-gluten wheats produced by genetic transformation. The U.S. baking industry requires consistently high-strength flours for efficient manufacturing of bread products. Bread loaf dough strength has been correlated with the presence of certain allelic variants of the glutenin seed storage proteins. In order to produce stronger gluten wheats, ARS researchers have transformed bread and pasta wheats with native wheat genes encoding high-molecular-weight glutenins. The flours from seeds of some of these lines combine improved dough elasticity with bread loaf volume potentials equal to their non-transformed parents. Many of these wheats have the same yield and agronomic properties as their parent variety. The differing effects of individually over-expressing two high-molecular-weight glutenins whose natural genes are always inherited together were demonstrated. Germplasm containing these transgenes that could be useful to bakers have been distributed to wheat breeders for evaluation and are currently being evaluated in several U.S. wheat breeding programs.

> Blechl, A., Lin, J., Nguyen, S., Chan, R., Anderson, O. D., Dupont, F. M. 2007. Transgenic wheats with elevated levels of Dx5 and/or Dy10 high-molecular-weight glutenin subunits yield doughs with increased mixing strength and tolerance. **Journal of Cereal Science**. 45:172-183.

Antioxidant from oat has beneficial health effects. Oats produce antioxidants called avenanthramides. In collaboration with scientists at the Jean Meyer Human Nutrition Laboratories, ARS scientists showed that the avenanthramide antioxidants produced by oats provided anti-atherosclerotic properties in model cell cultures. In addition, field trials demonstrated a negative relationship between crown rust incidence and avenanthramide production in oat cultivars, suggesting a correlation with disease resistance in the host plant. Recombinant inbred lines are now being developed to identify molecular markers to assist with genetic manipulation of the avenanthramides.

> Guo, W., Wise, M.L., Collins, F.W., Meydani. M. 2008. Avenanthramides, polyphenols from oats inhibit IL-1b-induced NF-kB activation in endothelial cells. **Free Radical Biology & Medicine**. 44:415-429.

> Nie, L., Wise, M.L., Peterson, D., Meydani, M. 2006. Mechanism by which avenanthramide-C, a polyphenol of oats, blocks cell cycle progression in vascular smooth muscle cells. **Free Radical Biology & Medicine**. 41:702-708.

Identification of acetylenic fatty acids with potent antifungal activity. Five 6-acetylenic acids with chain lengths from 16 to 20 carbons were isolated by NCNPR scientists from the plant *Sommera sabiceoides*. Two of these represent newly isolated fatty acids. These acetylenic acids were evaluated for their in vitro antifungal activities against several clinically important fungal pathogens and compared with known drugs. The compounds showed various degrees of antifungal activity against the 21 test strains. One showed minimum inhibitory concentrations comparable to several of the control drugs. Testing with cell lines and in mice suggests these agents are well tolerated. The low levels of toxicity in vitro and in vivo and significant antifungal potencies make these 6-acetylenic acids attractive for further development as antifungal agents.

Li, X.C., Jacob, M.R., Khan, S.I., Ashfaq, M.K., Babu, K.S., Agarwal, A.K., Elsohly, H.N., Manly, S.P., Clark, A.M. 2008. Potent in vitro antifungal activities of naturally occurring acetylenic acids. **Antimicrobial Agents and Chemotherapy** 52:2442-2448.

Identification of mechanisms of action of the antifungal alkaloid sampangine. Sampangine is a plant-derived alkaloid that exhibits strong fungicidal activity against human and plant pathogens. NCNPR scientists used transcriptional profiling coupled with analyses of yeast mutants to elucidate its mechanism of action. Sampangine produces a transcriptional response indicative of hypoxia, altering the expression of genes known to respond to low-oxygen conditions. These responses affect heme biosynthesis or catabolism. This potentially important target pathway for disruption in fungi may be exploited for fungicide or anti-fungal drug development.

Agarwal, A.K., Xu, T., Jacob, M.R., Feng, Q., Lorenz, M.C., Walker, L.A., Clark, A.M. 2007. Role of heme in the antifungal activity of the azaoxoaporphine alkaloid sampangine. **Eukaryotic Cell**. 7:387-400.

Discovery and characterization of new cyclopentenedione antifungal agents. The limitations of current antifungal drugs, increased incidence of systemic fungal infections, and rapid development of drug resistance highlight the need for the discovery of novel antifungal agents. Coruscanones A and B, two new antifungal cyclopentenedione derivatives, were isolated from the plant *Piper coruscans.* Coruscanone A showed potent in vitro antifungal activity against *Candida* and *Cryptococcus.* Structure-activity relationship studies indicated the precise structural component responsible for the antifungal activity of this new class of compounds. Understanding the mechanism of action of this new antifungal agent provides novel insights for design of effective antifungal drugs and fungicides.

Babu, K.S., Li, X.C., Jacob, M.R., Zhang, Q., Khan, S.I., Ferreira, D., Clark, A.M. 2008. Synthesis, antifungal activity, and structure-activity relationships of coruscanone A analogues. **Journal of Medicinal Chemistry**. 49:7877-7886.

RESEARCH COMPONENT 3: Plant Biotechnology Risk Assessment

Genetic engineering offers tremendous promise for improving crop production and protection, making production systems more efficient and sustainable, and providing high-value and high-quality products needed by the world's burgeoning population of consumers. These products can range from foods with enhanced nutrients to biomedical reagents. Methods used for genetic engineering of crop plants continue to require improvement, and the principles that determine the risks of transgenic plants in the environment need to be better elucidated. Research that integrates product development with risk assessment is essential to develop data that will help guide regulatory decisions on management of transgenic crops in a manner that builds public confidence in the safety of products derived from biotechnology.

The need for biotechnology risk assessment research conducted by the public sector was first identified by National Academy of Science (NAS) committee findings (2000, 2001, 2002). In April 2000, a NAS Committee published a report, which concluded that pest-protected genetically engineered organisms entailed no more risk than conventionally bred plants. However, the NAS Committee identified issues that could be addressed through biotechnology risk assessment research, especially on long-term genetic and ecological issues. Prominent research needs addressed unwanted gene flow, development of resistance in pest populations, and potential non-target effects of genetically engineered protectants.

Much of the initial data establishing safety of genetically engineered products came from companies commercializing the materials. The appearance of a conflict of interest and the desire for transparent processes and data available to the public led to support of more risk assessment data generated by public institutions.

Congressional appropriations that responded to the NAS reports and public concerns were made in 2003 and 2004 to ARS for biotechnology risk assessment research. As a result, Component 3 was added to the National Program 302 Action Plan for this cycle. New biotech risk assessment research was initiated at Albany, CA, Ames, IA, Beltsville, MD, and Ithaca, NY. A portion of the new appropriations were used to add or strengthen biotechnology risk assessment research objectives in ARS projects in other National Programs. Research results from those non-NP 302 projects (listed in Appendix 2) relevant to biotech risk assessment are included in this report.

Objectives for the new projects to address Problem Area 3A were chosen according to priorities identified at the NP 302 Customer and Stakeholder Workshop, for needs in advancing ARS biotech germplasm, and in consultation with regulatory agencies, particularly APHIS/Biotechnology Regulatory Services. Problem Area 3B was also added to the NP 302 Action Plan to address the needs identified by the National Academy of Sciences for expanded research on Interaction of Transgenic Plants with their Environment. ARS only received funding to initiate one dedicated NP 302 project (Madison, WI) that addresses Problem Area 3B. ARS projects in other national programs have developed biotech risk assessment objectives relevant to Problem Area 3B and their relevant results are included in this report.

ARS also supports short-term biotech risk assessment research grants through the BRAG (Biotechnology Risk Assessment Grants) Program. This USDA competitive grants program is co-managed by USDA CSREES and ARS. ARS funding is provided by a 2% assessment on all ARS biotechnology research projects as mandated in the 2002 Farm Bill. ARS and CSREES National Program Leaders have conducted joint workshops with the regulatory agencies (APHIS/BRS, FDA, and EPA) to identify research priorities for the BRAG short-term grant program and the longer-term NP 302 projects.

In addition to ongoing planning and coordination during this cycle of NP 302, the USDA-ARS Office of National Programs and Area Offices organized and led many workshops focused on specific biotechnology risk assessment research issues relevant to the needs of agriculture. A partial list of workshops appears in Appendix 1. These workshops enable ARS and other biotech risk assessment researchers to communicate their most recent data and to target high priority research needs.

Engaged ARS Locations: Albany, California; Ames, Iowa; Beltsville, Maryland; Corvallis, Oregon; Ithaca, New Yokr; Kearneysville, West Virginia; Lincoln, Nebraska; Madison, Wisconsin; Stoneville, Mississippi.

Problem Area 3A: Improving and Assessing Genetic Engineering Technology

Problem Statement: The utility of plant transformation protocols often is limited by low recovery of transformed cells, unpredictable expression of transgenes, inability to control transgene expression, residues of unneeded selectable marker-genes, and limited ability to introduce multiple genes. ARS research will characterize the genetic changes that accompany transformation, determine the mechanisms that alter gene expression and metabolic processes, and elucidate the biological consequences of those alterations. Target traits for transformation technology improvement include resistance to Fusarium head blight, resistance to plum pox virus, nematode resistance, all seed traits and fruit traits.

Research Needs: 1) Methods will be developed that reliably and reproducibly insert single copy genes into a host genome, that limit gene expression, and that remove transgenes from harvested materials once their usefulness is over. 2) Methods will be developed for "pyramiding" multiple genes so that multigenic traits can become feasible targets for transfer. 3) Baseline data will be collected on the changes in global gene expression patterns when plants undergo through a vegetative or sexual reproductive process, for comparison to changes that accompany recombinant DNA manipulation. The deviation of global gene expression patterns from normal will be correlated with plant performance, so that unacceptable levels of variability can be defined.

Expected Outputs: 1) Improved gene constructs and plant genetic transformation systems that efficiently incorporate genes and enable their stable expression, and that allow expression of multiple genes and limit expression to specified tissues. 2) New processes placed in the public domain to the extent possible, promoting public access to them. 3) Data that define genetic and epigenetic effects of transformation and comparison of those effects to similar effects of natural reproductive processes, so that thresholds of acceptability can be identified.

Problem Area 3A: Accomplishment Summary

This section summarizes the overall achievements in Problem Area 3A with a focus on scientific impact and potential benefits. Selected accomplishments follow this summary.

One goal in Problem Area 3A is to develop gene components, gene delivery systems, and improved protocols for the development of biotech products and varieties, when no solution is possible through conventional breeding, that address important agricultural problems, and for which biotechnology support from the private sector is limited.

The potential for gene flow from transgenic to nontransgenic crops, and to wild species of plants and animals, can be better managed if marker genes, and other nonplant DNA sequences, can be removed from the genome. NP 302 scientists developed a new recombinase-based technology for the removal of selectable marker genes *in planta*. ParA recombinase, from the *E. coli* plasmid RK2, was shown to mediate precise, heritable deletions of target DNA sequences that were flanked by ParA recognition sites *in planta*. This technology also enables the stacking or "pyramiding" of transgenes through repeated transformation and selection. Evaluation in crop plants is continuing. Should a patent be issued, this technology will be available for non-exclusive license for the production of marker free plant varieties.

Problem 3A accomplishments include significant contributions to the limited collection of well-characterized promoters in the public domain for the quantitative and qualitative control of transgene expression. NP 302 scientists characterized in detail the activity of organ-specific promoters from many systems *in planta*. Promoters were developed that target only the generative and sperm cells in pollen, or are green tissue-specific, root-specific, flower-specific, or leaf-specific. Problem Area 3A accomplishments include synthetic promoters that were generated using enhancer elements in a combinatorial strategy. The analysis of promoter element interactions generated new knowledge for combining promoter sequence elements to modulate gene expression. Plant derived promoters and synthetic promoters were released to researchers and stakeholders. Target applications, requested by stakeholders and regulatory agencies, include restricting transgene expression to plant tissues that are not consumed as food, minimizing the possibility of horizontal gene flow by the excision *in planta* of vector DNA and selectable marker genes, disease resistance, and the production of foreign proteins. Selected promoters are now being used to test candidate genes for combating Fusarium head blight and for use in gene flow mitigation.

Concerns expressed by some consumers about transgenic crops resulting from the presence of nonplant DNA sequences and the production of nonplant proteins can be addressed, in part, by the development of transformation vectors and marker sequences derived from plant DNA sequences. Problem Area 3A accomplishments include the development and testing of a rice DNA based “intragenic” or pDNA transformation vector and transcriptional control sequences. Intragenic transformation events were generated and are being evaluated.

Publicly supported research for the development of biotechnology based solutions for the control of pests and diseases in crops like barley and wheat are extremely important for these commodities and their stakeholders. Low transformation efficiency of barley germplasm used for the development of new varieties, has been a significant roadblock for the development of transgenic solutions for Fusarium head blight. NP 302 scientists developed a highly efficient barley transformation protocol for use on recalcitrant germplasm. Tranformation efficiencies of up to 25% were achieved, making the improved method the most efficient barley transformation system available. This in turn enables direct use of barley for molecular genetic studies that were previously possible only in model plants. For example, barley transformation is now being used to assess candidate genes for Fusarium head blight resistance.

Transgenic seeds are an important platform for the production and storage of foreign proteins. In soybean, where up to 40% of total seed mass is protein, the accumulation of a foreign protein using a strong seed-specific storage protein gene promoter (e.g. glycinin) and ER retention sequence tag (KDEL) was approximately 1.6% (w/w) of total seed protein. NP 302 scientists achieved enhanced levels of foreign protein production – up to 7% (w/w) – by combining the glycinin promoter and ER retention sequence tag with collateral seed protein rebalancing, using a β-conglycinin deficient seed protein mutant. This is a breakthrough achievement for the production of foreign proteins for pharmaceutical and industrial use, including enzymes for the conversion of cellulose and other cell wall components to biofuels.

ARS is prepared for the plum pox virus. Plum pox virus (PPV) is a devastating disease of stone fruit trees in Europe and also an important testcase for biotechnology risk assessment. Aphids are the primary vector. No natural resistance to PPV is available for breeding purposes. ARS researchers developed a genetically engineered plum, “HoneySweet”, that is highly resistant to PPV using gene silencing. Risk assessment analyses were performed for 16 years. The risk of crossbreeding is low and fruit composition is not affected. Thus, 'Honeysweet' illustrates the safe and effective application of biotechnology against a quarantine pest, and an effective strategy for reducing the use of pesticides to control natural vectors. 'Honeysweet' was deregulated by USDA/APHIS (Federal Register Doc. E7-13649) in 2007. The FDA agreed with the deregulation in 2009. However, the application remains under EPA review.

Some consumers have concerns that food harvested from transgenic crops may present a potential health risk because they have unanticipated changes in composition or quality, relative to conventionally produced products. Regulatory agencies noted that baseline information, about natural variation in selected commodities was needed when the NP 302 Action Plan was developed. In response, NP 302 scientists in Problem Area 3A generated data and produced methods that established a baseline for genetic variation in selected commodities. For example, in tomato, researchers demonstrated great natural variation for 24 heirloom varieties, breeding lines, and ripening mutants using gene expression analysis and metabolite analyses using NMR and LC-MS. In soybean, high levels of natural, genotype-specific, variation was documented for seed protein levels and seed protein composition for diverse wild and cultivated non-transgenic soybeans using proteomic approaches. One line from Nepal had more total protein, more cysteine, and more arginine than current commercial varieties. A baseline for seed protein content and composition was established for equivalency assessments of transgenic and non-transgenic lines. For barley, NP 302 scientists worked with an international consortium (also supported by USDA-CSREES) to generate the "Barley Gene Atlas", a summary of the developmental expression of 22,000 barley genes that covers fifteen tissues, from seed-to-seed, using the Barley1 GeneChip. This atlas is an important resource for the worldwide research community, and is available as a reference resource for regulatory agencies. In potato, NP 302 scientists, with funding from APHIS, and in collaboration with the University of Idaho, completed an assessment of the total genetic diversity of potato virus Y in every potato seed field in the USA and Canada using PCR and a bioinformatics pipeline. The natural genetic diversity revealed was very high. This is an important step in the production of diagnostic tools for monitoring the spread of viruses in potato by regulatory agencies and for the development of biotech solutions. Together, these results serve as a basis for assessing the effects of transgene insertion on global gene expression and metabolism that enables researchers and regulators to assess transgenic products for unanticipated effects on biotechnologically derived traits. A wide range of natural diversity within crop species was found that exceed reported effects from transgene insertion.

Selected Accomplishments:

Output 1: *Molecular Technology for Improved Genetic Engineering of Crop Plants*

Novel recombinase functions in planta. The imprecision of genetic engineering as currently practiced in plants has raised concerns about the random nature of transgene integration, which can often generate multi-copy insertions, unexpected DNA rearrangements, and invariably, the retention of marker genes in the resultant plant genome. To address these concerns, ARS researchers, Albany, CA, have identified seven new site-specific recombination systems that function in eukaryotic cells. One novel recombinase called ParA was shown to mediate precise, heritable deletions of a plant transgene that was flanked by the ParA recognition sites. This technology removes unwanted transgenic DNA from plants and is publicly available. Each of these seven recombinases has been shown to function in yeast and these are be used to develop new gene stacking technology in plants.

Thomson, J.G., Ow, D. W. 2006. Site-specific recombination systems for the genetic manipulation of eukaryotic genomes. **Genesis** 44: 465-476.

Thomson, J.G., Yau, Y.-Y., Blanvillain, R., Chiniquy, D., Thilmony, R., Ow, D.W. 2009. ParA resolvase catalyzes site-specific excision of DNA from the *Arabidopsis* genome. **Transgenic Research** 18:237-248.

Ow, D.W., Thomson, J. New site-specific recombinases for use in eukaryotic cells. U.S. Patent application 20060046294, published March 2, 2006 (pending).

Ow, D.W. Methods for the replacement, translocation and stacking of DNA in eukaryotic genomes. U.S. Patent 6,936,747, Issued August 30, 2005.

Enhancement of foreign protein accumulation in soybean. The use of crop plants to produce foreign proteins at industrially useful levels has been limited by the yield of protein produced per unit mass of harvested plant materials. When normal seed storage protein accumulation in soybean is suppressed using RNAi technology, the seed compensates for the suppression of the normal storage protein synthesis by increasing the synthesis of other seed proteins to maintain content. By expressing a foreign protein gene to mimic the compensating protein's allele, the accumulation of the foreign protein can be significantly enhanced. Soybean seeds have been generated that contain a foreign protein at levels that have commercial application. This new method to produce high-levels of foreign proteins such as industrial and biofuel enzymes in seeds is a major breakthrough. There is excellent potential to use this new technology in a low cost production platform (soybean seeds) to lower the production cost of industrial enzyme products and subsequently significantly lower production costs.

Schmidt, M.A., Herman, E.M. 2008. Proteome rebalancing in soybean can be exploited to enhance foreign protein accumulation. **Plant Biotechnology Journal**. 6: 832-842.

Herman, E. M., Schmidt, M.A. 2008. Improved protein storage in plants. Provisional patent application # 3536553 filed.

Tools for precise control of transgene expression. A limitation of current transformation technology is a dearth of well-characterized organ-specific promoters. Indeed, most regulatory elements currently used for crop biotechnology exhibit widespread expression throughout the plant. In order to confine transgene expression to only when and where it is needed, ARS researchers have identified four new organ-specific promoters in plants. Two are from rice, one of which is expressed only in green tissues and another that is root-specific. In addition, a promoter from barley was shown to be flower specific, and one from peach was shown to be primarily leaf specific. None of the four was active in edible plant parts. These molecular tools have been made available to researchers in government, academia and industry to allow organ-specific control of gene expression. Use of regulatory elements that are precisely expressed, can be exploited to eliminate the expression of foreign proteins in edible plant parts.

Bassett, C.L., Callahan, A.M., Artlip, T.S., Scorza, R. and Srinivasan, C. 2007. A minimal peach type II Chlorophyll a/b-binding protein promoter retains tissue-specificity and light regulation in tomato. **BMC Biotechnology** 7:47 (published online doi:10.1186/1472-6750-7-47).

Somleva, M. N., Blechl, A. E. 2005. The barley *Lem 1* gene promoter drives expression specifically in outer floret organs at anthesis in transgenic wheat. **Cereal Research Communications** 33: 665-671.

Thilmony, R., Guttman, M., Chiniquy, D., Blechl, A. 2006. pGPro1, a novel binary vector for monocot promoter characterization. **Plant Molecular Biology Reporter** 24:57-69.

Efficient barley regeneration conditions developed. Barley tissue culture and transformation have been limited by genotype restrictions on regeneration capacity. Optimization of tissue culture medium and growth conditions pre-transformation improved regeneration in a wide variety of genotypes. Levels up to 25% of explants producing transgenic plants were achieved, making the improved method the most efficient barley transformation system available. This improved transformation efficiency allows direct use of barley for a variety of molecular genetic studies that were previously possible only in model plants. These new barley transformation methods are now being exploited to assess candidate genes for Fusarium head blight resistance.

Dahleen, L.S., Manoharan, M. 2007. Recent advances in barley transformation. **In Vitro Plant.** 43:493-506.

Gene promoter useful in *Gladiolus*. Flower bulb crops such as *Gladiolus* and lilies are susceptible to viruses, *Fusarium*, and nematodes. It is increasingly difficult to control these pests and pathogens as fewer pesticides are available, and there are no resistant cultivars that can be used in breeding. Genetic engineering to develop resistant cultivars requires gene promoters that will drive high levels of transgene expression, but promoters from cereals are not useful in *Gladiolus*. ARS researchers showed that a polyubiquitin promoter from *Gladiolus* directs high levels of transgene expression in *Gladiolus*, as well as in several dicots. Transgenic *Gladiolus* can be engineered with transgenes that confer resistance to pathogens and pests.

Kamo, K.K. and Joung Y.H. 2007. A polyubiquitin promoter isolated from Gladiolus and its expression in Gladiolus plants. **Acta Hort** 736:251-258.

Synthetic plant-functional promoters. ARS scientists produced, tested, and released synthetic promoters to researchers and stakeholders. A detailed quantitative analysis of the function and interaction of individual transcriptional promoter elements was also performed to better understand how different regulatory sequence elements interact to control gene expression. This work adds to a limited collection of well-characterized plant promoters that are in the public domain, and provides novel insight that contributes to an improved ability to construct and employ gene regulatory systems for plant genetic engineering.

Cazzonelli, C.I., Velten, J. 2008. In vivo characterization of plant promoter element interaction using synthetic promoters. **Transgenic Res** 17:437-57.

Development of methods for transformation protocols in Rosaceae and a model biotechnology risk mitigation system for Rosaceae. ARS researchers have developed a genetically engineered plum variety "HoneySweet" that is highly resistant to plum pox virus (PPV). The virus has been detected in Pennsylvania, New York, and Michigan, Canada, and is a major problem in parts of Europe. No natural genetic resistance is available for use in tradition resistance breeding. Thus, ARS has taken the proactive step of developing a PPV-resistant, biotech tree and doing the extensive biotechnology risk assessment required to allow genetically engineered trees to become available if needed. Gene silencing of the viral coat protein gene has been used in the successful development of a commercially acceptable plum with plum pox protection. An objective of this research has been to develop a model biotechnology risk mitigation system and efficient genetic transformation protocols for Rosaceae. Extensive development of methods for biotechnology risk mitigation and assessment were made. Trees were successfully assessed according to authorized restrictions in the greenhouse in the U.S. and in Spain, Poland, and Romania where PPV is indigenous. Comprehensive research was completed determining low risk of crossbreeding and confirming that the fruit content had not been affected. The durability of PPV resistance in 'HoneySweet' is reflected through more than 10 years of field tests. Impact: Research (over 16 years) with 'Honeysweet' has demonstrated that this clone and the resistance mechanism that it represents is: 1) an important tool to demonstrate the successful deployment of biotechnology against a quarantine pest, 2) a safe product of biotechnology, and 3) a useful strategy for avoiding the use of pesticides to control natural aphid vectors of PPV. The deregulation of 'Honeysweet' in the USA by USDA/APHIS (Federal Register Doc. E7-13649, July 12, 2007) corroborates the utility of these findings. FDA agreed with the deregulation in 2009, and the application remains under EPA review.

Petri, C., Webb, K.K., Hily, J., Dardick, C.D., Scorza, R. 2008. High transformation efficiency in plum (*Prunus domestica L.*): a new tool for functional genomics studies in *Prunus* spp. **Molecular Breeding**: 22:581-591.

Output 2: ***Assessment of Potential Unintended Consequences of Transformation***

Methods for non-targeted metabolomic analysis of variation in tomato lines. Some consumers have the perception that food products from transgenic plants represent a risk because they may have unanticipated changes in composition or quality, relative to conventionally developed products. To address the potential for unintended consequences, ARS researchers have examined variation in tomato fruit quality and composition using gene and metabolite expression analyses. A diverse panel of 24 lines, including heirloom varieties, breeding lines and ripening mutants is being used to estimate the boundaries of normal variation in fruit quality and composition. Methods were tested for non-targeted fingerprinting of metabolites in methanolic extracts with NMR and LC-MS. This information on the natural range of factors for quality and composition provides baseline data for assessment of the effects of transgenes in tomaoto. This new information regarding the genetic and chemical bases for fruit quality in tomato will be useful to the breeding community.

Hoekenga OA. 2008. Using metabolomics to estimate unintended effects in transgenic crop plants: problems, promises and opportunities. **Journal of Biomolecular Techniques** 19:159-166.

Proteomic analysis of natural variation in soybean seed proteins. Soybean is the second most important cash crop in the U.S. and provides an inexpensive source of protein for humans and animals. Concerns about the biosafety of transgenic soybeans have been expressed by some consumers. The natural variation in the composition of seed protein was assessed using proteomic approaches on a diverse set of wild and cultivated non-transgenic soybeans. High levels of genotype-specific variation were evident in the storage proteins, glycinin and ß-conglycinin. One line from Nepal had more of the amino acids cysteine and arginine, as well as more total protein, compared to the U.S. commercial cultivars. In addition, wild soybeans have more of the three major soybean allergenic proteins and two anti-nutritional proteins than cultivated soybeans. A baseline for seed protein content and composition has been established for equivalency assessments of transgenic and non-transgenic lines, and is now available as a reference resource for regulatory agencies. Additionally, Nepalese soybean was identified as a potential germplasm resource for the improvement of protein content and quality and as a preferable alternative for swine and poultry feed.

Mohmoud, A.A., Natarajan, S.S., Bennett, J.O., Mawhinney, T.P., Wiebold, W.J., Krishnan, H.B. 2006. Effect of six decades of selective breeding on soybean protein composition and quality: A biochemical and molecular analysis. **Journal of Agricultural and Food Chemistry.** 54:3916-3922.

Xu, C., Caperna, T.J., Garrett, W.M., Cregan, P., Bae, H., Luthria D., Natarajan, S.S. 2007. Proteomic analysis of the distribution of the major seed allergens in wild, landrace, ancestral and modern soybean genotypes. **Journal of the Science of Food and Agriculture**. 87:2511-2518.

An atlas of gene expression in barley. In recent years, access to complete genomic sequences, coupled with rapidly accumulating data related to RNA and protein expression patterns, has made it possible to examine how genes contribute to complex phenotypes. Using the Barley1 GeneChip, ARS scientists worked with an International team of cereal scientists from five US Universities, in addition to institutions from five countries on four continents to evaluate in parallel the expression of 22,000 genes in fifteen tissues from seed-to-seed in barley development. The "Barley Gene Atlas", also supported by USDA-CSREES Initiative for Future Agricultural Systems (IFAFS), provides a unique opportunity to integrate detailed information on individual genes into a unified developmental framework that is available to the worldwide research community and a comparative dataset for those investigating genes or regulatory networks in other plant species**.** The information derived from these determinations provides baseline data for determining the effects of transgene insertion in barley.

Druka, A., Muehlbauer, G., Druka, I., Caldo, R., Baumann, U., Rostoks, N., Schreiber, A., Wise, R., Close, T., Kleinhofs, A., Graner, A., Schulman, A., Langridge, P., Sato, K., Hayes, P., McNicol, J., Marshall, D., Waugh, R. 2006. An atlas of gene expression from seed to seed through barley development. **Functional and Integrative Genomics** 6:202-211.

Assessment of genetic diversity of potato virus Y. Pathogens of potato are kept in check by seed certification programs. However, cultivars that are symptomless carriers of viruses have hampered the success of such programs. Assessing the diversity of viruses is the first step in producing diagnostic tools to allow regulatory agencies to monitor the problem and to design biotech solutions. Funded by APHIS and in collaboration with the University of Idaho, the genetic diversity of potato virus Y (PVY) in every potato seed field in the USA and Canada was determined using a PCR and bioinformatics pipeline. The genetic diversity is high overall, indicating that multiple diagnostic tools would be required to monitor PVY, and strategies for engineering resistance must take this broad genetic spectrum into account. The results were used in developing the Canada/US Bi-National Necrotic Potato Virus Management Plan. The methodology is also being used to diagnose PVY in seed potatoes.

Baldauf, P., Gray, S.M., Perry, K. 2006. Biological and serological properties of potato virus y isolates in northeastern US potatoes. **Plant Disease**. 90:559-596.

Problem Area 3B: Interaction of Transgenic Plants with Their Environment

Unbiased and rigorous information is needed to guide regulatory agencies that oversee the deployment of transgenic crops. The possibility of unintended ecological effects of transgenes needs to be maintained as low as possible, using the likelihood of effects from non-transgenic crops as a benchmark for comparison. These include such concerns as deleterious effects from the introgression of transgenes, effects of plant-incorporated protectants, or induced weediness or invasiveness. ARS research will focus on ways to accomplish appropriate recommendations of the National Research Council for biological confinement of transgenic material.

Research Needs: 1) The evaluation of gene flow from transgenic crops needs to be accompanied by analysis of the ecological effects and the persistence of those genes. 2) Methods will be developed to eliminate transgenes from pollen or, like male sterility, otherwise prevent transgenes from being transmitted during reproduction. The molecular basis of pollen-stigma compatibility will be characterized, so that pollen from transgenic plants can be made incompatible with potential female receptors.

Expected Outputs: 1) Greater knowledge and technology to remove transgenic DNA from pollen, or other approaches likely to help contain transgene flow in the field and control gene spread; and 2) Ability to characterize the nature and likelihood of persistent changes in ecosystems as a result of introducing new transgenic plants, in support of science-based regulation of transgenic crops.

Problem Area 3B: Accomplishment Summary

This section summarizes the overall achievements in Problem Area 3B with a focus on scientific impact and potential benefits. Selected accomplishments follow this summary.

Genetically engineered crops have revolutionized the management of insect pests by providing growers “in the bag” protection and by drastically reducing the need for chemical insecticides. These benefits, however, are not recognized in many countries, especially in Europe, where some question the safety of these crops on non-target organisms, e.g. Bt-maize and monarch butterflies. ARS scientists have taken a leading role in providing regulators, especially the U.S. Environmental Protection Agency (EPA), with the data necessary to make science-based decisions on the environmental safety of these crops. The approach focuses on the formulation and testing of clearly stated risk hypotheses, using formal decision guidelines to progress between testing stages (or tiers). Such information provides guidance to regulatory agencies that are currently developing their own nontarget risk assessment guidelines for transgenic crops. The knowledge and models generated by ARS scientists provide important reference data for the evaluation of next generation products that contain increasing numbers of transgenes.

New methods were developed to eliminate transgenes from pollen and sperm and thus prevent gene flow from transgenic crop plants to nontransgenic crops and to the environment. The ParA recombinase, mentioned previously in Problem Area 3A, was shown to mediate precise, heritable deletions of plant target sequences that are flanked by ParA recognition sites. This technology was developed for the removal of unwanted transgenic DNA from plants and is publicly available. NP 302 scientists used sperm and pollen EST sequences and expression profiling data to identify new promoters for the targeted expression of genes in pollen and sperm for the prevention of gene flow from transgenic crops to nontransgenic crops and to wild plant species. Some of these results were reviewed in Problem Area 3A. One promoter is active only in the generative cell (a sperm cell progenitor) and in mature sperm cells. A second is active only in the vegetative cell. Candidate genes were identified genetically for use in preventing transgenes from being transmitted during reproduction. *Ds* insertional mutagenesis experiments revealed many new genes that are essential for pollen-mediated gene flow to occur. The genes identified affected fertilization, pollen tube-embryo sac interactions, pollen tube polarity or guidance, pollen germination, and gametogenesis. The crop orthologs for these sequences are candidates for functional analysis, and ultimately for gene flow control using the pollen-specific promoters and RNAi.

Overall, NP 302 has contributed significantly to greater knowledge and technology to remove transgenic DNA from pollen, or other approaches likely to help contain transgene flow in the field and control gene spread.

ARS scientists working in Problem Area 3B conducted extensive research on the nature and likelihood of persistent changes in the ecosystem as a result of transgenic crops. For example, ARS scientists analyzed the impact of Bt-toxin producing corn on the ecosystem. European corn borer (ECB) and western root worm (WCR) can develop resistance to Bt-toxin, just as they have to chemical insecticides, and insect resistance management is critical for maintaining the efficacy of Bt technology. Tools and DNA marker assays, including SSR and SNP markers, were produced by NP 302 scientists for characterizing persistent changes in populations of WCR and ECB. ARS scientists developed and deployed marker assays that are now being used by many laboratories to study WCR and ECB populations and the genetics of resistance to Bt-toxin in these two pests.

ARS maintains European corn borer and western rootworm laboratory populations for conducting research on these important pests. For example, a colony of Bt-toxin resistant ECB was established through artificial selection in order to determine the genetics of Bt resistance in this pest. Analysis of resistance inheritance in this colony indicates there are multiple genes, not previously identified, involved in the resistance trait. Mapping of the resistance loci will enable identification of resistance genes, better resistance prevention, and monitoring assays and tactics.

Six ARS laboratory populations of the western corn rootworm are important resources used for risk assessment experiments, used to achieve controlled infestations, to study WCR behavior, to analyze mechanisms of insecticide resistance, and to analyze genetic control. DNA sequence based surveys showed that there was no detectable loss of genetic variation, when compared to wild populations, except in one population that had been maintained for 200 generations with no input of wild insects. This information will help researchers design and interpret results for all experiments conducted with these colonies.

In the field, genetic markers were used to characterize gene flow between many populations of ECB in the Corn Belt, and the magnitude of immigration within populations over generations. Individual adults commonly travel hundreds of kilometers, with a large percentage of populations consisting of migrants from many km distant. Resistance to Bt-corn will be slow to develop in ECB, but if established, it will spread very quickly over great distances, making effective mitigation problematic. ARS scientists worked with a biotech seed production company to determine the optimum mixture of commercial Bt-corn seed and non-transgenic corn seed in the field to prevent Bt-resistance from arising. In a separate experiment, NP 302 researchers determined that there was minimal impact of the transgenic Bt-corn on the ground beetle population.

For Bt-cotton, nontransgenic crops – corn, soybean, grain sorghum and peanuts – produced more bollworm, the most important cotton pest in the U.S., and thus outperformed nontransgenic cotton as a bollworm refuge for insect risk management. Conclusions from these ARS determinations resulted in the elimination of structured refuge requirements for Bt-cotton, saving growers up to $70/acre in structured refuge costs.

ARS researchers working in Problem Area 3B analyzed the effects of Bt-cotton, maize and potato on the abundance and interactions of non-target arthropods including predators, parasitoids, detritivores, omnivores, and herbivores. Nontarget insects were largely unaffected by Bt-crops when compared to non-Bt control crops. By contrast, Bt crops harbored higher densities of predators, parasitoids, detritivores, omnivores, herbivores when compared to non-Bt crops sprayed with insecticides for target pest control. ARS data also showed that the impact of Bt-corn pollen exposure on Monarch butterfuly larvae over the entire range of the Corn Belt is very small, and unlikely to affect monarch populations in North America. This information was useful for all stakeholders interested in the potential nontarget effects of transgenic plants. The EPA is using the data to monitor and review insect resistance management plan for biotech corn growers.

The nature and likelihood of persistent changes in ecosystems as a result of introducing new transgenic plants was also determined for sorghum. In the U.S. and Africa, sorghum is grown in close proximity to wild relatives. NP 302 scientists showed that sorghum flowering is synchronous with wild relatives in the U.S. and Africa, and that F1 progeny can produce more seed than either parent. The potential for gene flow from transgenic to weedy species is high in the U.S. and Africa confirming that transgenic sorghum lines must be designed to prevent production of viable pollen. Results of this research were provided to USDA/APHIS Biotechnology Regulatory Services for use in establishing transgenic sorghum confinement protocols in support of science-based regulation of transgenic crops.

Selected Accomplishments:

Output 1: *Methods to Eliminate Transgenes from Pollen*

Novel recombinase functions in planta. The imprecision of genetic engineering as currently practiced in plants has raised concerns about the random nature of transgene integration, which can often generate multi-copy insertions, unexpected DNA rearrangements, and invariably, the retention of marker genes in the resultant plant genome. To address these concerns, ARS researchers have identified seven new site-specific recombination systems that function in eukaryotic cells. One novel recombinase called ParA was shown to mediate precise, heritable deletions of a plant transgene that was flanked by the ParA recognition sites. This technology removed unwanted transgenic DNA from plants and is publicly available. These seven recombinases shown to function in yeast are the basis of developing gene stacking technology in plants.

Thomson, J.G., Ow, D. W. 2006. Site-specific recombination systems for the genetic manipulation of eukaryotic genomes. **Genesis** 44: 465-476.

Thomson, J.G., Yau, Y.-Y., Blanvillain, R., Chiniquy, D., Thilmony, R., Ow, D.W. 2009. ParA resolvase catalyzes site-specific excision of DNA from the *Arabidopsis* genome. **Transgenic Research** 18:237-248.

Ow, D.W., Thomson, J. New site-specific recombinases for use in eukaryotic cells. U.S. Patent application 20060046294, published March 2, 2006 (pending).

Ow, D.W. Methods for the replacement, translocation and stacking of DNA in eukaryotic genomes. U.S. Patent 6,936,747, Issued August 30, 2005.

A collection of *Ds* insertion mutants associated with defects in pollen, sperm, and male gametophyte development. 67 *Ds* insertion lines showed reduced transmission through the male gametophyte. About half of these mutations are male gametophytic-specific. For 16, the *Ds* element was inserted in or close to coding regions. The mutants were classified into five groups: defective in early stages of gametogenesis; defective in later stages affecting pollen germination; pollen tube growth, polarity or guidance; pollen tube–embryo sac interactions; and fertilization. The isolated mutants carry *Ds* insertions in genes with diverse biological functions and potentially specify new functions for several unannotated or unknown proteins.

Boavida, L.C. Shuai, B., Yu, H-J., Pagnussat, G.C., Sundaresan, V. and McCormick, S. 2009. A collection of *Ds* insertions associated with defects in male gametophyte development and function in *Arabidopsis thaliana*. **Genetics** 181: 1369-1385, published online Feb. 24, 2009; 10.1534/genetics.108.090852.

Borges, F., Gomes, G., Gardner,R., Moreno, N., McCormick, S., Feijo, J.A., Becker, J.D. 2008. Comparative transcriptomics of *Arabidopsis thaliana* sperm cells. **Plant Physiology** 148: 1168-1181, published July 30, 2008; 10.1104/pp.108.125229.

Yang, H., Kaur, N., Kiriakopolos, S. and McCormick, S. 2006. EST generation and analyses towards identifying female gametophyte-specific genes in *Zea mays* L. **Planta** 224: 1004-1014.

Reduced outcrossing associated with dehydration sensitivity of pollen. Reproductive success has always been correlated primarily with pollen availability. ARS scientists have found that other physiological parameters are key to pollination success. Gene flow in irrigated and dryland cotton varieties have been evaluated and found to be related to the dehydration sensitivity of the pollen and the shape of the flower petals. Flowers with cupped petals and dehydration resistant pollen exhibited the greatest degree of out-crossing under dryland conditions. Conversely, flowers with open petals and dehydration sensitive pollen exhibited reduced out-crossing. These findings provide new information, which can serve as the basis for developing new hypothesis to evaluate in developing cotton with reduced gene flow.

Output 2: *Evaluation of mechanisms of gene flow from transgenic crops to sexually compatible species*

Determination of best practices using seed mixtures to preserve the effectives of the Bt transgene product. ARS scientists have worked with a biotech seed production company to determine the optimum mixture of commercial Bt corn seed and non-transgenic seed to plant. In a separate experiment ARS researchers determined that there was minimal impact of the transgenic Bt corn on the ground beetle population population.

Agricultural Research Magazine (http://www.ars.usda.gov/is/ar/archive/jan03/corn0103.htm?pf=1).

Transgene spread from transgenic sorghum to wild relatives in experimental plot. Sorghum is grown in close proximity to wild relatives. The architecture of its flowering structure makes it particularly amenable to outcrossing and it was shown that flowering is synchronous with wild relatives. Transgene spread from Sorghum to naturally occurring wild relatives and weedy species was shown to occur in experimental plots in several different field locations. In two out of three F1 populations from crosses between a transgenic line and accessions from Africa, the F1 population produced more seeds than either parental line, suggesting that selectively neutral or advantageous crop alleles would persist in wild sorghum populations following hybridization. Analysis of baseline level of gene flow within and among US populations of the compatible weedy species shattercane, indicated that gene flow is rapid within and slow among populations. For monitoring transgene flow to the weedy relative shattercane, male sterility genes are being introduced into it. The knowledge gained demonstrated that transgenic sorghum lines must be designed to prevent production of viable pollen. Results of this research have been used to provide input to USDA/APHIS Biotechnology Regulatory Services in establishing transgenic sorghum confinement protocols. The lead ARS scientist partnered with Dr. S. Koehler, BRS, to develop a presentation at the International Conference on Sorghum for Biofuel, Houston, TX, Aug. 19-22, 2008. That presentation is posted at:
http://www.ars.usda.gov/meetings/Sorghum/presentations/pedersen.pdf The presentation includes APHIS/BRS guidelines for assessment of transgenic sorghum.

Tesso, T., Kapran, I., Grenier, C., Snow, A., Sweeney, P., Pedersen, J.F., Marx, D., Bothma, G., Ejeta, G. 2008. The potential for crop-to-wild gene flow in Sorghum in Ethiopia and Niger, a geographical survey. **Crop Science** 48:1425-1431.

Tang, H.V., Pedersen, J.F., Chase, C.D., Pring, D.R. 2007. Fertility restoration of the sorghum A3 male-sterile cytoplasm through a sporophytic mechanism derived from sudangrasss. **Crop Science.** 47:943-950.

Evaluation of impact of insect pollinators on gene flow patterns. Evaluation of gene flow from transgenic crops to compatible species mediated by insect-mediated pollen movement requires an understanding of how diverse insect pollinators move genes and whether specific insect pollinators have a different impact on gene flow. The model system of columbine was used because it can be manipulated to allow differentiation of seeds produced from pollination by honeybees or by hawkmoths. Microsatellite analysis of the genotype of pollen parents and progeny demonstrated that specific insect pollinators do indeed have a differential impact on gene flow via pollen. In addition, the level of aggregation and density of the planting differentially affects gene flow mediated by the two pollinators. The information will be used for constructing reliable models of transgene flow from transgenic crops. Design of transgenic cropping systems must take in to account differential dispersal of transgenic pollen by available pollinators.

Brunet, J.**,** Sweet. H.R. 2006. Impact of insect pollinator group and floral display size on outcrossing rate. **Evolution** 60:234-246.

Development of forage grass transformation technology and assessment of forage grass pollen spread. Spread of transgenes from genetically modified forage grasses to other plant species poses a potential risk to native and managed ecosystems. Genetic transformation of tall fescue cell suspension cultures was achieved and areas where efficiency can be improved were identified. Appropriate housekeeping genes for use in quantitative gene expression analyses were identified and developed in

Lolium. Quantitative information on pollen emission, survival, and dispersal from a forage grass were obtained from field studies and simulation modeling, indicating that pollen-mediated gene flow is likely at distances of 2 to 3 km from a source field and possible at distances up to 15 km. Methods for transformation of tall fescue and appropriate housekeeping genes for quantitative expression analyses have been identified and developed. The potential geographic scale of pollen-mediate gene flow was identified, providing essential baseline information to inform biotechnology risk assessments.

Baldwin, J.C., Dombrowski, J.E., Martin, R.C., Banowetz, G.M. 2007. Differentially expressed genes associated with post-harvest processing in *Lolium temulentum* L. **Plant Science**. 173:73-83.

Pfender, W., Graw, R., Bradley, W., Carney, M., Maxwell, L. 2007. Emission rates, survival, and modeled dispersal of viable pollen of creeping bentgrass. **Crop Science**. 47:2529-2539.

Output 3: *Characterization of the Likelihood and Nature of Persistent Changes in the Ecosystem as a Result of Introducing Transgenic Plants*

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* is the most destructive pest of corn in the USA, and is now present in several European countries. The European corn borer (ECB), *Ostrinia nubilalis*, is a serious pest in the Corn Belt, costing farmers yield losses from $15 to $50 per acre of corn. Losses from the western corn rootworm and European corn borer are presently controlled, in part, using transgenic Bt-toxin producing corn and nontransgenic refuges. However, ECB and WCR populations can develop resistance to the Bt toxin, just as they have to many conventional chemical insecticides. Therefore, insect resistance management (IRM) is of paramount importance to maintain the efficacy of Bt technology. This research is supported in part by Funds from BRAG.

Simple-sequence repeat (SSR) and single-nucleotide polymorphism (SNP) markers were developed and validated for use with the western corn rootworm and the European corn borer for use in population and genetic studies. DNA sequence based markers are essential tools for the genetic analysis of Bt resistance and for population studies of targeted pests. ARS scientists have developed and deployed marker assays that are now being used in many laboratories to study western corn rootworm and European corn borer populations and the genetics of resistance to Bt toxin in these two pests.

Kim, K.S., Coates, B.S., Hellmich II, R.L., Sumerford, D.V., Sappington, T.W. 2008. Isolation and Characterization of Microsatellite Loci from the European Corn Borer, *Ostrinia nubilalis* (Hubner) (Insecta: Lepidoptera: Crambidae). **Molecular Ecology Resources** *8*:409-411.

Kim, K.S., Stolz, U., Miller, N.J., Waits, E., Guillemaud, T., Sumerford, D.V., Sappington, T.W. 2008. A core set of microsatellite markers for western corn rootworm (*Coleoptera: Chrysomelidae*) population genetics studies. **Environmental Entomology** *37*:293-300.

A colony of Bt-toxin resistant European corn borer was established through artificial selection in order to determine the genetics of Bt resistance in this pest. Analysis of resistance inheritance in this colony indicates there are multiple genes involved. Candidate genes, known to cause Bt resistance in other insects, are not directly responsible for resistance in the selected ECB population. Genetic crosses were performed, creating families for diagnostic marker development and for genetically mapping the resistance genes. Mapping of resistance loci will enable identification of resistance genes, better resistance prevention, and monitoring tactics.

No loss of genetic diversity detected in laboratory colonies of western corn rootworm. Western corn rootworm raised in ARS laboratories are an important resource for risk assessment experiments, used to achieve controlled infestations, to study WCR behavior, to analyze mechanisms of insecticide resistance, and to analyze genetic control. It is important to know how much genetic variation can be retained during the establishment, maintenance, and purposeful selection of laboratory populations. Based on DNA sequence based surveys of selected gene loci in six ARS laboratory populations (one main population, four regional, and one nondiapausing), there was no detectable loss of genetic variation, except in the nondiapausing population that was maintained for 200 generations with no input of wild insects, that may have lost about 25% of its original variation. This information will help researchers design and interpret experiments conducted with the colonies, and help choose which colonies would be best to use for insecticide resistance selection experiments.

> Kim, K.S., French, B.W., Sumerford, D.V., Sappington, T.W. 2007. Genetic Diversity in Laboratory Colonies of Western Corn Rootworm (Coleoptera: Chrysomelidae) including a Nondiapause Colony. **Environmental Entomology** *36*:637-645.

Gene flow is promoted by long-distance dispersal of the European corn borer. Developing an effective insect resistance management (IRM) strategy for European corn borer (ECB) in transgenic Bt corn depends on the extent, frequency, and patterns of movement by adults, but very little information on these parameters has been available for this pest. Genetic markers were used to characterize gene flow, between many populations of ECB in the Corn Belt, and the magnitude of immigration within populations over generations. Individual adults commonly traveling hundreds of kilometers, with a large percentage of populations consisting of migrants from many km distant. Resistance to Bt corn will be slow to develop in ECB, but if established, it will spread very quickly over great distances, making effective mitigation problematic.

> Reardon, B.J., Sumerford, D.V., Sappington, T.W. 2006. Dispersal of newly-eclosed European corn borer adults (Lepidoptera: Crambidae) from corn into small-grain aggregation plots. **Journal of Economic Entomology** *99*:1641-1650.

Minimal gene flow between papaya orchards was detected. In Hawaii, transgenic papaya resistant to Papaya ringspot virus (PRSV) was developed and commercially released in 1998. The transgenic lines express the coat protein gene of PRSV. This approach has proven to be the most successful strategy to controlling PRSV damage in commercial fields. ARS scientists examined the flow of the transgene from commercial transgenic orchards to adjacent non-transgenic orchards. Initial studies found, distance-dependent, minimal transgene flow in this hermaphroditic tree species. More extensive analysis of seeds from each fruit is underway, as each fruit has about 600 seeds. Studies of the timing of optimal pollination during flower development are ongoing. Loss of production of papaya was stemmed by the release of transgenic papaya and by reducing viral load in commercial fields, transgenic papaya have positively impacted the production of non-transgenic papaya. The distance dependency of transgene flow has been used for certification programs.

> Fuchs, M., Gonsalves, D. 2007. Safety of virus-resistant transgenic plants two deades after their introduction: lessons from realistic field risk assessment studies. **Annual Review Phytopathology.** 45:173-202.

Development of Bt-resistance in targeted pests of cotton is pest- and host-transgenotype specific. Development of any Bt-toxin resistance in targeted pests jeopardizes the effectiveness of the Bt-toxin transgene, which has proven to be beneficial for growers and the environment. Regulatory agencies work to mitigate the development of resistance by requiring modified cropping systems with Bt crop refuges and monitoring of shifts in susceptibility of important pests targeted by Bt. Methods to standardize the monitoring test for cotton pests have been developed and monitored by ARS researchers. Resistance to Bt toxin in tobacco budworm, the most important U.S. cotton pest, has not been found over more than 10 years of assessing Bt-toxin cotton production in the U.S. Since Bollgard transgenic cotton was commercially introduced in 1996, structured non-Bt cotton refuges have been required on a percentage of the acreage planted to cotton on a particular farm for the purpose of preventing resistance development to the Bt technology. Contributions of crop hosts, including Bt and non-Bt cotton, field corn, soybeans, grain sorghum and/or peanuts, to the bollworm population was estimated in Arkansas, Georgia, Louisiana, Mississippi and North Carolina in order to determine whether bollworm production from non-cotton crop hosts adequately supplemented that of the structured non-Bt cotton refuge. In nearly all instances, non-cotton crop hosts collectively produced many more bollworm than non-Bt cotton, which suggested that the structured non-Bt cotton refuge played a minor role in delaying resistance development to Bt cotton in bollworm. In addition, a gossypol detection technique was used to estimate the proportion of the tobacco budworms that developed on cotton or non-cotton hosts in the same five-state area, and results indicated that tobacco budworms from non-cotton hosts made a significant contribution to the general population. Overall conclusions from these ARS determinations resulted in the elimination of structured refuge requirements for Bollgard II cottons saving growers up to $70/acre in structured refuge costs. Research publications and information have been provided to regulatory agencies to provide documented scientific evidence for their use in reviewing policies and required protocols for biotech cotton production.

Blanco, C.A., Perera, O.P., Boykin, D., Abel, C.A., Gore, J., Matten, S.R., Ramirez-Sagahon, J.C., Teran-Vargas, A.P., 2007. Monitoring Bacillus thuringinesis-susceptiblity in insect pests that occur in large geographies: how to get the best information when two countries are involved. **Journal of Invertebrate Pathology.** 95:201-201.

Blanco, C.A., Storer, N.P., Abel, C.A., Jackson, R.E., Leonard, R. 2007. Baseline Suscepitibility of the Tobacco Budworm (Lepidoptera: Noctuidae) to the CrylF Toxin from Bacillus thuringiensis. **Journal of Economic Entomology.** 101(1): 168-173

Bt expressing crops have little to no impact on non-target species. The broader impacts of Bt toxin-expressing crop plants on the ecological function of non-target species within agroecosystems were studied by examining the effects of Bt cotton, maize and potato on the abundance and interactions of non-target arthropods. A number of functional groups or guilds (predators, parasitoids, detritivores, omnivores, herbivores) are largely unaffected by Bt crops when compared to non-Bt control crops. However, in comparison with non-Bt crops sprayed with insecticides for target pest control, Bt crops harbored higher densities of members of most of these guilds. Predator to prey ratios were generally unchanged by either Bt crops or the use of insecticides. A dietary toxicity assay for Bt-corn transgenic material on non-target beetles was developed, and showed no effect of Bt tissue on a group of beetles. The decisions of diverse stakeholders regarding the safety of transgenic insecticidal crops can be guided by these results. EPA is using the data to monitor and review insect resistance management plan for biotech corn growers.

Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Royce, B.J., Watrud, L.S. 2008. Bt Crop Effects on Functional Guilds of Non-target Arthropods: A Meta-Analysis. **PLoS One** 3:e2118.

Bt corn pollen has negligible impact on Monarch butterfly larvae. Transgenic corn hybrids expressing the Bt toxin are an increasingly popular tactic for managing the European corn borer (ECB) in North America. A consortium of scientists in several States and in Canada conducted a formal risk assessment of the impact of Bt corn pollen on monarch butterfly populations. Studies on the toxic effects of Bt corn pollen and the degree to which monarch larvae would be exposed to active amounts of Bt pollen on its host plant, the common milkweed found in and around cornfields, suggested that the impact of Bt corn pollen from current commercial hybrids on monarch butterfly populations is negligible. All the scientific information on acute toxicity and exposure supports the conclusion that Bt corn pollen does not pose an unreasonable risk to monarch populations. In addition, chronic exposure of monarch larvae throughout their development to Bt corn pollen is detrimental to only a small fraction of the breeding population because the risk of exposure is low. Genes expressed in the gut tissue of ECB larvae were sampled, and genes previously shown to be involved in resistance to Bt were identified, as well as many new genes that are candidate genes involved in Bt resistance mechanisms. Research results indicated that the impact of Bt corn pollen exposure over the entire range of the Corn Belt, is very small and likely would not affect monarch populations in North America. This information was useful for all stakeholders interested in the potential nontarget effects of transgenic plants. In on-going research the ARS scientists are continuing to define the effects of biotechnology products and other genetic and ecological factors on the management of insect pests of corn and corn ecosystems. Research results are helping regulatory agencies assess biotechnology strategies and enabling corn producers to continue to effectively use biotechnology strategies for pest protection. Ultimately, use of the biotechnology strategies decreases operational costs for corn producers by eliminating the need for costly conventional insecticides to control Bt-resistant larvae.

Dively, G.P., Rose, R., Sears, M.K., Hellmich II, R.L., Stanley-Horn, D.E., Calvin, D.D., Russo, J.M., Anderson, P.L. 2004. Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. **Environmental Entomology.** 33:1116-1125.

Anderson, P.L., Hellmich II, R.L., Sears, M.K., Sumerford, D.V., Lewis, L.C. 2004. Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. **Environmental Entomology.** 33:1109-1115.

Prasifka, J.R., Hellmich, R.L., Dively, G.P., Higgli, L.S., Dixon, P.M. and Duan, J.J. 2008. Selection of nontarget arthropod taxa for field research on transgenic insecticidal crops: Using empirical data and statistical power. **Environmental Entomology.** 37:1-10.

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APPENDIX 1 – SELECTED SUPPORTING INFORMATION AND DOCUMENTATION FOR ACCOMPLISHMENTS AND IMPACT OF NP 302 RESEARCH

Planning and Coordination for NP 302

NP 302 Customer and Stakeholder Workshop, St. Louis, MO, May 2004
NP 302 Research Planning and Coordination Workshop, San Francisco, CA, Nov. 2004 (all lead SYs and National Program Leaders participated.)

Components 1 and 2 – Relevant Workshops

Crop Germplasm Committees, 2004-2009 (annual meetings of over 30 separate committees for individual crops)
National Fusarium Head Blight Forums (U.S. Wheat and Barley Scab Initiative), 2004-2009 with satellite meetings on wheat, barley, and oat genomics and genotyping priorities
ARS Sclerotinia Strategic Planning & Research Forums), 2004-2008
ARS-USB Soybean Genomics Workshops, 2004-2008
ARS Floriculture & Nursery Research Initiative Researchers Meeting (2004-2008)
National Citrus Genomics Workshops (2004-2005)
Joint NSF-USDA database reviews for MaizeDB (crop genome database) 2004, 2006
Technology Roadmap Temperature Fruit Genomics, Genetics, and Breeding Workshop, 2004
ARS-APC Peanut Genome Workshops, 2004, 2005, 2007
International Sunflower Research Conference
National Soybean Rust Research Workshop 2004-2008
Legume Crop Genome Initiative Workshop, 2004
U.S. Wheat Summit (2006, 2007)
U.S. Wheat Genomics Meeting (2007, 2008)
ARS Wine/Grape Industry Workshop, 2005
Horticultural Crops Research Unit Customer/Stakeholder Workshop, Corvallis, OR, S. Schneider and G. Wisler, NPLs (2006)
Automation and Engineering for Specialty Crops Workshop, Arlington, VA, NPLs, J. Steiner, S. Schneider, and counterparts from USDA-CSREES , NASA, (2007)
ARS/National Grape and Wine Initiative Workshop, Kennewick, WA, S. Schneider, NPL, leader (2007) and Beltsville, MD (2008, 2009)
Post-Genomic Sequencing Visioning Workshop, National Corn Growers Assoc., 2006
N. American Wheat Workers' Workshop, Saskatoon, SK, Canada, 2007
ARS National Sugarcane Workshop (2007), G. Wisler, NPL, leader
National Plant Breeding Workshop, 2007
National Wheat Improvement Committee Meetings, and separate meetings for the National Barley and Oat Improvement Committees, 2004-2008)
International Sorghum for Bioenergy Workshop, Houston, TX (2008)
Oat SNP Planning Workshop, ARS and General Mills, Minneapolis, MN (2009)
American Sugarbeet Growers Association (ASGA) Meeting, (2009)
American Society for Sugarbeet Technology (ASSBT), 2009
Pacific Basin Agricultural Research Center (PBARC) Customer/Stakeholder Workshop, Hawaii, M. Kretsch, S. Schneider, P. Bretting, Strickman, NPLs, (May 2008)

USDA Research, Education, and Extension Specialty Crops Research Initiative Implementation Planning Team, S. Schneider ARS lead (2008-2009)

Public Research and the Regulatory Review of Small-Market (Specialty) Biotechnology-Derived Crops Workshop, Nov. 2004, (Organizers: Natl. Center for Food and Agricultural Policy, USDA-CSREES, USDA-ARS, USDA-APHIS)

Component 3 – Biotech Risk Assessment Research Planning and Coordination

Agricultural Biotechnology Risk Analysis (AGRA) Task Group (2004-2007)
2007 Report on Cross Agency Cooperation in Agricultural Biotechnology Risk Analysis Research in the Federal Government (John Radin, ARS leader)

USDA Biotechnology Coordinating Group (2002 – present), Kay Simmons, ARS representative to interagency biotech group

Biotechnology Risk Assessment Research Workshops (2003, 2004, and 2005) – ARS and CSREES organized with APHIS-Biotechnology Regulatory Services, led workshops to develop an initiative to develop a pathway for genetically engineered specialty crops. The three USDA agencies co-sponsored the workshops in 2004 and 2005 to identify the barriers and suggest appropriate activities to overcome them, and advanced a proposal to create an organization like IR-4 that would facilitate advancement and approval. That idea has subsequently been transferred to the private sector for advancement.

Mexican/U.S. Workshop on Plant Biotechnology and Biosafety/Risk Assessment Research Organizers: CINVESTAV and USDA-ARS (Kay Simmons, U.S. Leader along with ten ARS researchers leading biotech risk assessment and mitigation projects). Date and Location: June 23-25, 2003; Irapuato, Mexico. Workshop Focus: Biotechnology approaches for crop improvement and risk assessment research. Crops of focus: maize, beans/legumes, potatoes, citrus, chili peppers. Agenda topics included Biotechnology Risk Assessment (gene flow and its consequences; effects on non-target organisms; safety issues)

Sino/USDA Workshop: Crop Genomics Applications to Plant Breeding and Biotechnology: A Seamless Approach from Research to new Varieties and Products in China and the U.S, Oct. 23-25, 2006, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China. Session 3 focused on Biotechnology Risk Assessment and Communication. ARS speakers included David Ow, Albany, CA, “Biotechnology Methods and Strategies” and Craig Abel, Stoneville, MS, “Biotechnology Risk Assessment in Cotton – Use of Alternative Hosts; and Yufa Peng, Institute of Plant Protection, CAAS, “Risk Assessment for Transgenic Crops” (sponsored by the U.S. State Department)

2nd Symposium for Agricultural Biotechnology Risk Analysis Research - a meeting to facilitate dialog between federally funded investigators and the scientists and administrators responsible for regulatory oversight of agricultural biotechnology products. Organized by USDA (Gail Wisler, ARS lead), EPA, and FDA. Dec. 5-6, 2007, Food and Drug Administration Building, College Park, MD

ARS Intragenics Workshop (Jan. 15-16, 2009), Organizers: Maureen Whalen and William Belknap, USDA, ARS, Western Regional Research Center, Albany, CA. This included 20 invited attendees from the international scientific and regulatory communities to discuss recent technical advances and pathways toward international commercialization of intragenic and related technologies. Participants were from the U.K., Netherlands, New Zealand, and the U.S. including APHIS/Biotechnology Regulatory Services. Development of a "tool box" for all native DNA transformation was targeted along with discussion of consumer concerns and regulatory issues.

Interagency Workshops

EU-US Taskforce on Biotechnology Research, Workshop on Biotechnology for Sustainable Bioenergy, Feb. 2008 (U.S. coordinators, Kay Simmons, ARS, and David Thomasson, DOE)

National Academy of Sciences, Committee on the National Plant Genome Initiative, Achievements and Future Directions (USDA-ARS NP 302 leaders (J. St. John, K. Simmons) worked with other leads from NSF, USDA-CSREES, DOE and others of providing summaries of national program accomplishments (2007-2008).

The Future of the National Plant Genome Initiative, Stadtler Workshop, Irvine, CA (led by the Interagency Working Group on the Plant Genome including ARS NP 302 leaders (J. St. John, K. Simmons).

USDA-CSREES Stakeholder Workshop on Plant and Pest Biology (2005 and 2007), K. Simmons invited panel speaker on USDA interagency cooperation in supporting plant biology research.

Relevant Publications

Economic Research Services report: Can technology transfer help public-sector researchers do more with less? K.D. Rubenstein and P.W. Helsey, The case of the USDA's Agricultural Research Service. AgBioForum 8:134-142 (2005) and ERS Report 15, Feb. 2006.

External Awards

Peer-reviewed research grants and stakeholder awards are a useful indicator of the quality and value of the NP 302 program. From 2004-2009, NP 302 National Program scientists were awarded 154 grants and awards that complemented and enhanced the objectives of the NP 302 Action Plan. Table 1 shows that 24 of the 43 NP 302 project teams reported receiving grants from NSF, DOE, NIH and/or CSREES (67 grants awarded in total). Twenty-two of the NP 302 project teams reported having awards from an industrial source or an international agency (30 awards in total) – evidence that their research is valued by stakeholders.

Table 1. Peer-reviewed research grants and awards given to NP 302 project scientists from 2004-2009.

	Industry	CSREES	NSF	DOE	NIH	International	Other
Total	57	30	24	7	6	15	15

Scientist Training

The training and preparing of young scientists for careers in agriculture is critical for sustaining our modern society. Table 2 shows that from 2004-2009, NP 302 National Program scientists trained and mentored at least 171 postdoctoral fellows, 124 graduate students and 342 undergraduate students. Together, the external research grants, awards and training of young scientists are important measures of the leadership and community stewardship that NP 302 research leaders and scientists are contributing to agriculture and related sciences.

Table 2. Scientists trained by NP 302 research leaders and scientists from 2004-2009.

	Postdoctoral Fellows	Graduate Students	Undergraduate Students
Total	171	124	342

Appendix 2: NP 302 Research Projects

ARS Project Code	Lead Scientist	Project Title	Problem Area	City & State
1230-21000-047-00D	Kathy Kamo	Genetic engineering of floral bulb crops for virus and nematode resistance	1B, 3A	Beltsville, MD
1265-21000-140-00D	Autar Mattoo	Molecular approaches to enhance plant nutrient content	2A, 2B	Beltsville, MD
1275-21000-180-00D	Lisa Rowland	Enhancement of blueberry, strawberry, and brambles through molecular approaches	1A, 1B, 2B	Beltsville, MD
1275-21000-223-00D	Savithiry Natarajan	Evaluation of the quality and safety of transgenic soybeans	3A	Beltsville, MD
1275-21220-221-00D	Ben Matthews	Genomics and proteomics approaches to broadening resistance of soybean to pests and pathogens	1B, 2B	Beltsville, MD
1275-21200-222-00D	Anna Smigocki	Molecular genetic approaches to pest and pathogen resistance in sugar beet, *Beta vulgaris* L.	2B	Beltsville, MD
1907-21000-024-00D	Leon Kochian	Genomic approaches for improving transport and detoxification of selected mineral elements in crop plants	1A, 1B, 2B	Ithaca, NY
1907-21000-025-00D	James Giovannoni	Genomics approaches for improving nutritional quality of food crop species	1B, 2A, 2C	Ithaca, NY
1907-21000-026-00D	Owen Hoekenga	Determination and characterization of unintended effects in genetically modified food crops	3A	Ithaca, NY
1931-21000-016-00D	Michael Wisniewski	Using functional genomics to improve stress and disease resistance in fruit trees	2B, 3A	Kearneysville, WV
3602-21000-005-00D	Karen Hudson	Identification and characterization of genes important during seed development in legumes	1B	West Lafayette, IN
3611-21000-020-00D	Donald Ort	Identifying and manipulating determinants of photosynthate production and partitioning	1A, 2A, 2B	Urbana, IL
3622-21000-027-00D	Michael McMullen	Genetic mechanisms and molecular genetic resources for maize	1B	Columbia, MO
3622-21000-028-00D	Mel Oliver	Genetic enhancement of soybean seed value by biotechnology	2C	Columbia, MO
3622-21000-029-00D	Jan Miernyk	Modification of seed composition for food, feed, and industrial applications of soybeans	2A, 2C	Columbia, MO
3625-21000-049-00D	Roger Wise	Functional genomics of cereal disease defense	1B, 3A	Ames, IA
3640-21000-024-00D	Carroll Vance	Functional genomics for improving nutrients and quality in alfalfa and soybean	1B, 2A, 2B	St. Paul, MN
3655-21000-030-00D	Johanne Brunet	Genetic exchange and gene flow risks from plants in agriculture	3B	Madison, WI

3655-21000-041-00D	Cynthia Henson	Physiological, biochemical and genetic regulation of carbohydrate metabolism in cereal tissues	2A, 2C	Madison, WI
3655-21000-044-00D	Mitchell Wise	Metabolism and analysis of cereal phytochemicals	2B, 2C	Madison, WI
3655-21430-008-00D	Ronald Skadsen	Improvement of barley seed quality through molecular and functional genomic analysis of gene expression	2A, 2B	Madison, WI
5320-21000-011-00D	Dennis Gonsalves	Molecular resources for the improvement of tropical crops	2A, 3B	Hilo, HI
5325-21000-002-00D	Ann Blechl	Molecular tools to minimize risk in genetically engineered crops	3A	Albany, CA
5325-21430-011-00D	Ann Blechl	Production of wheat germplasm with enhanced ethanol baking quality	2C	Albany, CA
5325-43000-026-00D	William Hurkman	Molecular analysis of effects of environment on wheat flour quality and allergenic potential	2A, 3A	Albany, CA
5335-21000-017-00D	Peter Quail	Molecular mechanisms of light perception, signaling & control of gene expression by the phytochromes	1A	Albany, CA
5335-21000-028-00D	Sarah Hake	Positional cloning in maize of genes that regulate plant architecture	1B	Albany, CA
5335-21000-029-00D	Jennifer Fletcher	Functional genomics of plant architecture	1A	Albany, CA
5335-21000-030-00D	Sheila McCormick	Molecular developmental genetics of pollen and pollen-pistil interactions in crop plants	1A, 1B, 3B	Albany, CA
5335-21000-031-00D	David Ow	Transgene management through site-specific recombination	3A, 3B	Albany, CA
5335-21430-006-00D	Athanasios Theologis	Molecular genetics of ethylene biosynthesis	1A, 1B	Albany, CA
5347-21000-009-00D	Michael Salvucci	Physiological and genetic basis of cotton acclimation to abiotic stress	2B	Maricopa, AZ
5358-12210-003-00D	Carolyn Scagel	Influence of root growth, development, and function on horticultural crop productivity and quality	2A, 2B	Corvallis, OR
5442-21000-031-00D	Lynn Dahleen	Genetic improvement of barley	2B, 3A	Fargo, ND
5442-21430-002-00D	Karen Fugate	Sucrose accumulation and retention in sugarbeets	2A, 2B, 3B	Fargo, ND
6208-21000-016-00D	John Burke	Characterization and enhancement of plant resistance to water-deficit and thermal stresses	2A, 2B	Lubbock, TX
6408-21410-006-00D	Stephen Duke	Discovery and development of natural product-based weed management methods	2B, 2C	Oxford, MS
6408-21410-007-00D	Stephen Duke (cooperative agreement)	Discovery and development of natural products for pharmaceutical and agrichemical applications	2C	Oxford, MS

6435-21000-013-00D	Sarah Lingle	Biochemical and genetic basis of sucrose partitioning in sugarcane stalks for sucrose and biofuel production	2A	New Orleans, LA
6435-21000-015-00D	Vice-Barbara Triplett	Molecular analysis of development to improve cotton fiber	2C	New Orleans, LA
6604-21000-002-00D	Diane Rowland	Physiological mechanisms that determine crop response to irrigation, disease and production practices	2B	Dawson, GA
6615-21000-009-00D	Prem Chourey	Functional genomics of agronomic traits in developing seed and pollen in maize and sorghum	2A	Gainesville, FL
6645-21000-026-00D	Robert Upchurch	Physiological/biochemical mechanisms associated with genetic alteration of soybean quality and productivity	2A, 2B	Raleigh, NC

Research Projects in Other National Programs Contributing to NP 302

Project ID	Lead Scientist	Project Title	Problem Area	City and State
5358-21000-039-00D	Gary Banowetz	Improvement of seed and end-use quality of cool season grasses	3A, 3B	Corvallis, OR
5358-21000-039-00D	James Dombrowski	Improvement of seed and end-use quality of cool season grasses	3A, 3B	Corvallis, OR
1931-21000-017-00D	Ralph Scorza	Genetic improvement of fruit crops through functional genomics, and breeding	3A	Kearneysville, WV
6402-22000-047-00D	Craig Abel	Resistance management and injury potential of lepidopterous pests to transgenic cottons	3B	Stoneville, MS
5447-21220-003-00D	Jonathan Lundgren	Pest biology, ecology, and integrated pest management for sustainable agriculture	3B	Brookings, SD
5447-21220-003-00D	B. (Wade) French	Pest biology, ecology, and integrated pest management for sustainable agriculture	3B	Brookings, SD
3622-21220-005-00D	Bruce Hibbard	Risk of western corn rootworm adaptation to transgenic corn	3B	Columbia, MO
1907-22000-018-00D	Stewart Gray	Management of nematodes and virus diseases affecting potato and grain crops	3A	Ithaca, NY
5440-21220-007-00D	Jeffrey Pedersen	Enhancement of sorghum for bioenergy, feed, and food value	3B	Lincoln, NB
3625-22000-016-00D	Richard Hellmich	Ecologically-based management of insect corn pests	3B	Ames, Iowa

Appendix 3: NP 302Publications

Codes	Project No. / Project Title / Lead Scientist (P) / SY Team	Publications
1B 3A	1230-21000-047-00D Genetic engineering of floral bulb crops for virus and nematode resistance Kathy Kamo (P) John Hammond	Kamo K, Jones B, Bolar J, Smith F (2005) Regeneration from long-term embryogenic callus of the Rosa hybrida cultivar Kardinal. In Vitro Cell Dev Biol-Plant 41:32-36.
		Kamo K, Gera A, Cohen J, Hammond J, Blowers A, Smith F, Van Eck J (2005) Transgenic Gladiolus plants transformed with the bean yellow mosaic virus coat-protein gene in either sense or antisense orientation. Plant Cell Rep 23:654-663. Joung YH and Kamo K. 2006. Expression of a polyubiquitin promoter isolated from Gladiolus. Plant Cell Reports 25:1081-1088 Hammond J, Hsu HT, Huang Q, Jordan R, Kamo K, Pooler M. 2006. Transgenic approaches to disease resistance in ornamental crops. J Crop Improvement 17:155-210 Joung H.Y., Cantor, M., Ellis, D.D. Kamo, K.K. 2007. Cryopreservation of Gladiolus shoot tips derived from cormels. Korean Journal of Horticultural Science 48:251-255. Joung, H.Y., Cantor, M., Kamo, K.K., Ellis, D.D. 2007. Cryopreservation of Gladiolus cultivars. Acta Horticulturae 760:225-232. Kamo, K. K. and Cantor, M. 2007. Genetic engineering for disease resistance in ornamental plants. In: Conservation of Horticultural Germplasm, pp. 36-39. Kamo, K.K. and Joung Y.H. 2007. A polyubiquitin promoter isolated from Gladiolus and its expression in Gladiolus plants. Acta Horticulturae 736:251-258. Kamo, K. and Joung, Y.H. 2007. Gladiolus. In: Biotechnology in Agriculture and Forestry Volume 61, pp. 289-298. Kamo, K. 2008. Transgene expression for Gladiolus plants grown outdoors and in the greenhouse. Scientia Horticulturae 117:275-280. Kamo, K. and Han, B. H. 2008. Biolistic-mediated transformation of Lilium longiflorum cv. Nellie White. HortScience 43:1864-1869.
2A 2B	1265-21000-140-00D Molecular Approaches to Enhance Plant Nutrient Content, Shelf-Life and Stress Tolerance Autar K. Mattoo (P)	Li, J-F., Qi, R., Qu, L-H., Mattoo, A.K., and Li, N. Cleavage of the carboxy-terminus of LEACS2, a tomato 1-aminocyclpropane-1-carboxylic acid synthase isomer, by a 64-kDa tomato metalloprotease produces a truncated but active enzyme. J. Integrative Pl. Biol. 47: 1352-1363. 2005.
		Mattoo, A.K., Sobolev, A.P., Neelam, A., Goyal, R.K., Handa, A.K., and Segre, A.L. NMR spectroscopy-based metabolite profiling of transgenic tomato fruit engineered to accumulate spermidine and spermine reveals enhanced anabolic and nitrogen-carbon interactions. Plant Physiol. 142: 1759-1770. 2006. Mattoo, A.K., and Abdul-Baki, A. Crop genetic responses to management: Evidence of root-to-leaf communication. In: Biological Approaches to Sustainable Soil Systems (N. Uphoff, A.S. Ball, E. Fernandes, H. Herren, O. Husson, M. Laing, C. Palm, and J. Thies, eds.), CRC Taylor & Francis, Boca Raton, FL, pp. 221-230. 2006 Edelman, M., and Mattoo, A.K. The D1 protein: past and future. In: Photoprotection, Photoinhibition, Gene Regulation and Environment (B. Demmig-Adams, W. Adams and A.K. Mattoo, eds.), Springer, Dordecht, the Netherlands. Pp. 23-38, 2006

Srivastava, A., Chung, S.H., Fatima, T., Datsenka, T., Handa, A.K., and Mattoo, A.K. Polyamines as anabolic growth regulators revealed by transcriptome analysis and metabolite profiles of tomato fruits engineered to accumulate spermidine and spermine. Plant Biotechnol. 24: 57-70. 2007.

Mattoo, A.K. Chung, S-H., Goyal, R.K., Fatima, T., Srivastava, A., Solomos, T., and Handa, A.K. Over-accumulation of higher polyamines in ripening transgenic tomato fruit revives metabolic memory, upregulates anabolism-related genes, and positively impacts nutritional quality. J. AOAC Intl., 90: 1456-1464. 2007.

Mattoo, A.K., and Handa, A.K. Higher polyamines resuscitate metabolic memory in fruit. Plant Sci. 174: 386-393. 2008.

Shukla, V., and Mattoo, A.K. Sucrose non-fermenting 1-related protein kinase 2 (SnRK2): A family of protein kinases involved in hyperosmotic stress signaling. Physiol. Mol. Biol. Plants 14: 91-99. 2008.

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Mattoo, A.K., Yachha, S.K., and Fatima, T. Genetic manipulation of vegetable crops to alleviate diet-related diseases. In: Improving the Health-Promoting Properties of Fruit and Vegetable Products (F.T. Barberan, F. & M.I. Gil Eds.), Woodhead Publ. Ltd., Cambridge, pp. 327-345. 2008.

Fatima, T., Rivera-Dominguez, Troncoso-Rojas, Tiznado-Hernandez, Handa, A.K., and Mattoo, A.K. Tomato. In: Compendium of Transgenic Crop Plants: Transgenic Vegetable Crops (C. Kole, and T.C. Hall, eds.), Vol. 6, Blackwell Publishing, Oxford, UK. Pp.1-46, 2008.

Nambessan, S., Handa, A.K., and Mattoo, A.K. Polyamines and regulation of ripening and senescence. In: Postharvest Biology and Technology of Fruits, Vegetables and Flowers (G. Paliyath, D.P.Murr, A.K. Handa, and S. Lurie, Eds.), Willey-Blackwell Publ., Ames, IA, pp. 319-340, 2008.

1A
1B
2B

1275-21000-180-00D

Enhancement of Blueberry, Strawberry, and Brambles through Molecular Approaches

Lisa J. Rowland (P)
Janet Slovin
Kim Lewers
James Polashock
Mark Ehlenfeldt

Alkharouf, N.W., Dhanaraj, A.L., Naik, D., Overall, C., Matthews, B.F., Rowland, L.J. 2007. BBGD: An online database for blueberry genomic data. Biomed Central Plant Biology 7:5. Log# 198263.

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Bassil, N.V., Njuguna, W., Slovin, J.P. 2006 EST-SSR markers from Fragaria vesca L. cv. Yellow Wonder. Molecular Ecology Notes 6:806-809. Log #190140

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Boches, P.S., Bassil, N.V., Rowland, L.J. 2005. Microsatellite markers for Vaccinium from EST and genomic libraries. Molecular Ecology Notes. 5:657-660. Log# 174824.

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Ehlenfeldt, M.K., Rowland, L.J. 2006. Cold-hardiness of Vaccinium ashei and V. constablaei germplasm and the potential for northern-adapted rabbiteye cultivars. Acta Hort. 715:77-80. Log# 157061.

Ehlenfeldt, M.K., Rowland, L.J., Ogden, E.L., Vinyard, B. 2007. Bud cold hardiness of Vaccinium ashei, V. constablaei, and hybrid derivatives and their potential for producing northern-adapted rabbiteye cultivars. HortScience 42:1131-1134.

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Evaluation of the quality and safety of transgenic soybeans

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Genomics and Proteomics Approaches to Broadening Resistance of Soybean to Pests and Pathogens

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Molecular Genetic Approaches to Pest and Pathogen Resistance in Sugar Beet, Beta vulgaris L.

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Genomic approaches for improving transport and detoxification of selected mineral elements in crop plants

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2C Genomics Approaches for Improving Nutritional Quality of Food Crop Species

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Using Functional and Applied Genomics to Improve Stress and Disease Resistance in Fruit Trees

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Genetic Mechanisms and Molecular Genetic Resources for Maize

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Genetic enhancement of soybean seed value by biotechnology

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Modification of seed composition for food, feed, and industrial applications of soybeans.

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Functional Genomics of Cereal Disease Defense

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Nick Lauter

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2B Functional Genomics for Improving Nutrients and Quality in Alfalfa and Soybean

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Bruna Bucciarelli

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Genetic exchange and gene flow risks from plants in agriculture

Johanne Brunet (P)

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2A 2B	3655-21430-008-00D Improvement of barley seed quality through molecular and functional genomic analysis of gene expression Ronald W. Skadsen (P) Charles An	Federico, M.L., Kaeppler, H.F., and Skadsen, R.W. The complex developmental expression of a novel stress-responsive barley Ltp gene is determined by a shortened promoter sequence. Plant Mol. Biol. 57:35-51. 2005.
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Molecular Resources for the Improvement of Tropical Crops

Dennis Gonsalves (P)

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Molecular Tools to Minimize Risk in Genetically Engineered Crops

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Production of Wheat Germplasm with Enhanced Baking Quality

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Molecular Analysis of Effects of Environment on Wheat Flour Quality and Allergenic Potential

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Molecular Mechanisms of Light Perception, Signaling & Control of Gene Expression by the Phytochromes

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Influence of Root Growth, Development, and Function on Horticultural Crop Productivity and Quality

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Discovery and Development of Natural Product-Based Weed Management Methods

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