USDA
AGRICULTURAL RESEARCH SERVICE

NATIONAL PROGRAM 302 –
PLANT BIOLOGICAL AND MOLECULAR PROCESSES

ANNUAL REPORT FY 2010

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National Program 302, Plant Biological and Molecular Processes, 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 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 2010 marked the fifth year of this 5-year cycle of research performance for National Program 302. The Action Plan provided guidance for NP 302 projects that led to many ground-breaking discoveries and important advances, some of which were described in the NP 302 Accomplishment Report 2004-2009, and 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 from this very successful National Program.

As of October 1, 2010, NP 302 has merged with National Program 301, Plant Genetic Resources, Genomics, and Genetic Improvement. The need to merge these two successful national programs was realized by National Program Leaders, scientists, and the external retrospective review panel that assessed the national program's accomplishments detailed in the NP 302 Accomplishment Report 2004-2009. The new National Program 301 will enable ARS scientists to better integrate research in biological and molecular processes with the development of plant genetic resources, genomic technologies, and computational expertise to promote innovation and creativity, and to develop project plans that rapidly and efficiently address four of the world's most critical problems – global food security, climate change, renewable energy, and improving human health.

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

Molecular control network governing early crop seedling establishment and photosynthesis deciphered. Seedling establishment is an important trait that impacts crop production globally as well as adaptation to global change. However, the genetic and molecular determinants for improving seedling establishment are not well defined. At germination a seed must activate dormant pathways, and commence vigorous growth in darkness toward the soil surface, where upon exposure to light the photoreceptor phytochrome promotes a second transformation into the photosynthetically active state. ARS scientists in Albany, California, revealed that light activated phytochrome binds to a cohort of PIF (Phytochrome-Interacting Factors) proteins inducing their rapid degradation, thus releasing genes responsible for photosynthesis and green seedling growth. This research establishes a foundation of basic information for the genetic determinants for improving crop stand establishment in target crops.

RNA controls seed production. Seed production and plant development are initiated by a double fertilization event during pollination that triggers both embryo and endosperm development. The coordination of these processes is highly susceptible to stresses associated with global change, including heat and water stress. ARS scientists in Albany, California, are genetically dissecting the cell and molecular processes responsible for the coordination of fertilization in plants. Genetic and molecular studies revealed that in male sperm cells, two genes work together to produce a natural double-stranded RNA molecule, that down-regulates the activity of a control gene that functions specifically in sperm. Plants whose sperm cells lack this important natural double-stranded RNA molecule rarely complete double fertilization and hence seed set is reduced. This discovery established a new paradigm for the role of natural double-stranded RNA molecules in coordinating plant development and seed production.

Overexpression of a blueberry gene protects against freezing. Losses in fruit quality and yield due to freezing can be devastating in blueberries. ARS scientists in Beltsville, Maryland, cloned and sequenced a blueberry gene called CBF that is important because it is responsible in many species for turning on genes that confer cold tolerance. CBF was introduced into blueberry and other species and was shown to increase freeze tolerance in non-acclimated plants. This research enables the development of new berry varieties with increased tolerance to cold through the use of molecular breeding approaches.

New genetic information on corn kernel quality traits. Information on the genes and the genomic regions that control corn quality – including kernel starch, protein, and oil content – is needed by researchers to improve the nutritional and product quality of corn products. ARS researchers in Columbia, Missouri, analyzed the kernel content of over 5,000 recombinant inbred lines from diverse types of corn. The lines were grown in seven locations, then researchers used 1.6 million molecular markers and statistical analyses to identify regions of the genome that control each of the three kernel traits. The research resulted in successful identification of many genetic variants that strongly affect each kernel trait. Notably, new corn lines with a wide range in oil quality were identified. These results provide a rich source of new tools and genetic resources to develop new corn lines and hybrids with a wide range of oil, starch, and protein content.

Discovery of a master regulator gene involved in barley disease defense. Cell death is a key component of disease resistance. Hence, new information about the molecular mechanisms of cell death can provide insight into how to protect plants from disease. ARS researchers in Ames, Iowa, have used a combined strategy of high-throughput transcript profiling with classical genetic analysis of cell death mutants to identify a barley gene that regulates cell death. The gene encodes a key ribosomal RNA processing protein, so the results suggest a role of rRNA processing genes in mediating plant defense responses. The gene appears to be a master regulator that controls the expression of dozens of other genes, which are necessary for plant survival, stature, and yield. This discovery, also supported by the National Science Foundation-Plant Genome Research Program, provides new information that can be exploited by plant scientists in designing more effective mechanisms to protect plants from disease.

A broccoli gene that impacts nutritional content in vegetable crops. Selenium is an essential trace mineral. Both biosynthesis and volatilization of selenium compounds affect the accumulation of the bioactive forms of selenium in crop plants. Broccoli accumulates high level of bioactive forms of selenium. To reduce selenium volatilization for producing healthy and more nutritious crops, ARS scientists in Ithaca, New York, isolated a novel broccoli gene whose product mediates selenium volatilization, and utilized this knowledge to manipulate the nutritional value of crops via reduced nutrient loss due to natural volatilization. The discovery opens up new avenues toward increasing the accumulation of bioactive compounds in plants.

Component II: Biological Processes that Improve Crop Productivity and Quality

Tracking peanut gene expression patterns. To sequence the genome of the peanut, a comprehensive tool for dissecting its complex genetic mechanisms is needed. ARS scientists in Lubbock, Texas, developed a tool to analyze changes in the expression of 49,205 peanut genes and tested the utility of this tool on a variety of peanut tissues. This is the first large-scale, publicly available tool for determining which specific peanut genes are active out of all genes that exist within the peanut plant. The results generated by this tool will provide starting points for in-depth studies on finding candidate genes that can be utilized in reverse genetics to assign gene functions and identify specific molecular mechanisms of peanut response to environmental signals, developmental stages, and yield quality characteristics.

Discovery of natural variation in ozone tolerance in soybean. Ozone, the atmospheric pollutant, is responsible for billions of dollars in lost crop production each year. The effects of ground level ozone on soybean photosynthesis, seed yield, and antioxidant production, was investigated in 10 different soybean cultivars by ARS scientists in Urbana, Illinois. Doubling background ozone decreased soybean yields on average by 17 percent, but the variation in response among cultivars and years ranged from 8 to 37 percent. Chlorophyll content and photosynthetic parameters were positively correlated with seed yield, while antioxidant capacity was negatively correlated with photosynthesis and seed yield, suggesting a trade-off between antioxidant metabolism and carbon gain. Ozone exposure response curves indicated that there has not been a significant improvement in the tolerance of commercial soybean cultivars to ozone in the past

30 years. The discovery of genetic variation in ozone tolerance in non- commercial soybean cultivars is an important step toward improving ozone tolerance in commercial soybeans through breeding.

Development of high oleic acid soybeans. Soybean oil can be improved for the nutritional value in human foods and for industrial biodiesel use through altering the fatty acid composition. Vegetable oils with high oleic acid contents are desirable for the health benefits of the monounsaturated fatty acids, which have recently made olive and canola oils very popular. High oleic acid content also dramatically improves oxidative and temperature stability of the oil, and improves cold flow properties in diesel engines. ARS scientists in Columbia, Missouri, identified and combined mutant alleles of two soybean fatty acid desaturase genes, resulting in high oleic acid-soybean oil. The researchers developed a technology to directly select the genes conferring the desired fatty acid profile, thus accelerating the rate at which new soybean varieties containing this important trait can be made available to producers.

Discovery of the two genes responsible for the soybean low phytate trait. Much of the phosphate in soybean seeds is present as a component of phytic acid, an anti-nutritional factor which impedes the bio-availability of inorganic elements in food and feed containing soybean. This presents contamination issues via run-off from animal waste disposal. Determination of the molecular genetic basis of a soybean line containing the low phytate trait by ARS scientists in Columbia, Missouri, resulted in the discovery of two mutant genes that lower phytate by acting in combination. An additional low phytate soybean line was also characterized and found to contain a dramatic mutation in one of the genes. A method was developed to directly select for any or all of the mutations discovered. Application of this method should simplify development and release of soybean varieties with improved nutritional quality as a trait important for feed and food applications. Such varieties would have a significant impact on the livestock protection industry.

Transgenic manipulation of sorgoleone production in sorghum. Sorghum plants produce a compound referred to as sorgoleone that could have beneficial properties for fighting weeds. ARS researchers in Oxford, Mississippi, have uncovered critical genes required for the synthesis of this compound, and are manipulating its levels in sorghum plants by modifying the expression of one of these genes. Such manipulation could be very useful in improving the weed-fighting capacity of sorghum by increasing sorgoleone production. Conversely, knocking out sorgoleone production could reduce problems encountered by farmers planting other sensitive crop species in the same field immediately following a sorghum crop, thus facilitating more efficient crop rotations.

Development of a method for selecting soybeans with reduced levels of a major protein allergen. Allergenic proteins can reduce the nutritional qualities of soybeans used in food and feed applications. ARS scientists in Columbia, Missouri, discovered that some soybean lines lack a major allergen because of a specific mutation in the gene encoding the protein. A method was developed to directly select for the mutant gene. Adoption of this method will accelerate the rate at which new soybean varieties containing this trait can be evaluated for improved nutritional properties and human health.

Component III. Plant Biotechnology Risk Assessment

A rapid reliable assay for directly comparing gene excision efficiencies. The adoption of genetically engineered crop plants has met with some consumer concerns about the presence of selectable marker genes (e.g. antibiotic resistance markers) in transgenic plants. To address these concerns and to provide new tools for biotechnology, ARS scientists in Albany, California, developed a plant cell assay to compare the efficiencies of site-specific marker gene excision mediated by recombinase enzymes. This assay requires only two days once the DNA constructions are in hand. It is being used to evaluate modifications of recombinase enzymes for improved activity in crop species. These novel recombination systems will help alleviate some consumer concerns by providing the biotechnology industry with convenient options to remove unwanted DNA prior to commercialization.

Gene flow and transgene escape and distinct pollinators of alfalfa. ARS researchers at Madison, Wisconsin, examined the impact of distinct pollinators on gene flow in alfalfa, a crop that can be visited by various bee species. Honeybees had the greater potential risk of carrying pollen from biotech alfalfa to non-biotech alfalfa than three wild bumble bee species. The potential for gene flow for honeybees was not affected by plant density, although for the three wild bumble bee species it was highest in high-density fields. In addition to managed pollinators (e.g., honeybees), the role of common wild pollinators on gene flow should be considered when determining the risk of transgene escape in an insect-pollinated crop.