Revised 3/13/2006

United States Department of Agriculture

Research, Education & Economics

Agricultural Research Service

National Program Staff

March 2006

Version 2.4

National Program Action Plan for the Peanut Genome Initiative

Application of Plant Genomics to Mitigate Peanut Allergy

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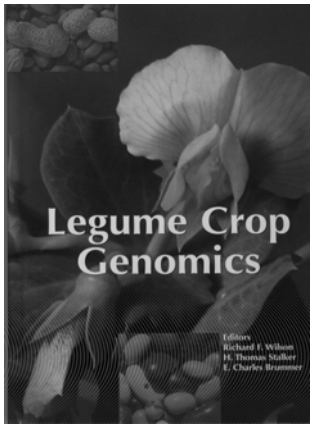
Executive Summary

Vision Statement: An infrastructure for genomic research with a coordinated research approach is needed to guide the effective development of peanut germplasm, genetic tools and bioinformation. Deployment and practical use of these genomic resources will help ensure the competitiveness of U.S. peanut producers in domestic and global markets.



Process & Development of Strategic Research Goals: On 22-23 March 2004, 26-scientists with expert knowledge of critical fields in genetics and plant molecular biology participated in a workshop hosted by the Peanut Foundation/American Peanut Council in Atlanta Georgia. These scientists reviewed the current status of peanut genomic research, which has been documented in the book entitled, *Legume Crop Genomics* published by AOCS Press under the auspices of the U.S. Legume Crop Genome Initiative (USLCGI). In affiliation with USLCGI and other stakeholders, the Peanut Genome Initiative (PGI) was launched at this workshop. A Strategic Plan was developed that outlined research goals objectives, performance measures and significant near-term milestones representing 'quantum leaps' in the advancement of this emerging science.

STRATEGIC GOALS OF THE PEANUT GENOME INITIATIVE (PGI)

1. Improve the utility of genetic tools for peanut genomics research by developing useful gene markers.
2. Improve the efficacy of technology for gene manipulation in genomes by developing useful transformation methods for functional genomic research in peanut.
3. Build a framework for assembling the peanut genome by identifying and integrating the positions of expressed genes on genetic, transcript and physical maps.
4. Improve knowledge of gene identification and regulation by providing baseline data and tools that facilitate the association of DNA-sequences in gene-rich regions of the peanut genome with a biological function.
5. Provide bioinformatic management of peanut biological information resources by establishing a state-of-art interactive system for public distribution of data and information.

The PGI steering committee, representing the broad interests of industry and the peanut research community, recommended that the infrastructure for future advances in peanut genomic research should be based on the solution of a finite problem that has National prominence, encumbers all aspects of genomic research, and builds upon current and relevant knowledge of regulatory mechanisms of plant biological systems. Based on these criteria, the application of plant genomics toward the mitigation of peanut allergy was selected as the demonstration project for PGI. The intent was to establish the framework for an interactive network within the peanut research community and to demonstrate the application of an integrated genomic, proteomic, bioinformatic and immunological approach initially toward the problem of peanut allergy. Success toward that goal will set the foundation for genomic solutions to other major problems such as: aflatoxin contamination, tomato spotted wilt virus, poor peanut flavor and product quality, and the efficient use of water by peanuts.

With stakeholder input, research priorities were identified and aligned with the five goals or components (Genetic Tools, Transformation Technology, Genome Sequencing & Gene Discovery, Functional Genomics & Proteomics, and Bioinformatics) of the PGI Strategic Plan. On June 28, 2004, the steering committee charged individuals to initiate team building toward achieving all performance measures for each Component, and a writing team to develop an Action Plan that defines those performance measures of the Strategic Plan that address the initial highest priority research program needs. The PGI Action Plan (v.1.) was adopted by the peanut research community at the American Peanut Research & Educational Science (APRES) meetings

in San Antonio, Texas on July 13, 2004. At the APRES meeting in Portsmouth, Virginia on July 12, 2005, the PGI workgroup agreed to amend the Action Plan to include ancillary performance measures specific to the Immunology of Peanut Proteins in Model Systems for methods of determining or differentiating allergenic potential among candidate allergen proteins. Revisions to that effect are included in the PGI Action Plan (v-2.3, September 2005), and supercede all prior versions of this plan.

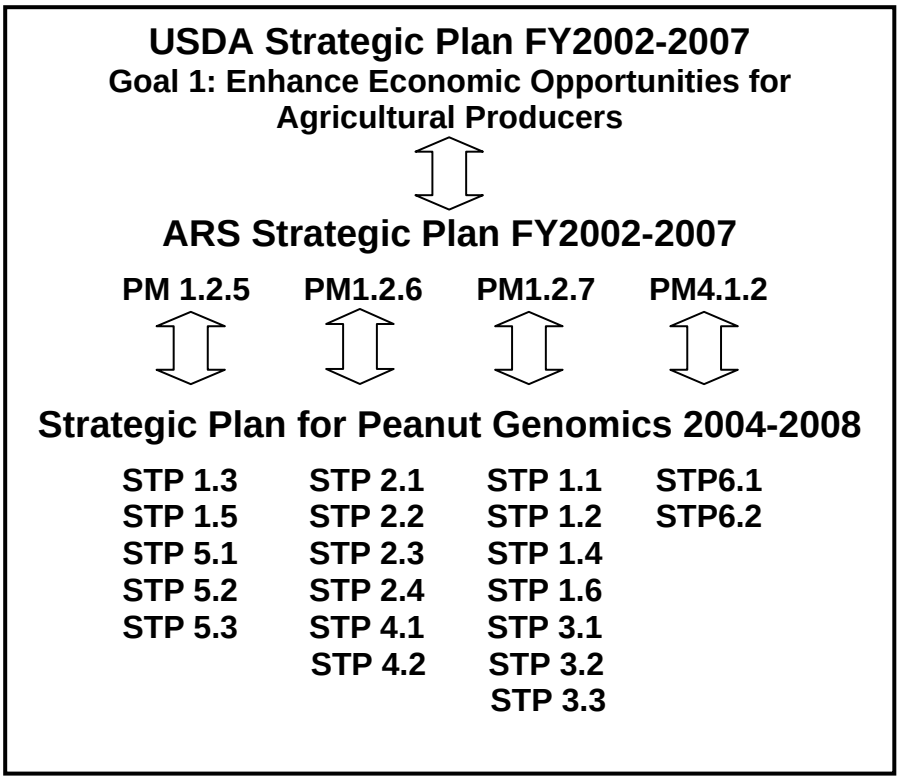
Relation of PGI Performance Measures to the ARS and USDA Strategic Plans: Outputs of this Initiative address Goal 1: *Enhance Economic Opportunities for Agricultural Producers* of the USDA Strategic Plan for FY2002-2007, and support the "Actionable Strategies" associated with the performance measures shown below from the *ARS Strategic Plan for 2003-2007,* Objective 1.2: *Contributions to the Efficiency of Agricultural Production Systems*, and Objective 4.1: *Promote Healthier Individual Food Choices…; Improve Human Health by Better Understanding Nutrient Requirements of Individuals and the Nutritional Value of Foods…*

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.

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.

Performance Measure 1.2.7: Identify genes responsible for plant product quality and resistance to disease, pests, and weather losses.

Performance Measure 4.1.2: Define functions, bioavailability, interactions and human requirements for known, emerging and new classes of nutrients.



Performance Plan for Application of Peanut Genomics Research: 2004-2008

STP	Performance Measure	Baseline 2004	Target in 2006	Target in 2008
1.1	Develop a molecular map of the peanut genome	Two maps developed: A diploid map between A-genome spesies; and a tetraploid map	Develop mapping populations for A- & B-genomes; populations for inbred line selection in A. hypogaea	Develop satruated gentetic maps of allergen genes in the A- and B-genomes
1.2	Develop a comprehensive set of genetic markers for peanut	Very few SNPs exist for peanut	Develop 100 new SNP markers for peanut	Develop 1000 new SNP markers for peanut
1.3	Develop DNA resource from the core-collection accessions	Screened core peanut germplasm collection for allergen-null genes	Extract and preserve DNA from sample plants	Establish a repository and distribution system for DNA samples
1.4	Association of phenotypic traits with molecular markers	RFLP, RAPD, AFLPs for a few pest, nematode, morphology, disease traits	Develop mapping populations for quality and yield-limiting traits	Position markers associated with variation in phenotypic traits on genetic maps
1.5	Develop genomic comparisons among legume species for allergens	Few comparisons among peanut species with molecular tools	Identify sequence tag sites in peanut for cross-species markers	Develop reconstituted genetic maps of the peanut genome with CMAP
1.6	Develop DNA marker assisted selection projects for enhancement of peanut germplasm	No MAS programs in place	Associations of DNA markers among genoypic and phenotypic variation for specific traits	Demonstrate the utility of MAS selection in peanut for quality and agronomic traits
2.1	Improve transformation efficiency and utility for peanut	Biolistic protocols are common. Agrobacterium-mediated protocols not repeatable in general application	Establish Agrobacterium-mediated protocols for high through-put transformation in peanut	Develop effective alternative protocols for high through-put transfromation of peanut
2.2	Improve transformation technology for peanut genomics	Ability to generate & evaluate transgenics expressing 10 constructs among entire community of tranformers	Develop & test new gene promoters, selectable markers & terminators in peanut	Establish high through-put peanut community transformation facilities with expanded capacity
2.3	Develop transgenic screens to understand gene function	Impractical to introduce BAC clones into peanut. Tagging systems developed in theory	Confirm utility of RNAi methods for targeted knockouts and modification of IgE bining sites.	Evaluate heterologous Ac/Ds and/or tDNA tagging systems
2.4	Develop Biotech Risk Assessment & Mitigation Strategies	No known commercial transgenic peanuts. Little data on gene flow or contamination problems	Identify major safety concerns and assess experimental approaches to mitigate allergens	Develop management and/or technological approaches for reducing risk of allergen mitigation
3.1	Advance discovery and knowledge of abundantly expressed genes	5000 expressed sequence tags (ESTs)	50,000 ESTs submitted to GenBank & publically available chromatograms	150,000 ESTs submitted to GenBank plus chromatograms
3.2	Advance discovery & knowledge of rarely-expressed genes, & regulatory sequences that determine gene expression	Virtually no data. Sequences of various allergen gene isoforms complete	Evaluate methodologies in standard genotypes that represent A, B, and AB genomes	Apply methods. Collect transcriptome sequence information from genotypes that represent diploid A and B genomes
3.3	Integration of diverse tool sets to visualize organizational structure of the peanut genome	A 6X BAC library exists for one Florunner component line	Further characterization of the Florunner BAC library and identification of gene-rich BACs	Florunner BACs anchored to detailed genetic map of the peanut genome. Initiate physical maps of diploid genomes
4.1	Proteomics research to characterize seed protein composition and function relavant to potential allergens	Virtually no proteomics research in peanut	Generate high-density proteome map of mature peanut seed with peptides that represent abundant seed proteins. Characterize allergen protein isoforms	Western blot proteomics to detect allergenic seed proteins. Generate a reference proteomic map for leaves, to target allergen proteins
4.2	Application of reverse genetics to explore gene function	Initial development of a TILLING population for mutations in genes encoding potential allergens	Establish facility for high-throughput TILLING in peanut. Develop M2 TILLING populations. DNA libraries.	Advance TILLING populations to M3 families. Large-scale screening for mutations in allergen genes
5.1	Establish collaborative relations with other legume research communities	No peanut presence in the trait and plant ontology efforts	Assure inclusion of peanut concepts in the plant and trait ontology efforts	Assure implementation of peanut specific genes and traits in controlled vocabularies for all plant bioinformation resources
5.2	Establish a peanut bioinformatics oversight committee	Establish a peanut bioinformation oversight committee. Designate developer of a Peanut Information Resource	Establish a curator from the peanut research communitiy who assures data and information content of the Peanut Information Resource	Initiate plans to integrate the Peanut Information Resource into LIS
5.3	Develop a peanut information resource (ArachisDB) as a component of the Legume Information System	There is no single Peanut Information Resource. Peanut genetic & genomic information is stored among diverse resources. A web enabled community bulletin board for peanuts was built and hosted as part of LIS	Map linkage & physical data in LIS. Port metadata into CMAP for comparison of genetic maps between peanut and other legumes. CMAP modified for seamless opertion with LIS. Establish automated linkage of sequence-based markers to EST & genomic data in LIS	All publically available sequences (EST and genomic), expression array and protein information from peanuts will be integrated with other legume information and fully supported with comparative analysis tools in a web-enabled environment
6.1	Develop reliable non-rodent animal models for bioassay and mechanism testing	The pig immune system is more similar to humans than mice. Data from a swine model of food allergy is directly relevant to human response	Test gene expression profiles associated with inflammation and allergy with 100-gene RT-PCR arrays to profile pig immunological response to *Trichuris suis*	Develop pure-line swine populations selected for hypersensitivity to peanut proteins, and validate method with biomarkers and monoclonal antibodies to peanut proteins
6.2	Determine the threshold & kinetics of absorption of sensitizing proteins and allergen peptides in humans	International allergen threshold protocol has been established for allergen detection in foods. There are no credible estimates of the maximum levels of peanut proteins that can elicit allergenic reaction in humans	Access kinetics of allergen absorption into the bloodstream of non-sensitive humans. Collect serum IgE from peanut sensitive indifiduals.	Screen genetically modified peptides and proteins from TILLING and RNAi-allergen gene deletion libraries. Estimates of the maximum threshold dose that elicits reactivity to peanut proteins.

Highest research priorities (listed in order of programmatic need)

STP 4.1: Proteomics research to characterize seed protein composition and function
Location: USDA-ARS, Plant Genetics Research Unit, Columbia, Missouri (Danforth Plant Science Center, St. Louis Missouri)
Lead Scientist: Dr. Eliot Herman
Funding Need & Collaboration: $500, 000 for construction of proteomic maps, and determining the effect of gene knock-outs and substitutions for allergen genes on peanut allergy and seed composition.

STP 3.1: Advance discovery and knowledge of abundantly-expressed genes in peanut
Location: USDA-ARS, Crop Protection & Management Research Unit, Tifton, Georgia
Lead Scientist: Dr. Baozhu Guo
Funding Need & Collaboration: $600, 000 for developing robust EST libraries for peanut, and funding collaborative research investigations to be conducted jointly with Dr. Maria Gallo at the University of Florida, Gainesville, Florida. Dr. Gallo will clone and sequence putative genes for allergens and disease resistance from EST libraries.

STP 4.2: Application of 'reverse genetics' to explore gene function
Location: University of Georgia-Tifton, NESPAL, Tifton, Georgia
Lead Scientist: Dr. Peggy Ozias-Akins
Funding Need & Collaboration: $400,000 for developing and maintaining TILLING populations for peanut, and funding for collaborative research to be conducted jointly with Dr. Niels Nielsen, USDA-ARS, Crop Production & Pest Control Research Unit, W. Lafayette, Indiana; and Dr. Soheila Maleki, USDA-ARS Food Processing & Sensory Quality Research Unit, New Orleans, Louisiana. Dr. Nielsen will be responsible for developing DNA libraries of polymorphic genes in peanut TILLING populations as a unique genetic resource for functional genomics research. Dr. Maleki will screen inbred progeny from TILLING populations, with anti-allergen antibodies and human IgE, for gene mutations affecting the expression of candidate allergen proteins.

STP 4.1: Proteomics research to characterize seed protein composition and function Location: USDA-ARS, Soybean & Nitrogen Fixation Research Unit, Raleigh, North Carolina.
Lead Scientist: Molecular Geneticist (in collaboration with Dr. Niels Nielsen)
Funding Need & Collaboration: $400,000 for discovery of candidate genes from EST libraries, development of micro-arrays to expedite breeding progress in the enhancement of peanut germplasm, and determination of the genetic and metabolic mechanisms that regulate seed composition and quality.

STP 6.1: Develop reliable non-rodent animal models for bioassay and mechanism testing
Location: USDA-ARS, Nutritional Requirements & Function Laboratory, Beltsville Human Nutrition Research Center, Beltsville, Maryland
Lead Scientist: Dr. Joe Urban
Funding Need & Collaboration: $500,000 for develop a predictable procedure to sensitize weaned pigs to proteins that mimic natural food hypersensitivity (clinical disease), and funding for collaborative research to be conducted jointly with Dr. Niels Nielsen, USDA-ARS, Crop Production & Pest Control Research Unit, W. Lafayette, Indiana. Dr. Nielsen will coordinate the development of pure-line swine populations selected for hypersensitivity to peanut proteins and the necessary monoclonal antibodies for standardization of the immune response in these pigs. Dr. Urban will characterize the immunological and physiological basis of peanut hypersensitivity in pigs and develop immune biomarkers to facilitate evaluation of disease expression.

STP 1.2: Develop a comprehensive set of genetic markers for peanut.
Location: USDA-ARS, Wheat, Peanut, & Other Field Crops Research Unit, Stillwater, OK.
Lead Scientist: Dr. Kelly Chenault
Funding Need & Collaboration: $400,000 for developing useful SSR and SNP markers of the peanut genome, and funding for collaborative research investigations to be conducted jointly with Dr. Mark D. Burow at Texas A&M University, Texas Agricultural Experiment Station, Lubbock, Texas. Dr. Burow will position DNA markers on genetic maps of the peanut genome.

STP 2.1: Improve transformation efficiency and utility for peanut
Location: Crop Science Department, North Carolina State University, Raleigh, North Carolina.
Lead Scientist: Dr. Arthur Weissinger
Funding Need & Collaboration: $400,000 for improved and more efficient transformation technology for functional genomic applications in peanut.

STP 1.4: Association of phenotypic traits with molecular markers
Location: USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, Georgia.
Lead Scientist: Dr. Corley Holbrook
Funding Need & Collaboration: $500,000 to establish a marker assisted breeding program for hypoallergenic peanuts, and funding for collaborative research investigations to be conducted jointly with Dr. Mark D. Burow at Texas A&M University, Texas Agricultural Experiment Station, Lubbock, Texas; and Dr. Soheila Maleki, USDA-ARS Food Processing & Sensory Quality Research Unit, New Orleans, Louisiana.. Dr. Maleki will phenotype germplasm resources with specific antibodies. Dr. Burow will develop useful recombinant inbred breeding populations for genome mapping of genetic traits in MAS breeding projects.

STP 5.3: Assure peanut genomic resources are included in Legume Information System
Location: USDA-ARS, Corn Insects & Crop Genetics Research Unit, Ames, Iowa.
Lead Scientist: Dr. Randy Shoemaker
Funding Need & Collaboration: $400, 000 for developing and maintaining ArachisDB, and funding for collaborative research to be conducted jointly with Dr. William Beavis at the National Center for Genomic Research, Santa Fe, New Mexico. Dr. Beavis will coordinate the integration of ArachisDB with LIS.

STP 6.2: Use thresholds and absorption kinetics to confirm the allergenic potential of proteins
Locations: USDA-ARS-SRRC, New Orleans, LA and USDA-ARS-HNRC, Beltsville MD
Lead Scientists: Dr. Soheila Maleki and Dr. David Baer
Funding Need & Collaboration: $500,000 for determining the kinetics of allergen absorption into the human blood stream, and to estimate threshold levels of specific peanut proteins with blood serum from peanut sensitized individuals. Dr. Baer will compare the rate of absorption of peanut protein isoforms, with suspected natural or mitigated allergenic potential, into the human blood stream and confirm the risk posed by fragments most likely to sensitize individuals to peanut. Dr. Maleki will characterize IgE binding and other allergenic properties of similar peanut proteins or peptides, and will determine the maximum threshold dose that elicits reactivity to peanut with clinical collaborators at Tulane, LA

Introduction

The competitiveness of U.S. peanut producers in domestic and global markets is threatened by losses in productivity and quality that are attributed to diseases, pests, environmental stresses and allergy or food safety issues. The USDA-ARS National Plant Germplasm System (NPGS), the world's largest collection of plant germplasm (ca. 450,000 accessions of 10,000 different species) typically provides the first line of long-term defense against those problems. The Peanut Germplasm Collection at Griffin, Georgia contains ca. 9900 accessions of 72 species from 106 countries. Natural genetic diversity among wild relatives and accessions of cultivated peanut provides the primary means to attain durable resistance or tolerance to major constraints such as peanut root-knot nematode, tomato spotted wilt virus, drought, and pre-harvest aflatoxin contamination. Even so, new technology is needed to facilitate more rapid discovery of genes that confer a remedy to these constraints and the incorporation of those genes into elite germplasm by conventional breeding methods in a timely manner. Genomic, proteomic and bioinformatic research can provide the genetic tools to effectively mine useful genes from the wealth of natural genetic diversity that exists in peanut. However, the infrastructure for future advances in peanut genomic research should be based on the solution of a finite problem that has National prominence, encumbers all aspects of genomic research, and builds upon current and relevant knowledge of regulatory mechanisms of plant biological systems. Based on these criteria, the application of plant genomics toward the mitigation of peanut allergy was selected as the demonstration project for PGI. The intent was to establish the framework for an interactive network within the peanut research community and to demonstrate the application of an integrated genomic, proteomic, bioinformatic and immunological approach initially toward the problem of peanut allergy. Success toward that goal will set the foundation for genomic solutions to other major problems such as: aflatoxin contamination, tomato spotted wilt virus, poor peanut flavor and product quality, and the efficient use of water by peanuts.

Peanut allergies are reported by more than 4 million Americans and are becoming an increasingly serious public health and food safety issue, especially for the 600,000 or more U.S. children who are affected. As little as ½ a peanut can cause a fatal reaction in severely allergic individuals. Many adults who survive peanut allergy in childhood do not outgrow their sensitivity to peanut, and may have life-threatening allergic reactions for the rest of their lives. There is no cure for peanut allergy, and it is difficult to avoid foods with peanut-ingredients. Development of agronomic peanut varieties that lack allergenic proteins may provide the most durable solution to this problem, which continues to increase in severity. Genomic tools and peanut bioinformatics are needed to implement effective strategies to achieve that goal in a timely manner.

Current information suggests that certain peanut proteins are the causal factor of peanut allergy. Nine polypeptides that constitute the major peanut seed proteins (*Ara-h1* to *Ara-h9*) appear to bind serum-specific *IgE* from peanut-sensitive people. *Ara-h1* is a vicilin-type protein. *Ara-h2, h6* and *h7* are conglutin-type proteins. *Ara-h3* & *h4* are legumin- type proteins. *Ara-h5* is a profilin; *Ara-h8* is a lipid transfer protein, and *ara-h9* has been identified as an oleosin. The relation of these proteins to individual gene families is unclear, but more than one gene probably is involved in the expression of each *Ara-h* protein. For example, at least two genes, each potentially with multiple copies, encode *Ara-h2*.

Although genomic techniques may be used to identify and 'knock-out' genes that encode specific *Ara-h* proteins, it is unclear which of those genes are most relevant to allergic reaction or how metabolic compensation in seed protein synthesis might alter allergic sensitivity to peanut. In conjunction with a genomic approach, alternative non-primate test systems that simulate human responses to peanut proteins are needed to evaluate the effects of genomic strategies to eliminate or modify peanut proteins. Clinical efforts also should be redoubled to develop accurate-as-possible estimates of the maximum threshold level for human response to peanut proteins, and to better understand how human absorption of undigested proteins that cause sensitization or anaphylactic reaction in allergic humans.

The *Strategic Plan for the Peanut Genomics 2004-2008* provides the peanut research community with a foundation for a comprehensive and integrated research approach toward this problem. Performance measures that have need for immediate implementation are documented in this Action Plan. In accord with the *President's Management Agenda*, this plan also defines the actions that will be taken to solve the problem, describes what is promised or will be produced, assigns accountability for the work to be accomplished, and provides a mechanism for peer review and assessment of research progress.

Action Plan for Peanut Genomics Research

Genetic Tools & Breeding Methods

Arachis hypogaea is a domesticated crop species that contains two different sets of chromosomes. These two genomes can be differentiated with DNA markers. Although a large amount of genetic variation is evident among accessions of cultivated peanuts, few molecular genomic tools are available to mine useful genes or improve the efficiency of genetic enhancement of peanut through plant breeding. These molecular markers are associated with resistance to only a few peanut diseases and insects. There is critical need to expand the scope of DNA marker coverage of entire peanut genomes, and to use DNA marker systems either from peanut and other legume crops to facilitate peanut crop improvement.

Goal: Improve the utility of genetic tools for peanut genomics research by developing useful gene markers.

High Research Priorities for Genetic Tools & Breeding Methods:

STP 1.2: Develop a comprehensive set of genetic markers for peanut. Simple-sequence repeat (SSR) markers and other types of molecular marker systems such as single nucleotide polymorphisms (SNP) are valuable genetic tools for the identification of useful polymorphisms (mutations) in genes in *A. hypogaea* and wild species of the genus *Arachis*. Markers have utility in the characterization of candidate genes for specific traits from raw DNA sequence data, mapping the organization of the peanut genomes, anchoring physical maps of the genomes to genetic maps, and in improving the efficiency and effectiveness of peanut breeding.

Research Need: DNA-markers to enable development of genetic maps and comparative analyses of genomic regions that may harbor genes associated with peanut allergy & agronomic traits. DNA markers for specific phenotypic traits will be discovered in a database of expressed sequence tags (EST), the expressed portion of the peanut genome. Selected EST will be associated with bacterial artificial chromosomes (BAC), created with segments of peanut DNA sequence, that contain specific allergen-related genes. 'Data-Mining' these EST and comparative genomic analyses with relevant DNA-probes will locate all members of allergen gene families in peanut, and genes for agronomic traits as well.

Outputs

- Useful sets of SSR and SNP markers for the identification of candidate genes for important genetic traits from genome sequences, genome mapping and translational genomic approaches (marker–assisted selection) in peanut breeding.

Resources

Location: USDA-ARS, Wheat, Peanut, & Other Field Crops Research Unit, Stillwater, Oklahoma
Lead Scientist: Dr. Kelly Chenault
Funding Need & Collaboration: $400,000 for developing useful SSR and SNP markers of the peanut genome, and funding for collaborative research investigations to be conducted jointly with Dr. Mark D. Burow at Texas A&M University, Texas Agricultural Experiment Station, Lubbock, Texas. Dr. Burow will position DNA markers on genetic maps of chromosomes in the peanut genome.

STP 1.6 Germplasm enhancement for quality and agronomic traits with molecular markers. A MAS system for selection for specific traits requires identification of germplasm with contrasting phenotypes, identification of markers closely associated with QTL (quantitative trait loci), and technologies to facilitate rapid/cost effective screening of large populations. Linkages of resistance genes to different molecular markers have demonstrated the value of selecting breeding lines with desirable traits. Further progress in improving the efficiency of peanut cultivar development is limited by the lack of more complete coverage of the gene-space in the peanut genome with appropriate molecular markers.

Research Need: Recombinant inbred line populations for validation of genetic maps, and Marker-Assisted-Selection (MAS) breeding programs for genetic enhancement of peanut agronomic traits & reduction of peanut allergens. Many of the most difficult traits to improve in a selection program are multi-genic. Gene families govern allergen protein expression. Genes that protect plants against pathogens often exhibit multiple components of resistance. Molecular markers will be used to identify untapped sources of resistance, and to develop segregating populations for inheritance studies and marker verification for crop improvement. MAS will be deployed to provide a more efficient method for combining multiple genes in a single genotype.

Outputs

- Useful genetic populations and methods for accurately mapping and positioning gene markers on genetic maps of the peanut genome.
- More efficient and effective breeding methods; and peanut germplasm enhanced for improved quality, flavor, reduced aflatoxin contamination, lower levels of allergenic proteins, and greater productivity.

Resources

Location: USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, Georgia
Lead Scientist: Dr. Corley Holbrook
Funding Need & Collaboration: $500,000 to establish a marker assisted breeding program for hypoallergenic peanuts, and funding for collaborative research investigations to be conducted jointly with Dr. Mark D. Burow at Texas A&M University, Texas Agricultural Experiment Station, Lubbock, Texas. and Dr. Soheila Maleki, USDA-ARS Food Processing & Sensory Quality Research Unit, New Orleans, Louisiana. Dr. Maleki will phenotype germplasm resources with specific antibodies. Dr. Burow will be responsible for developing useful recombinant inbred breeding populations for genome mapping of genetic traits in MAS breeding projects.

Anticipated Impact: Adequate supply of useful DNA-markers and genetic maps will accelerate discovery of gene rich QTL in the peanut genome. Gene markers and genetic maps will enable the design of more effective breeding strategies. Gains in breeding efficiency will enable the simultaneous ‘stacking’ or ‘pyramiding’ of multiple genes governing many desired traits that influence peanut allergy, quality, and productivity in elite peanut germplasm.

Plant Transformation Technology

Successful protocols have been developed for peanut transformation using both microprojectile bombardment and *Agrobacterium*-mediated gene transfer. However, it is unlikely that all U.S. laboratories capable of peanut transformation, working in concert, could analyze more than about 10 genes per year. There is critical need to expand the capacity of transformation systems to provide genome information that is essential to the timely achievement of technical advances in peanut enhancement, and the control and manipulation of genes that encode peanut allergens..

Goal: Improve the efficacy of technology for gene manipulation in genomes by developing useful transformation methods for functional genomic research in peanut.

High Research Priority for Plant Transformation Technology:

STP 2.1 Improve transformation efficiency and utility for peanut. *Agrobacterium*-based transformation has certain advantages compared with "naked DNA" transfer procedures, such as microprojectile bombardment. Current protocols for inserting or deleting genes in peanut are limited by low transformation efficiency, and increased time in tissue culture. There is no adequate test system to identify the best recipient genotypes for specific agronomic goals.

Research Need: Protocols for higher efficiency in the recovery of transformed tissues. Peanut transformation has shown significant improvement and enabled production of cultivars with transgenic traits. However, the greatest need for research to enhance transformation efficiency is for applications in functional genomics. Transformation technology is essential for the discovery of gene function via changes in phenotype attributed to the deletion or insertion of specific genes. To meet pending demand for this technology, improvements will be made in areas that help ensure greater efficiency and effectiveness of peanut transformation.

Outputs

- Greater peanut transformation capacity to accelerate the characterization of candidate sequences (genes and regulatory elements) for potential allergens that are generated by a large-scale peanut genomics program.

Resources

Location: Crop Science Department, North Carolina State University, Raleigh, North Carolina.
Lead Scientist: Dr. Arthur Weissinger
Funding Need & Collaboration: $400,000 for improved and more efficient transformation technology for functional genomic applications in peanut.

Anticipated Impact: Evaluation of existing transformation approaches will determine the optimal protocol that delivers greater efficiency and capacity. Advances in transformation capacity that are gained from the development of new methods will enable transformation of larger DNA sequences (e.g., from BAC libraries), tagging strategies that facilitate the cloning of important genes by interrupting normal function and by marking genes with identifiable molecular sequences.

Genome Sequencing & Gene Discovery

The nuclear genome of the cultivated peanut is approximately 3×10^9 bp, similar to the size of the human genome. The peanut genome contains about 50,000 genes that collectively determine growth and development, response to biotic and abiotic factors in the growth environment, crop productivity and product quality. A detailed analysis of genomic regions containing genes and associated regulatory elements (promoters and enhancers) is essential to understanding the full genetic potential of peanut as a healthful and profitable food crop. Polyploidy, resulting from the joining of two genomes from ancestors with divergent evolutionary history, is a consideration in crafting research strategies for cultivated peanut. Because two or more copies of many genes typically are present, specialized approaches must direct the accurate alignment and assignment of the sequence for each gene isoform to the proper chromosome in assembly of the peanut genome from a random assortment of DNA sequence fragments.

Goal: Build a framework for assembling the peanut genetic genome by identifying and integrating the positions of expressed genes on genetic, transcript and physical maps.

High Research Priority for Genome Sequencing & Gene Discovery:

STP 3.1: Advance discovery and knowledge of abundantly-expressed genes in peanut.
Sequencing of clones from cDNA of an expressed sequence tag (EST) database is the most efficient approach for gaining genomic information in peanut. ESTs can be used for gene discovery, genome annotation, comparative genomic analyses, and markers for genes that will lead to new cultivars with improved traits such as hypo-allergenicity and disease resistance. Currently, only about 5,000 peanut ESTs exist in the public domain. Several hundred thousand EST sequences from high-quality cDNA libraries representing a diverse range of tissues at different developmental stages are needed to obtain adequate estimates of the organization of the peanut genome. Original chromatograms of these sequences also permit extraction of additional information such as SNPs from raw sequence data.

Research Need: Adequate EST libraries to generate useful DNA-markers that are associated with peanut allergenicity & agronomic traits. EST libraries provide insight to specific portions of the genome that contain genes of interest. A database containing about 200,000 ESTs (single-pass 5' sequence) will be constructed from flowering and peg/pod/seed tissues, from tissues responding to pathogens causing diseases, specifically the tomato spotted wilt (TSWV), leaf spots, Sclerotinia blight, and *Aspergillus*-aflatoxin, and to drought/heat stress. These will then be used to generate a set of 20,000 unigene clones that will be subjected to 3' sequencing. Selected ESTs will be associated with BACs for gene discovery, and the development of molecular markers and genetic maps of the peanut genomes.

Outputs

- Useful EST libraries from specific peanut organs exposed to various environmental/ experimental conditions during various stages of plant development.
- Identification and characterization of candidate genes for allergens and agronomic traits from peanut EST sequences.
- Bacterial Artificial Chromosome (BAC) libraries with DNA sequences associated with allergen genes.

Resources

Location: USDA-ARS, Crop Protection & Management Research Unit, Tifton, Georgia.
Lead Scientist: Dr. Baozhu Guo
Funding Need & Collaboration: $600, 000 for developing robust EST libraries for peanut, and funding collaborative research investigations to be conducted jointly with Dr. Maria Gallo at the University of Florida, Gainesville, Florida. Dr. Gallo will clone and sequence putative genes for allergens and disease resistance from EST libraries.

Anticipated Impact: A database of expressed sequence tags (EST), the expressed portion of the peanut genome, will facilitate gene discovery and development of DNA markers. Use of these markers, on microarray chips to select progeny with desired gene combinations from segregating populations, will enhance the efficiency of peanut variety development

Functional Genomics & Proteomics

Proteins are the structural and enzymatic products encoded by genes. Examination of gene expression on a genomic scale at the mRNA level using micro-arrays does not provide information on post-translational modifications that affect the abundance and activity of gene products (proteins). High-resolution protein separation on two-dimensional gels followed by mass spectrometry of excised protein spots allows the rapid profiling of gene expression and protein metabolism. The use of probes created from known gene sequences or deduced from protein structure ('reverse' genetics) is an effective approach to discover and determine the function of the corresponding unknown gene sequence in peanut.

Goal: Improve knowledge of gene identification and regulation by providing baseline data and tools that facilitate the association of DNA-sequences in gene-rich regions of the peanut genome with a biological function.

High Research Priorities for Functional Genomics & Proteomics:

STP 4.1 Proteomics research to characterize seed protein composition and function.

Proteomics is the extensive characterization of proteins in biological organisms. The extent of genetic variability among peanut seed protein profiles has not been explored with high-resolution methods. Few attempts have been made to characterize changes in protein composition and allergenicity in the seed of peanuts with genetically inserted or deleted genes that mediate expression of allergenic proteins and other agronomic traits.

Research Need: Genetic regulation of allergen synthesis, structure, and composition. The diversity of allergen genes will be determined from ESTs representing all major peanut seed proteins. With clinically relevant serum, IgE-binding proteins will be identified for individual members of gene-families and correlated by sequence with the corresponding cDNAs. Homogenous purification of potential allergens will help characterize and distinguish proteins according to anaphylactic risk. Genes for specific high-risk allergen proteins will be eliminated by 'RNAi' or similarly effective technology. Annotated maps of the proteome will be developed to interpret immunological and metabolic changes in proteins that accumulate in compensation for 'deleted' allergens.

Outputs

- Annotated high-density proteomic maps of developing & mature peanut seed of cultivars and germplasm exposed to various biotic and abiotic stresses.
- Annotated fingerprint patterns for allergenic proteins among experimental germplasm accessions of the USDA Peanut Germplasm Collection.
- A reference proteomic map from peanut leaf tissue.
- Micro-arrays with relevant DNA markers to expedite breeding progress in the development of elite peanut varieties.
- Knowledge of the genetic and metabolic regulation of peanut seed protein profiles.
- Genetic resources exhibiting unique gene insertions or deletions that influence the levels of potential allergenic proteins in peanut.
- An index of the allergenic potential of specific peanut proteins.

Resources
Location-1: USDA-ARS, Plant Genetics Research Unit, Columbia, Missouri (Danforth Plant Science Center, St. Louis, Missouri)
Lead Scientist: Dr. Eliot Herman
Funding Need & Collaboration: $500, 000 for construct proteomic maps, and determining the effect of gene knock-outs and substitutions for allergen genes on peanut allergy and seed composition.

Location-2: USDA-ARS, Soybean & Nitrogen Fixation Research Unit, Raleigh North Carolina.
Lead Scientist: Molecular Geneticist (in collaboration with Dr. Niels Nielsen)
Funding Need & Collaboration: $400,000 for discovery of candidate genes from EST libraries, development of micro-arrays to expedite breeding progress in the enhancement of peanut germplasm, and determination of the genetic and metabolic mechanisms that regulate seed composition and quality.

STP 4.2 Application of 'reverse genetics' to explore gene function
EST sequencing projects will provide an abundance of DNA sequence information from expressed genes, some of whose functions can be predicted based on sequence similarity. The prediction of function would not, however, fully describe the role of a particular gene in peanut growth and development. Gene function could be definitively determined by the creation of a series of mutant alleles with evaluation of resultant phenotypes. Since cultivated peanut is a tetraploid, and gene function may not simply be additive with respect to the combination of two putative progenitors of *A. hypogaea* into one species, it would be most informative to create mutants in cultivated peanut. An effective genome-based approach to exploring the function of genes is called TILLING (Targeting Induced Local Lesions in Genomes). This method relies on high-throughput screening at the DNA level for mutations in genes. A TILLING population for peanut should be developed as a public resource.

Research Need: Peanut TILLING populations exhibiting useful mutations in allergen and other agronomic genes. Assigning gene function to DNA-sequences is hindered by a lack of polymorphism (spontaneous mutations) within the peanut genome. Natural mutations in genes may be induced throughout the genome via chemical agents or high-energy radiation. TILLING is an approach that uses induced mutations in DNA-sequences to identify the function of genes. TILLING will accelerate recovery of genes that may abate peanut allergy, and the development and use of precise DNA-markers in 'marker-assisted' breeding programs to select genotypes with specific mutations in members of allergen gene-families.

Outputs
- Unique genetic resources for peanut that include DNA libraries of induced mutations in genes for allergenic proteins and agronomic traits.
- Germplasm and breeding lines with beneficial mutations in genes that govern the expression of allergens and other agronomic traits.

Resources
Location: University of Georgia-Tifton, NESPAL, Tifton, Georgia
Lead Scientist: Dr Peggy Ozias-Akins
Funding Need & Collaboration: $400,000 for developing and maintaining TILLING populations for peanut, and funding for collaborative research to be conducted jointly with Dr. Niels Nielsen, USDA-ARS, Crop Production & Pest Control Research Unit, W. Lafayette, Indiana; and Dr. Soheila Maleki, USDA-ARS Food Processing & Sensory Quality Research Unit, New Orleans, Louisiana. Dr. Nielsen will be responsible for developing DNA libraries of polymorphic genes in peanut TILLING populations as a unique genetic resource for functional genomics research. Dr.

Maleki will screen inbred progeny from TILLING populations, with anti-allergen antibodies and human IgE, for gene mutations affecting the expression of candidate allergen proteins.

Anticipated Impact: Advances in functional genomics and proteomics will provide assurance that targeted allergenic potential is arrested and will facilitate the assessment of possible collateral alteration of other potential allergens. These resources will enable strategies for reducing the allergenic potential of peanut, and to provide novel germplasm for biological characterization of metabolic and regulatory mechanisms governing protein synthesis in peanut seed. The use of gene markers on micro-array chips, to select progeny with desired gene combinations from segregating populations, will enhance the efficiency of peanut variety development.

Bioinformatics

Bioinformatics consists of data management (acquisition, storage, integration and dissemination) and data interpretation (data analysis, visualization and biological modeling). The scope of bioinformation includes DNA sequences, RNA expression levels, protein interactions, map positions and QTL. However, until robust databases are developed for peanut, bioinformation for peanut must be leveraged from other plant and legume species. Ability to find gene products that can be associated with biochemical pathways or networks, enables the discovery of the molecular basis for phenotypic traits from related species. The peanut bioinformatic database should be a component of the Legume Information System (LIS). LIS, a Congressionally mandated joint collaboration between USDA-ARS and the National Center for Genomic Research (NCGR), was conceived to be a comparative legume resource, and a first step towards leveraging bioinformation from model plants to gain insights into crop species. LIS is a data management system that includes an annotation pipeline of data analysis tools and legume (soybean, peanut, alfalfa, pea, dry beans) data bases developed by multiple groups.

Goal: Provide bioinformatic management of peanut biological information resources by establishing a state-of-art interactive system for public distribution of data and information

Highest Priority Performance Measures

STP 5.3 Assure Peanut Genomic Resources are included in the Legume Information System (LIS). A distinct peanut information resource would facilitate the storage and use of bioinformation for peanut. The success of a biological information resource is assured if the research community served exercises a sense of ownership in the system. For example, there is greater potential for high quality peanut information and enhanced comparative genomics approaches if peanut researchers control an information resource that is distinct to the genus *Arachis.* Subsequent or concomitant incorporation of peanut data into LIS does not preclude such ownership, but greatly enhances the utility of the peanut information resource.

Research Need: Development of ArachisDB. Bioinformatics involves management and interpretation of data from DNA sequences, forms of gene expression, protein interactions and the relationships of these data with traits such as allergens. With advances in Peanut Genomics a distinct information resource will facilitate the storage and use of bioinformation for peanut. Incorporation of peanut information into LIS will not only ensure that peanut research is enhanced with related information from other legumes, it will also ensure that the diversity of allergen genes and potential allergens are identified among all food legumes.

Outputs

- A state-of-art interactive bioinformatics resource for peanut, and other legumes.
- Advanced methods for comparative genomic analyses.

Resources
Location: USDA-ARS, Corn Insects & Crop Genetics Research Unit, Ames, Iowa.
Lead Scientist: Dr. Randy Shoemaker
Funding Need & Collaboration: $400,000 for developing and maintaining ArachisDB, and funding for collaborative research to be conducted jointly with Dr. William Beavis, National Center for Genomic Research, Santa Fe, New Mexico. Dr. Beavis will coordinate the integration of ArachisDB with LIS.

Anticipated Impact: Since inception, the development of LIS has been guided by the legume research communities. Future advances also require collaborative relations with other plant bioinformation groups to ensure representation in plant community defined ontologies and controlled vocabularies. Recognizing the power of comparative genomics among species to identify candidate genes, unique genes, and evolutionary relationships among genes for crop improvement, a Peanut Information Resource (ArachisDB) should be developed and should become a component of LIS

Immunology of Peanut Proteins in Model Systems

Hypersensitivity to dietary peanut proteins has not been critically evaluated. The expression of clinical disease follows a pattern of peanut sensitization and transitory hypersensitivity to subsequent exposure, but the generalized intestinal response is common to several different forms of food hypersensitivity. Some investigations suggest that neonatal immune development with the production of anti-peanut protein IgG/IgM antibodies and subsequent complement-mediated epithelial cell injury is responsible, while others indicate hypersensitivity based on IgE-mediated immediate intestinal anaphylaxis. It is essential to define dietary peanut intolerance in order to develop appropriate control strategies.

Goal: Provide reliable model systems that enable accurate and quantitative prediction of the potential response of sensitized humans to peanut allergens.

Highest Priority Performance Measures

STP 6.1: Develop reliable non-rodent animal models for bioassay and mechanism testing

A survey of 55 immunological variables in pig, human and mice, showed that the pig immune system is more similar to humans than mice for >80% of the variables compared. Thus, data derived from a swine model of food allergy is more directly relevant to human than data derived from rodents. A practical pig model to study peanut food allergy will provide procedures that alleviate or reduce clinical disease, and provide a means to evaluate food intolerance to other legumes and extrapolate to human food allergy.

Research Need: Genetically pure swine populations selected for hypersensitivity to peanut proteins, and molecular tools for quantitative interpretation of assay results. There is no validated animal model to evaluate or predict the allergic risk of humans to native and genetically-modified proteins. Refined diagnostic tools and resources will be used to characterize novel or genetically modified proteins to ascertain potential for eliciting or mitigating human response to candidate allergens, and to improve prevention and/or intervention strategies for treatment of food allergy.

Outputs

- Tools for modern molecular immunological and physiological measurements of food allergy response in pigs.
- Techniques such as artificial insemination, frozen semen and embryo transfer that facilitate the reproducible selection and preservation of hypersensitive pig populations.
- Molecular strategies to identify peanut genes with large effects on the allergic response in sensitized humans.

Resources

Location: USDA-ARS, Nutritional Requirements & Function Laboratory, Beltsville Human Nutrition Research Center, Beltsville, Maryland
Lead Scientist: Dr. Joe Urban
Funding Need & Collaboration: $500,000 for develop a predictable procedure to sensitize weaned pigs to proteins that mimic natural food hypersensitivity (clinical disease), and funding for collaborative research to be conducted jointly with Dr. Niels Nielsen, USDA-ARS, Crop Production & Pest Control Research Unit, W. Lafayette, Indiana. Dr. Nielsen will coordinate the development of pure-line swine populations selected for hypersensitivity to peanut proteins and the necessary monoclonal antibodies for standardization of the immune response in these pigs. Dr. Urban will characterize the immunological and physiological basis of peanut hypersensitivity in pigs and develop immune biomarkers to facilitate evaluation of disease expression.

STP 6.2: Use threshold estimates and absorption kinetics to confirm the allergenic potential of peanut proteins. International allergen threshold protocol has been established for allergen detection in foods. However, additional investigations are needed to develop greater confidence levels in estimates of the maximum levels of peanut proteins that can elicit allergenic reaction in humans. Such information will guide genomic strategies for developing peanut cultivars with reduced allergenic properties.

Research Need: Absorption kinetics for natural and genetically modified proteins or peptides that elicit allergenic response in model systems. Quantitative time-course assessment and kinetics of absorption into the blood serum of digested or undigested peanut proteins (identified as potential allergens by response in swine and/or IgE binding properties) will be performed with human subjects who are non-peanut sensitive. Allergenic potential of the protein products of natural and modified isoforms of specific plant genes will be compared and assayed for sensitization capability using blood serum that contains allergen specific antibodies (IgE) from peanut sensitive humans. Food challenge studies will be conducted by accredited medical staff using different doses of disguised peanut proteins in a double blind-placebo experimental design with a statistically significant number of patients. Results of threshold dose and peanut protein absorption/sensitization studies will be correlated with corresponding dose responses in the swine-model to develop appropriate genomic strategies for developing peanut cultivars with reduced allergenic potential.

Outputs

- Immunoassays for improved detection of potential allergens in crop germplasm.
- Databases on the digestibility and kinetics of absorption of different allergenic and non-allergenic proteins into the blood stream following ingestion.
- Immunological tools to screen products of TILLING populations for mutations in potential allergen genes.
- Estimates of the threshold doses for peanut allergic individuals..

Resources
Locations: USDA-ARS-SRRC, New Orleans, LA and USDA-ARS-HNRC, Beltsville MD
Lead Scientists: Dr. Soheila Maleki and Dr. David Baer
Funding Need & Collaboration: $500,000 for determining the kinetics and nature of allergen absorption into the human blood stream, and to estimate threshold levels of specific peanut proteins with blood serum from peanut sensitized individuals. Dr. Baer will compare the rate of absorption of peanut protein isoforms, with suspected natural or mitigated allergenic potential, into the human blood stream and confirm the risk posed by fragments most likely to sensitize individuals to peanut. Dr. Maleki will characterize IgE binding and other allergenic properties of similar peanut proteins or peptides, and will determine the maximum threshold dose that elicits reactivity to peanut with clinical collaborators at Tulane, LA

Anticipated Impact: Quantitative model systems for evaluating the human response to peanut proteins will provide clinical researchers with robust resources (including reagents such as an anti-pig IgE detection antibody and immune biomarkers for immediate-type hypersensitivity) that complement other models used by federal regulatory agencies for food allergen prediction and testing. This technology also will enable knowledgeable decisions in genomic strategies for achieving beneficial modifications in the seed protein composition of commercial peanuts. Determination of threshold doses for peanut allergy and other legumes will provide useful guidance to avoid allergen contamination during the manufacture of food products.

Collaborating Research Locations & Organizations

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