National Program 302 - Plant Biological and Molecular Processes

Assessment Report

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The USDA-ARS National Program (NP) 302 has a laudable vision to conduct scientific research leading to future advances in crop production, quality and safety. It serves as a bridge between fundamental research for basic understanding of plant function and development of safe biotech applications. NP302 integrates with other National Programs, such as NP301: Plant Genetic Resources, Genomics & Genetic Improvements; and NP303: Plant Diseases, in contributing to the ARS Strategic Plan Objective: Increase the Efficiency of Domestic Agricultural Production and Marketing System. The three research components in NP302: 1) functional utilization of plant genomes, 2) biological processes that improve crop productivity and quality, and 3) plant biotechnology risk assessment, were identified by a Customer and Stakeholder Workshop in 2004. Subsequent progress is evaluated here by an eight-member review panel from academia, government, industry and regulatory sectors with considerable expertise in plant biology and biotechnology research. In general, these research components are well thought out and carefully planned by ARS project teams, and significant progress has been made in this five-year cycle.

Research Component 1 (Functional utilization of plant genomes) has extensively utilized the abundant genomic and genetic information in both model and crop plants for identification of novel genes and processes related to important plant functions such as vegetative and reproductive growth, responses to light, and disease resistances. The quality of research outputs is excellent and several ARS scientists in this Component are leaders in their field of expertise.

Some activities of Research Component 2 (Biological processes that improve crop productivity and quality) overlap slightly with Research Component 1 in investigating functions of genes and metabolic processes mediating plant development and productivity. The roles of several regulatory genes in tomato fruit ripening and regulation of an important hormone receptor kinase via protein tyrosine phosphorylation have been identified. This Research Component also broadly addresses plant responses to the environment, including both biotic and abiotic stresses.

Research Component 3 addresses the need for biotechnology risk assessment conducted by the public sector. Novel recombinase-based transgene stacking and removal technologies which have been developed in this effort could be extremely useful in the production of future transgenic plants. However, addressing the environmental effects of transgenic organisms is directly useful for risk assessment.

Overall, it is felt that most NP302 projects have achieved their projected goals, and resulted in a good number of high quality publications and intellectual properties.

Specific evaluations regarding each Problem Area are summarized below.

Component 1. Functional Utilization of Plant Genomes: Translating Plant Genomics into Crop Improvement

<u>Problem Statement 1A: Advancing From Model Plants to Crop Plants</u>

Anticipated Impacts:

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

Review Team Rating: High Impact

Review Team Assessment:

An outstanding set of outputs have emerged from this portion of the national objective. The review team concurred that cornerstone advances were obtained, and that these were well-targeted for impacting subsequent research. The Quail and McCormick groups have made especially high-impact contributions, extending from mechanisms of pollen tube growth to plant responses to daylength and light quality. Products from this objective included multiple testable hypotheses highly relevant to agriculture.

Accomplishments exceeded both sets of projected outputs (first at the gene level, then at the gene-family and gene-network level). In addition to the knowledge obtained, new approaches were tested, methods developed, and materials generated that will allow still greater successes for future efforts on this front. Creativity and innovation were implicit in this objective, and were evident at multiple levels.

The parament use of model plants was reflected in the achievements reported. These included gene discovery, analysis of function, and use of these to target work on orthologous genes in crop species. The value of model plants also extended to that of testing hypotheses originating from work done on agriculturally-important crops. In this way, findings were validated in another system and relevance extended to diverse species.

A key success of this group and a promising approach for the future has involved development of multiple model systems, particularly for solving specific problems. A highly promising example was provided by the work with *Sporobolus* (a grass related to maize), in which a defined set of transcription factors were found to regulate drought-stress responses in a tissue-specific manner (Cho et al. 2008).

Success of this overall objective is still greater than reported here due to broad-based efforts in this direction by diverse projects across the US.

Problem Statement 1B: Applying Genomics to Crop Improvement

Anticipated Impacts:

- 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.
- Voluminous expansion of genomic information on the function and regulation of gene systems that govern the expression of important traits in agricultural crops.
- Expanded macro-array and micro-array capabilities to visualize functional changes in gene expression during the development of agronomic crops species.
- Proteomic technologies to extend genomic understanding to the level of gene products.

Review Team Rating: High Impact

Review Team Assessment:

Projects addressing this Problem Area have delivered sound science and valuable technologies. This body of work provides an outstanding opportunity for synergy by expansion of these projects across crops, from corn to berries to peanuts.

Some specific genes have been identified that meet the Problem Area objectives as articulated: specifically, genes contributing to fungal resistance (*BLUEFENSIN1*) and aluminum tolerance (*ALMT1*) in cereals have been identified and characterized. Although it is unclear how important the *Corngrass1* and the *Tasselseed4* genes are to corn biomass and seed yield traits described as the objective of the Problem Area 1B, the technical work is outstanding and is critical to enhancing our understanding of the molecular basis of corn plant development.

Genes associated with important traits in berries and melons are being identified through the development of EST libraries; this work and resource will be invaluable in the development of genomics tools for improvement of these crops.

The maize-teosinte populations certainly contribute to the expansion of maize genetic diversity and provide insight to the regulation of gene systems. The panel raised concerns about the value of this work towards the enhancement of grain nutritional quality. These populations are promising as a resource for the improvement of agronomic properties and disease tolerance in maize. However, the panel questioned whether this work would be valuable for the enhancement of grain nutritional quality.

Likewise, the berry research above will certainly benefit from application of some of the association mapping technology developed for the corn projects.

Excellent progress has been made in cataloging of genes expressed at different stages of barley development, and the application of gene expression tools in peanut will mesh nicely with application of association mapping technology (Impact 2) and with proteome maps (Impact 4).

The approaches used to develop the genetic mapping tools above from some of the maize-teosinte backcross populations could be used here to accelerate peanut breeding, since the key nutritive and anti-nutritive proteins can be readily identified. This provides not only an opportunity for developing and utilizing peanut genome information, but also an excellent broader opportunity for synergy opportunity to NP302 researchers.

Component 2. Biological Processes that Improve Crop Productivity and Quality

Problem Statement 2A: Understanding Growth and Development

Anticipated Impacts:

- Improved knowledge of how genes and gene networks that mediate crop development and productivity are regulated.
- Improved knowledge of how metabolic processes that mediate crop quality and productivity of agricultural commodities are regulated.
- Technology to enhance flavor, nutritional value, or other quality traits of plant products.

Review Team Rating: High Impact in most areas; achieved expected outcomes in most areas.

Review Team Assessment:

ARS researchers in NP 302 have made significant advances towards achieving a deeper understanding of genes and metabolic processes regulating growth and development of crop plants, and in exploiting this knowledge. This problem area was one of the most successful of the program.

The research of Giovanni and colleagues on genes and networks controlling ripening in tomatoes has been very successful and has high impact. The researchers have been productive with patent filings and generation of high impact publications (15 in 2008, listed in Appendix 2). Their findings appear to apply to ripening in other fruit crops and so have broad applicability. The research will impact our understanding and capacity to regulate accumulation of bioactive secondary natural products.

The research on gene expression in nodules of *Medicago* is considered important though not novel. It will be a challenge to reduce the list of eighty genes to a more manageable number as targets for genetic modification for improvement of nitrogen fixation in legumes.

Understanding the role of polyamines in metabolic processes controlling regulation of ripening in tomato has considerable potential for metabolic engineering and the research has provided new insights into the biochemistry of ripening processes.

The research on tyrosine phosphorylation in receptor kinase signaling is considered highly novel and a breakthrough in the field of plant hormone signaling. This is one of the most significant accomplishments of the NP 302 program.

The work on the role of amyloplasts in production of starch versus protein is highly significant and considered by the panel to be an important area of research. The identification of potential regulatory steps is very exciting and could lead to strategies to manipulate the ratio of starch to protein in a major food crop.

Reduction of phytic acid in soybean meal has been achieved in practice through the addition of phytase enzymes to the meal. The panel felt that effective reduction of phytic acid levels by genetic means is not likely to be widely used in commercial agriculture.

Problem Statement 2B: Understanding Plant Interactions with Their Environment

Anticipated Impacts:

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

Review Team Rating: Medium-High Impact

Review Team Assessment:

The NP 302 researchers have made significant breakthroughs in gaining a deeper understanding of how plants respond to biotic and abiotic stresses; this knowledge is being exploited to identify genes that could be used as markers and to potentially confer stress tolerance to crop plants.

The discovery of the role of the *esk1* transcription factor in *Arabidopsis* is an important finding representing a new approach to improve cold tolerance of plants in the absence of acclimation.

The work on blueberry *CBF* demonstrates that this gene has utility in improving cold tolerance in fruit crops. The study on cold-induced transcription factors in peach trees could provide useful markers.

The panel recognized the potential of advances made in high temperature stress lab and field studies in a number of plants. A substantial body of research has been accomplished on the mechanism of Rubisco activase function. This work is considered to be of high impact and could lead to markers and means to improve heat tolerance in crop plants. The identification of phosphatidic acid as a potential biochemical marker for heat stress tolerance in maize is also a valuable contribution. The refinement of an infrared (IR) thermometer was considered to be a useful technical accomplishment.

Studies on changes in gene expression related to carbon dioxide levels, mulch and phosphorous availability have led to a deeper understanding of response mechanisms and have potential for new marker development.

The research on the biosynthesis and role of sorgoleone, an allelochemical from sorghum is scientifically interesting and could potentially provide a means to control synthesis of the chemical. However, the panel felt that the potential of this natural product as an herbicide was less clear.

The NP 302 research studies on insect resistance mechanisms was considered to be very solid, with significant advances being made in barley breeding efforts to combat the Russian wheat aphid, and sugar beet root maggot. The identification of a peptide elicitor inceptin, with activity at the femtomole level, represents a major technological accomplishment and a novel avenue to improve biotic stress tolerance.

The research on biotic stresses induced by fungi and nematodes provided new information on crop plant responses and will likely lead to development of strategies in breeding resistance to diverse pests.

Problem Statement 2C: Developing High-Value Products

Anticipated Impacts:

- Tools and methods to identify specific genes that mediate secondary end-product traits desired by consumers, such as nutritional and pharmaceutical compounds.
- 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.

Review Team Rating: High Impact

Review Team Assessment:

Genetic and biochemical approaches were effectively used to identify pathways and genes that mediate end-product traits. The researchers used a novel combination of genomics and biochemistry for the discovery and elucidation of the sorgoleone biosynthetic pathway in sorghum. This is expected to have high impact, although the Report may have overstated the significance of allelopathy for disease control in sorghum crop rotations. The use of forward genetics to discover a gene controlling carotenoid accumulation in cauliflower is an excellent example of how genomics will help us understand and discover non-pathway genes important for the control of nutritional and other metabolites.

A multi-disciplinary approach involving map-based cloning was applied to wheat to identify genes important for both grain quality and agronomic traits. The technical approach was novel and cutting edge, particularly considering the challenges associated with wheat genome size and hexaploidy. The work was appropriately focused on genes important for the stated Problem Area.

The panel recognized the high quality of the work to understand and improve soybean grain quality. The work using transgenesis to knock-out one of the allergens found in soybeans received much publicity and represents an excellent use of biotechnology towards grain improvement. Similarly, the expression of antigens to immunize animals against disease is a creative use of biotechnology towards increasing grain value. The modification of both source and sink to increase the sulfur amino acid content of soy represents solid work to improve feed efficiency and further enhance work done in the private sector. Similarly, the work using transgenesis to reduce the linolenic acid content of soybean represents solid work; however, this work may be more advanced in the private sector at this time.

In general the review panel questioned the role of the USDA in creating transgenic soybeans for modified grain quality. Regardless of the quality of the work, the challenges in technology transfer of transgenic traits for adoption by farmers is quite high. Industry participants on the panel suggested that the costs and quality control issues associated with commercialization of these traits reduce the probability of successful technology transfer necessary to benefit US agriculture.

The panel recognizes the work in developing non-transgenic approaches to soybean grain quality through the use of molecular markers as particularly well suited for technology transfer to seed companies and farmers' fields. The characterization of the *fad3* locus mutation responsible for the formation of linolenic acid and the resulting molecular markers are excellent examples of how USDA developed technology can benefit US agriculture; this technology has been adopted by industry in the commercialization of low linolenic soybeans.

Wheat is a crop where the USDA can have major impact on crop improvement; the USDA researchers and collaborators are in a position of technical leadership. The panel strongly supports the continued public sector funding of wheat genetics and molecular biology to complement to the low levels of funding for similar research in the private sector.

Work with oat antioxidants is on target for the goals of Problem Area 2C of NP302, as is work with acetylenic fatty acids, alkaloids, and cyclopentenedione (potential antifungals).

Overall, the panel lauds the strong scientific quality, impact and practical contribution of USDA work towards the solution of Problem Area 2C.

Component 3. Plant Biotechnology Risk Assessment

<u>Problem Statement 3A: Improving and Assessing Genetic Engineering Technology</u>

Anticipated Impacts:

- 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.
- New processes placed in the public domain to the extent possible, promoting public access to them.
- 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.

Review Team Rating: High Impact

Review Team Assessment:

The proposed objectives were met. Since the focus of Component 3 was on risk assessment, use of the terms "risk" and the relationship to "risk assessment" would benefit from clarification. In an area of research and work that *per se* is already controversial, broad use of the word 'risk' to include different aspects of environmental evaluation should be more specifically and appropriately applied to avoid *a priori* implying a problem. For applications of genetic engineering technologies developed, it is important that technology transfer activities take place among interested parties, and that multidisciplinary teams be formed with that purpose in mind. Use of this information as part of enhancing educational programs might also be useful to pursue. The panel felt this is of particular importance to assure the public has access to factual information.

For improved gene constructs and plant genetic transformation systems, recombinases were identified that could be used to mediate precise heritable deletions and insertion of a plant transgenes. At this point, seven recombinase systems work in yeast, with one also demonstrated to function *in planta*.

New processes placed in the public domain and promoting public access to them included work on enhancement of foreign protein accumulation in soybean; development of efficient barley regeneration conditions; providing synthetic plant-functional promoters; development of transformation protocols for genetic engineering of plants in the Rosaceae family for protection from plum pox virus with research completed (tested during 16 years); development of atlas of gene expression in barley (expression pattern of 22,000 genes); and the assessment of genetic diversity of potato virus Y for the development of diagnostic tools. Much of the work was useful in applying the tools of genetic engineering to address specific problems. The most directly applicable was that on developing plum pox virus resistant plum trees. In several other cases, however, technology transfer is highly encouraged.

To address genetic and epigenetic effects of transformation so thresholds of acceptability can be identified, several areas of research occurred including that four new organ-specific promoters were identified for precise control of transgene expression; a gene promoter useful for use in the protection in *Gladious* species from viruses, *Fusarium*, and nematodes was developed; metabolomic analysis of tomato lines to understand the range of variation of factors related to quality and composition was done; and proteomic analysis of natural variation in soybean seed proteins was was achieved. Some of the research seems to be more relevant to other Components of NP302, and enhancing the appropriate linkages with other researchers and forming teams to assure technology transfer would be useful. However, the baseline information on nutrition and composition is very useful for food safety evaluation.

Since the area of risk assessment is of interest to many of stakeholders, it will be helpful to leverage the value of public research in addressing safety issues and to encourage increased involvement from APHIS regulators and the private sector to assure maximum value of the research.

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

Anticipated Impacts:

- 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. 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.

Review Team Rating: Medium High Impact

Review Team Assessment:

USDA, ARS has received Congressional appropriation to respond to NAS reports and public concern for biotechnology risk assessment research. In response, USDA, ARS has added and strengthened biotechnology risk assessment research through NP 301 and NP 302. The agency has also reached out to a wider audience through workshops on biotechnology risk assessment conducted by the agency over the past four years.

Including risk assessment research within NP302 is important. For this past period, the specific outputs requested at the beginning of the program were met, along with the anticipated impacts: technologies were advanced that potentially could reduce or eliminate movement of transgenes to areas where they are unwanted, and data were developed for regulators regarding the environmental consequences of the use of transgenic plants. Implied objectives to address multiple needs were laudable and benefitted from work accomplished through direct collaboration across the research programs and with other research agencies. Recombinase research allowing deletion of marker transgenes is useful for removing proteins not associated with the product trait. Baseline research on gene flow and the impact of pollinators on gene flow patterns are both directly relevant to the factors risk assessors must take into account in certain situations.

Research on the impact of the current Bt traits is a highly relevant topic of this program, since factors affecting the development of effective insect resistance management practices ensure the viability of high-social-value species such as monarch butterflies. This research has been useful for verification or modification of regulatory assessment approaches. In fact, studies conducted by ARS and others suggest no impact on the biodiversity of insect and plant communities in growing areas.

The movement of transgenes beyond growing areas and into the environment continues to be of public concern. Although the ecological consequence of gene transfer cannot be generalized, research has not demonstrated adverse environmental consequence associated with the commercial release of insect resistant and herbicide tolerant transgenic plants to date.