

**USDA
AGRICULTURAL RESEARCH SERVICE
NATIONAL PROGRAM 302
Plant Biological and Molecular Processes
ANNUAL REPORT FY 2008**



National Program 302 Plant Biological and Molecular Processes

FY 2008 Annual Report

National Program 302, Plant Biological and Molecular Processes (NP 302), includes much of the Agricultural Research Service's fundamental research with plants that is necessary to understand the biological principles for plant growth and development. The research focuses on mechanistic understanding of specific plant processes and properties. The knowledge and tools developed can be used in an integrated research approach to achieve practical improvements in the functions and properties of crops that address consumer concerns.

The Action Plan for this National Program was rewritten in 2004 with stakeholder input to ensure that the components of the program were relevant to current needs of U.S. agriculture.

NP 302 is comprised of the following three components:

- Functional Utilization of Plant Genomes: Translating Plant Genomics into Crop Improvement, with research aimed at bridging the gap between fundamental DNA-based science and the applications of that science for crop improvement.
- Biological Processes that Determine Plant Productivity and Quality, encompasses mechanisms of plant growth and development, disease resistance, photosynthesis, productivity, and environmental responses that relate molecular events and processes to attributes of the whole organism. It also includes research to identify and enhance nutritional quality of foods.
- Plant Biotechnology Risk Assessment, houses research of two types: identification and evaluation of possible unwanted environmental or genetic consequences of genetic engineering, and development of new technology to mitigate or eliminate unwanted consequences.

The year 2008 marked the third year of the 5-year cycle of research performance for National Program 302. The Action Plan provided guidance for NP 302 projects that has led to many important scientific discoveries and advances, some of which are described below. By no means do these selected accomplishments capture the important achievements of the entire research program. Rather, they highlight the type of activities carried out under this program and the type of benefits that result.

Component I: Functional Utilization of Plant Genomes: Translating Plant Genomics into Crop Improvement

In the past five years, DNA sequencing of reference plant genomes such as Arabidopsis, Brachypodium, and crop plants (rice, corn, sorghum, and soybean) has contributed to an exponential gain in information about plant genes and gene expression. These efforts have produced extensive sequence data, vast EST resources and large 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.

Problem Statement IA: Advancing From Model Plants to Crop Plants

Extensive genomic information derived from model plants, such as Arabidopsis, has significantly advanced knowledge about plant genomes. 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.

Genetic pathways controlling plant architecture identified. Using the model plant Arabidopsis, ARS scientists in Albany, California, identified the role of an essential gene (called "EMB1611") in the regulatory pathway that controls plant shoot growth. They elucidated how the gene regulates shoot growth through maintenance of a down-stream signaling pathway and also demonstrated that the EMB1611 gene sustains young plant tissue cells in an actively dividing state. Plant scientists can exploit this new information to manipulate similar genes in crop plants that affect shoot growth. In the long-term this advance could lead to increased yields, benefiting domestic farmers as well as export markets.

Problem Statement IB: Applying Genomics to Crop Improvement

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 identify opportunities for crop improvement.

Genome assembly accelerated with new bioinformatic tools. The genome of an organism contains all of the hereditary information encoded in the DNA. Transposable elements (TEs) are mobile segments of DNA that produce the repetitive segments and are found throughout the genome. ARS scientists in Ames, Iowa, developed new bioinformatic software, designated TEnest, that will accelerate the assembly of the genome. This new software enables researchers to identify repeat insertions in the genome, calculate their ages since integration into the DNA, and model their evolution. Bioinformatic tools such as TEnest dramatically increase the speed and efficiency with which genomes are assembled and provide insight into the evolutionary history of the plant through time. Understanding the genome of agricultural crops may allow scientists to develop crops capable of growing in arid conditions and with resistance to pests. To date, TEnest has been applied to maize, barley, wheat, and rice. The tool is available for use both as a Web server on the Resource for Plant Comparative Genomics, www.PlantGDB.org, and as a standalone downloadable version available from www.Wiselab.org.

High-density genetic map developed for the nested association mapping population. Comparison of quantitative agronomic trait experiments in corn is hindered by the ad hoc manner in which different mapping populations are used. In collaboration with USDA colleagues at Ithaca, New York, and Raleigh, North Carolina, and with support from the National Science Foundation, ARS scientists in Columbia,

Missouri, developed the nested association mapping (NAM) genetic population of corn, consisting of 5,000 recombinant inbred lines and with maximal allelic diversity. They completed a high-resolution, composite genetic map consisting of 1106 SNP (single-nucleotide polymorphism) loci, which serves as the basis for locating genes for important agronomic traits. Using the NAM genetic map, scientists have identified and mapped about 50 quantitative trait loci (QTLs) for flowering time in maize. The NAM genetic resource is the most powerful genetic tool for the dissection of the genetic basis of quantitative traits (traits involving collections of genes) for any species. These populations will become the trait analysis standards and help convert QTL analysis from a single experiment format to an integrated genomics activity.

DNA markers developed for blackberry. There is need to develop reliable DNA markers for genetic mapping in blackberry. ARS scientists at Beltsville, Maryland, in collaboration with scientists at Clemson University, sequenced 2,678 blackberry genes, from which 673 molecular markers were developed. These are the first blackberry gene sequences and markers to be made publicly available. This work lays the foundation for use of these markers by other scientists in genetic studies on blackberry.

Stress gene sequences obtained and analyzed for strawberry. There is a need to identify genes that help plants grow and produce fruit under stress conditions such as cold, heat, and drought. ARS scientists at Beltsville, Maryland, obtained new sequences from approximately 10,000 genes expressed in strawberry under stress conditions and deposited them into GenBank, making them publicly available. In an analysis of all expressed gene sequences from strawberry that are currently in the public domain, they identified 13,449 unique gene sequences, which represent the foundation for the genomic study of environmental stress on production of flowers and fruit in strawberry and other members of the *Rosaceae* family. These sequences can be used by scientists for molecular marker development, for examining expression of genes in response to stress, and for testing the function of genes that may increase plant tolerance to environmental stresses.

DNA markers identified for genes that confer resistance to fire blight disease in apple. Fire blight is a destructive disease of apple and pear trees that is estimated to cost the U.S. fruit industry over \$100 million a year in crop losses and disease control. ARS scientists at Kearneysville, West Virginia, working in collaboration with scientists from Cornell University, Pennsylvania State University, and HortResearch, New Zealand, identified gene-specific DNA markers for fire blight resistance in apple. Scientists mapped DNA markers derived from the candidate resistance genes, segregating for apple seedlings with fire blight resistance. To date, of 28 candidate fire blight resistance gene markers that have been mapped, 6 have co-located with known genetic loci for fire blight resistance. This research will facilitate new methods of marker-assisted selection to efficiently breed superior apple cultivars with fire blight resistance.

A 40,000 gene apple microarray developed for functional genomic studies of *Rosaceae* species. In order to better understand and manipulate plant development and important economic traits, it is essential to identify the coordinated induction or repression of numerous genes that are involved in various biochemical pathways. In collaboration with the University of Illinois, and with additional support from the Cooperative States Research, Education, and Extension Service (CSREES), an apple microchip was developed by ARS researchers at Kearneysville, West Virginia, consisting of 40,000 gene probes that can be used to better understand which genes and which biochemical pathways are involved in growth, fruiting, fruit quality, disease and stress resistance, and other characteristics. This tool will allow scientists to study the genetic regulation of traits important to the apple industry and will also be useful for pear, peach, plum, apricot, and other Rosaceous plant species.

Component II: Biological Processes that Determine Plant 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.

Problem Statement IIA: Understanding Growth and Development

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.

Role for tyrosine signaling identified in plant receptor kinases. Tyrosine phosphorylation, long thought to be a hallmark of animal but not plant receptor signaling, was shown to play an unexpected role in brassinosteroid signaling in *Arabidopsis* species. ARS scientists at Urbana, Illinois, showed for the first time that the brassinosteroid receptor, known as BRI1, was autophosphorylate on tyrosine as well as serine and threonine residues. Importantly, the tyrosine phosphorylation was shown to play a specific role in hormone signaling in vivo. These results overturn a long-standing dogma and suggest new approaches to manipulate plant growth and development.

Pollen tube growth shown to require interactions of pollen receptor kinases and RopGEF protein. Pollen grows by tip growth and is regulated by a protein called Rop GTPase. Rop in its GTP-bound form is active, and in its GDP-bound form it is inactive, and a protein called Rop-GEF (guanine exchange factor) switches Rop between its active and inactive forms. RopGEFs in plants and animals are completely different in their amino acid sequence, but ARS scientists at Albany, California, showed that the plant and animal proteins have protein domains that perform similar functions. They also showed that this inhibition is released when the RopGEF interacts with a pollen receptor kinase, a protein that receives signals from the female tissue, where the pollen tubes grow. This understanding of how proteins control pollen tube growth is important for agriculture, since pollen tube growth is required before seeds can form.

New plant hormone studied. Ethylene is an important hormone for fruit ripening and many aspects of plant growth. In order to determine its mode of action, ARS scientists in Albany, California, constructed a non ethylene producing *Arabidopsis* plant. Its phenotype suggests that ACC, the precursor of ethylene, may also be a plant growth regulator, and additional experiments are in progress to verify this observation. An understanding of the true role of ethylene and the precursor to ethylene could have a major impact on the fruit industry.

Physiological changes associated with urea sprays defined. Spraying leaves of container-grown nursery plants in the autumn with urea alters uptake and use of other mineral nutrients, but little information is available on the physiological changes induced by urea sprays on nursery crops, and there is no information on whether this practice alters uptake of other nutrients. ARS scientists at Corvallis, Oregon, determined that spraying container-grown *Rhododendron* nursery plants with urea in the autumn alters uptake and allocation of nutrients other than Nitrogen (N), demonstrating that urea sprays increased uptake and storage of some specific nutrients in some cultivars and also caused losses of other nutrients (e.g., potassium). Thus, the influence of urea sprays in the autumn on uptake of nutrients has potentially positive benefits for growth the following spring; however, when using urea sprays in the autumn as a N management technique, plant demand for other nutrients in the spring may also be higher, and spring

fertilizer practices may need adjustment to ensure optimal growth. Knowledge from this accomplishment will be used by growers and by extension personnel to develop fertilizer management strategies that improve plant quality, minimize production costs, and encourage use of environmentally sustainable practices.

Relationship between nitrogen and fertilizer studied. Application of nitrogen (N) fertilizer late in the growing season can decrease cold hardiness and increase the potential for cold injury to buds and stems; however, the specific relationships between plant N status and fertilizer formulations have not been well investigated. ARS scientists at Corvallis, Oregon, in cooperation with researchers at Mississippi State University, Oregon State University, and Washington State University, determined the influence of N rate and N form on autumn and winter cold tolerance of buds and stems of green ash (*Fraxinus pennsylvanica*). Both N rate and form influenced cold tolerance, especially hardiness levels during mid-winter and the de-acclimation period in February. Results indicated that stems and buds of plants fertilized with urea formaldehyde-based fertilizer were more cold tolerant than stems and buds of trees fertilized with ammonium nitrate-based fertilizer, suggesting that trees at a similar N status can withstand different levels of cold depending on the type or form of fertilizer used during production; consequently, fertilizer component selection may be more important than application rate for production of container-grown nursery crops in climates prone to winter injury. This new knowledge will be used by growers, fertilizer manufacturers, and extension personnel to develop fertilizer management strategies that improve plant quality for nursery stock prone to winter injury, thus increasing profitability.

Problem Statement IIB: 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.

Genetically engineered Gladiolus and Ornithogalum tested for virus resistance. Cucumber mosaic virus (CMV) is one of the most economically limiting diseases of cucumber. ARS scientists identified four transgenic plants lines of Gladiolus that were resistant in vitro to CMV strain S (serotype 2) and four lines that were resistant to CMV serotype 1. Future challenges will continue.

Sugar beet resistance gene identified. The sugar beet root maggot is one of the most devastating insect pests of sugar beet and is found in two-thirds of all U.S. sugar beet fields. Ineffective cultural practices and harmful chemical insecticides are the primary available measures for its control; therefore, a strong impetus exists for the development of effective and environmentally friendly control measures. ARS scientists at Beltsville, Maryland, identified sugar beet genes that are associated with root responses to root maggot feeding in both susceptible and moderately resistant sugar beet varieties. One gene in particular was identified, cloned, reconstructed for over-expression in plants, and introduced into a sugar beet root model system and a model plant. Preliminary studies suggest that this gene increases the plants’ resistance levels to a number of plant pests. Scientists will use this information to increase their understanding of plant resistance mechanisms in sugar beet and to develop new approaches for increasing root pest and disease resistance without the use of pesticides.

Protein complex associated with disease resistance discovered. If the proteins responsible for protecting plants from diseases or enhancing pathogens’ ability to cause diseases were known, this knowledge might accelerate the improvement of cultivars through breeding or transgenic technology. ARS scientists at Beltsville, Maryland, discovered a group of plant proteins that may play key roles in solving problems of plant disease resistance to pathogens. Comparisons between plants that are naturally resistant and plants naturally susceptible to certain pathogens revealed a group of proteins that contribute to the resistance response, while a separate group of proteins was found that help the fungus survive within

susceptible plants. These discoveries have helped to identify disease resistance proteins that could eventually be used to protect susceptible plants and are of keen interest to both public and private organizations that are searching for new genes to fight diseases such as soybean rust.

Novel protein identified that interacts with a sorghum aluminum (Al) tolerance protein, AltSB. Acid soils comprise large areas of land throughout the world, often containing toxic forms of aluminum (Al) that damage and inhibit root systems. To improve crops for agriculture on acid soils, there is interest in characterizing the molecular and physiological basis for plant tolerance to Al toxicity. In evaluations aimed at characterizing a major sorghum Al tolerance gene, ARS scientists at Ithaca, New York, identified a gene that encodes a citrate transporter (AltSB), which detoxifies aluminum. They also identified a unique metal-binding protein that binds very tightly to this citrate transporter, promoting aluminum tolerance. Plant scientists can now exploit this information to develop new testable approaches to improve plant aluminum tolerance and yields in acid soils.

Rice responses to elevated CO₂ and O₃ conducted. The response of rice to rising CO₂ and O₃ has been assessed in a number of individual experiments, but the mean response of rice to these elements of climate change is unknown. ARS scientists at Urbana, Illinois, conducted a meta-analysis of all research to date on rice responses to rising atmospheric CO₂ and rising tropospheric O₃. On average, elevated CO₂ (627 ppm) increased rice yields by 23 percent, while elevated O₃ (62 ppb) decreased yields by 14 percent. This synthesis provides a best estimate of the response of one of the most important food crops on earth to climate change. These results will assist plant scientists in better understanding the effects and interactions of elevated carbon dioxide and ground-level ozone on crop yields.

Dehydrin gene expression studied in peach flowers. Last year, nearly 98 percent of the South Carolina peach crop was lost due to cold snaps occurring in late March and early April. The flower is the part most sensitive to cold damage and represents a potential target for genetic manipulation. ARS scientists in Kearneysville, West Virginia, constructed plant vectors carrying peach dehydrin genes under the control of a carpel-specific promoter from petunia. Since dehydrins have been shown to have cryoprotective activity (protection against freezing), boosting expression only in the carpels may protect flowers from frost damage. Development of these new experimental genetic resources now enable plant researchers to determine if expression of one or more dehydrins in the carpels of fruit trees can provide increased protection against late spring frost.

Problem Statement IIC: Developing High-Value Products

Plants are increasingly being recognized as sources of compounds that have important roles in nutrition, medicine, or industrial products. Frequently, new phytochemicals are produced as organisms interact with each other, but many materials of interest are produced without external stimulus. ARS research on natural products will help develop new high-value feedstocks for biobased products and pharmaceuticals.

Molecular marker assays developed for low allergen soybeans. Previous research indicated that accessions from the ARS soybean germplasm collection contain low levels of the major allergen, P34. ARS scientists at Columbia, Missouri, successfully characterized P34 gene sequences from different soybean accessions and discovered the mutation responsible for the low allergen trait, and subsequently developed molecular marker assays for its direct selection. As a result, breeding programs can take advantage of the molecular markers to rapidly develop low allergen soybean varieties. This technology has been released to breeders publicly for the development of allergen-free soybeans.

Transcription factor regulating ripening characterized. While a number of ripening-associated genes have been isolated from tomato and other species, few "master regulator" genes have been identified to date. After identifying one such gene, the ripening-inhibitor (RIN) transcription factor, ARS scientists in Ithaca, New York, initiated an effort to expand understanding of fruit ripening control and to develop new gene targets for breeding and genetic engineering. The researchers identified a new regulator that apparently acts in the control cascade of RIN and impacts downstream ripening traits, including accumulation of

nutritionally important carotenoids and important quality attributes such as softening and ascorbate (vitamin C) accumulation. Regulatory analysis of this gene suggests that it represents a control mechanism operating independently of the ripening hormone ethylene control and thus may represent a regulatory target common to fruiting species that do not require ethylene for ripening (e.g., strawberry, grape, citrus). Ongoing analysis will reveal whether or not this gene may prove useful as a tool in regulating post-harvest shelf life, texture, and nutrient quality in fruit crop species.

Component III: 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. However, the science of recombinant DNA, both creating the genetically engineered plants and evaluating their impact is in an early stage. Methods used for genetic engineering need to be improved, 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 needed 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.

Problem Statement IIIA: Improving and Assessing Genetic Engineering Technology

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.

New selection marker identified for transgenic plants. Concerns about the widespread use of antibiotic resistance genes as selectable markers for genetic transformation in plants have motivated researchers to find alternative selection procedures. ARS scientists at Lubbock, Texas, identified a gene coding for a protein of a naturally occurring heat protection system of plant cells and developed a method to select transgenic plants without the use of antibiotic resistance genes. This new gene, which is involved in heat tolerance, allows transformed cells, embryos, and plants to survive a high temperature challenge; studies showed that non-transformed tissues did not survive the heat challenge. This technology eliminates the need to use antibiotic resistance genes in selecting genetically modified plants.

Wheat transformed with "clean gene" constructions. Commercialization of transgenic wheat has not yet occurred, in part because some consumers have concerns about the presence of foreign genes and proteins in the food supply. ARS researchers in Albany, California transformed wheat with linear DNA that only contained the sequences needed for expression of new traits. No antibiotic resistance genes were introduced into these transformants. These results show that wheat can be transformed with linear DNAs, making it possible to construct wheat transformants that contain only wheat DNA.

Derivative plant lines generated to test interchromosomal recombination. Due to linkage drag, the introgression of transgenes from laboratory to field varieties requires numerous backcrosses. To expedite this breeding process, ARS scientists in Albany, California tested the possibility of using site-specific recombination to break the linkage drag. They introduced recombinase genes into a parental plant line to derive two different alleles, such that recombination between the two alleles can reveal the efficiency of interchromosomal recombination. Working out the efficiency of interchromosomal site-specific recombination helps evaluate this technology for use in plant breeding.

Problem Statement IIIB: 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.

Crop-to-wild gene flow potential in sorghum documented. Baseline data on physical proximity and flowering synchrony of cultivated sorghum and wild and weedy relatives is needed for policy makers and regulators to make decisions on testing and deployment of genetically modified sorghum. ARS researchers at Lincoln, Nebraska, with funding from the U.S. Agency of International Development and in cooperation with researchers from Purdue University, Ohio State University, and the University of Nebraska, have completed a survey of sorghum-producing regions in Ethiopia and Niger. The results indicate that opportunity exists for cultivated sorghum to hybridize with wild and weedy sorghum relatives. This assessment documents proximity and flowering synchrony, and will expand available data on genetically modified sorghum in the environment.