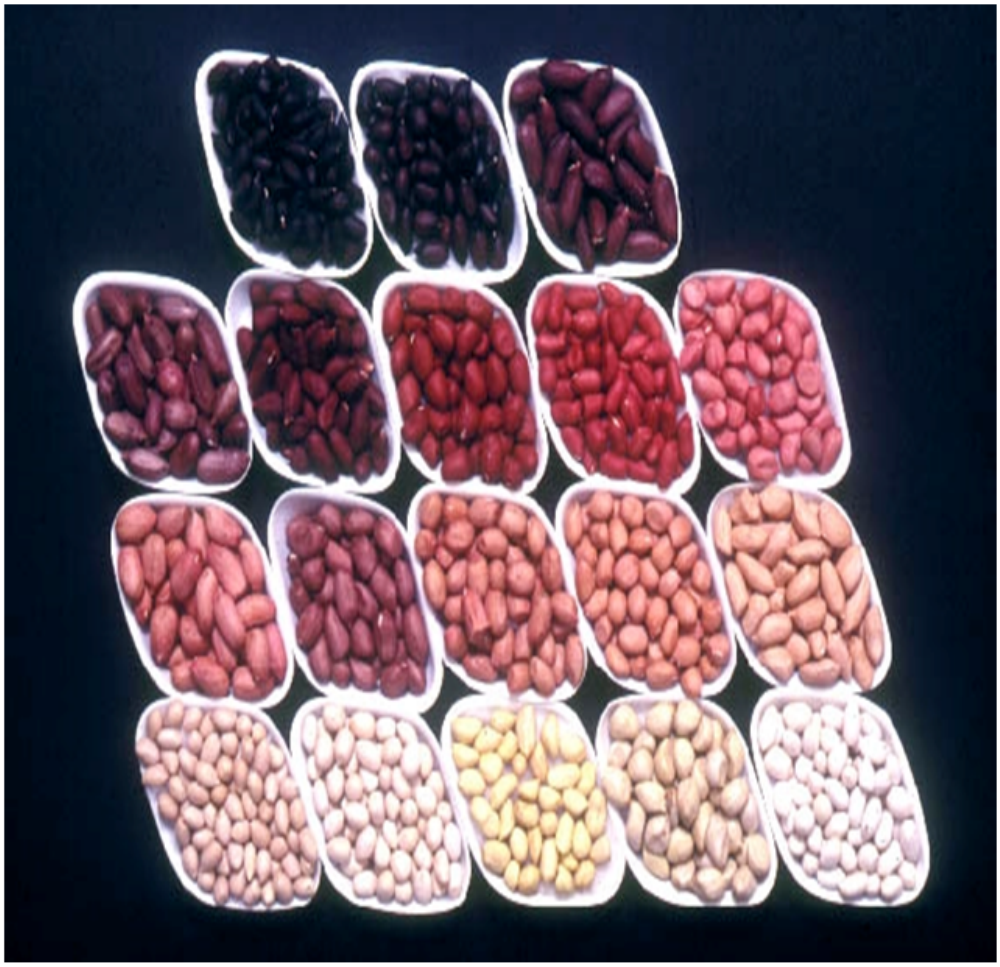
International Strategic Plan for the Peanut Genome Initiative

2008-2012

Improving Crop Productivity & Protection, Product Safety & Quality

Version 1.2 March 2008



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Executive Summary

Vision Statement: An integrated research approach will guide the effective development of trait enhancement technologies, disease management systems, genomic resources, and agronomic germplasm for profitable peanut production. Strategic deployment of these resources will help ensure the competitiveness of peanuts and peanut products in domestic and global markets.

Process & Development of the Strategic Plan: The second annual meeting of the international peanut research community entitled, *Advances in Arachis Through Genomics and Biotechnology: An International Strategic Planning Workshop* was held in Atlanta, Georgia on October 24-26, 2007. The conference was organized by the leaders of the Peanut Genomics Initiative in association with the Peanut Foundation, the International Crops Research Institute for the Semi-arid Tropics (ICRISAT), and representatives of three institutes in China (Shandong Academy of Agricultural Sciences, Henan Academy of Agricultural Sciences, and Guangdong Academy of Agricultural Sciences). Seventy-three participants included researchers with expertise in genomics, transformation technologies, genetics, plant pathology, food science, agronomy, entomology, and plant germplasm preservation. The international community was represented by scientists and administrators from South America (Brazil, Argentina), Asia (China and India), and Africa (Benin, Mali, Nigeria, Kenya, and South Africa). The workshop was sponsored by the American Peanut Council and The Peanut Foundation. Financial support was received from: Bayer CropScience Inc., the Georgia Peanut Commission, MARS Inc., J.M. Smucker Inc., the National Peanut Board, North Carolina State University, the Peanut Company of Australia, the Peanut Foundation, USAID Peanut Collaborative Support Program, USDA Agricultural Research Service (ARS), and USDA Cooperative State Research, Education and Extension Service (CSREES).

Genomics and biotechnology are new areas of science for peanut research. Indeed, Dr. Howard-Yana Shapiro, Global Director-Plant Science & External Research at MARS Inc. pointed out in his key-note address at the opening ceremony at the Carter Center that 'peanut' is conspicuously absent from the multitude of species represented in the programs for the Plant & Animal Genome Conference or related national or international meetings. Yet, knowledge of genome architecture is needed to facilitate the identification of useful DNA markers, genes, and peanut genotypes that mediate or harbor important traits, such as: resistance to tomato spotted wilt virus (TSWV), nematodes and preharvest aflatoxin contamination (PAC); tolerance to drought or water stress; and improved flavor and nutritional quality. Furthermore, genomic tools that identify genes controlling these traits will facilitate marker-assisted breeding and transgenic strategies for accelerating the selection of superior varieties. However, peanut researchers have not entered the genomics arena as a community. Dr. Shapiro elaborated further on the benefits of collaborative working relations and the need to establish an international peanut research community as a necessary step to leverage resources and gain visibility as a contender in the competition for genomics funding. This thyme was seconded by two additional key note addresses. Dr. Frank McGill, Professor Emeritus, University of Georgia, described major threats to the peanut industry and motivated participants to respond to problems with solutions, no matter how difficult the task; and Dr. Fuhe Luo, President, Guangdong Academy Agricultural Sciences (GAAS) reminded participants that *Advances in Arachis through Genomics* was a another committed step toward bringing elite members of the international peanut community together in a manner that fosters research collaboration on high priority issues. These presentations issued a mandate to build an interconnected framework for the application of genomics and biotechnology to major problems in peanut production in a transparent and accountable manner. Such action would effect the implementation of an integrated international peanut research network to help ensure an adequate supply of safe and nutritious peanuts for food, feed and fuel.

Accordingly, the objectives of the workshop were to:

1. provide a global forum for communication between stakeholders and the peanut research communities in the U.S., China, South America, India, Australia and Africa
2. draft a strategic plan to define and guide implementation of an integrated multi-disciplinary international peanut research program
3. initiate the development of credible processes for ensuring program relevance, quality and productivity, and
4. establish processes that help bridge the gap between R&D and commercial utilization of the fruits of genomic and biotechnology on an international scale.

These objectives were achieved by large measure throughout the workshop. The technical program for the workshop in Atlanta opened with presentations summarizing the status and scope of peanut research in Africa (Dr. Liezel Herselman, University of Free State), China (Dr. Xuan Qiang Liang, GAAS), India (Dr. Rajeev Varshney, ICRISAT), South America (Dr. David Bertioli, Catholic University & EMBRAPA) and the U.S. (Dr. Steven Knapp, University Georgia). These updates were followed by plenary papers on advances in legume crop genomics and plant transformation technology by eminent leaders in the cool and warm season legume research community. Topics included 1) sequencing cool and warm season legume genomes (Dr. Scott Jackson, Purdue University), the Legume Information Network (Dr. Greg May, National Center for Genomic Research), high throughput SNP discovery and genotyping (Dr. Perry Cregan, USDA-ARS, Beltsville, MD), evolution of legume defense genes (Drs. Varma Penmetsa and Doug Cook, University of California-Davis), and transformation and functional genomics of legume crops (Dr. Monica Schmidt, Danforth Plant Science Research Center). Plenary papers on relevant applications of these tools and their potential impact on the peanut industry were presented in the areas of marketing and trade policy (Dr. Andrew Rude, USDA-Foreign Agricultural Service), increasing the supply of peanut oil for food and biofuel feedstocks (Dr. Richard F. Wilson, Oilseeds & Bioscience Consulting), and mitigating food allergy and improving the nutritional value of peanuts and peanut products (Dr. Stacie Jones, University of Arkansas for Medical Sciences, Arkansas Children's Hospital).

Scientists and stakeholders then adjourned to concurrent sessions to develop a five-year strategic plan for integrating a broad range of scientific disciplines toward solutions of major consumer concerns on an international scale. Input captured by session facilitators helped define research priorities for what needed to be done, and established timelines for delivery of anticipated products in the following areas:

Production and Productivity. Major emphasis was placed on strategies to lower production costs for high quality peanuts. Specific objectives included: 1) use of elite genetic stocks and cultural practices to improve productivity, 2) management of soil fertility, irrigation and post-harvest handling, 3) cultural management practices to limit diseases and pests that lower productivity, and 4) improved plant water-use efficiency to alleviate drought and temperature stresses.

Product quality and safety. Major emphasis was placed on strategies to address issues that impact marketing and consumer preferences for peanuts and their products. Specific objectives included: 1) elimination of pre-harvest aflatoxin contamination, 2) management of immunological, nutritional, and digestibility properties of peanut protein, 3) enhancing levels of peanut constituents associated with health benefits, and 4) enhancing peanut composition for bioenergy applications.

Disease and pest management. Major emphasis was placed on multi-tactical and economical disease and pest management strategies for peanut. Performance measures included: 1) optimized fungicide and pesticide application schedules, 2) decision criteria for disease and pest management, and 3) improved understanding of the epidemiology of peanut pathogens.

Gene discovery and genome analysis. Major emphasis was placed on the development of genomic tools and technologies to identify genes that mediate the biological regulation of productivity, production, and quality traits. Specific performance measures included: 1) developing DNA sequence resources for characterization of genome structure, 2) determining genetic diversity and DNA polymorphism in genomes, 3) developing transcriptional tools and technologies for characterizing gene function, and 4) establishing bioinformatic resources and comparative genome analysis tools.

Genetics and germplasm enhancement. Major emphasis was placed on ensuring an adequate supply of agronomic and high-quality peanut cultivars for commercial production. Specific performance measures included: 1) enhanced understanding of genetic diversity and genomic variation in *Arachis*, 2) improved methods to develop genetic resources and useful traits, and 3) improved selection efficiency through use of mapping populations and high throughput genotyping methodologies.

Plant transformation technology. Major emphasis was placed on plant transformation technologies to better manipulate genetic traits in agronomic germplasm and for functional analyses of the peanut genome. Specific objectives included: 1) optimized peanut transformation and regeneration protocols, 2) improved and useful transformation vectors, 3) transgenic breeding lines with useful and stable traits, and 4) biotech risk assessment and mitigation strategies.

Based on this input the following individuals volunteered to draft the Strategic Plan for the International Peanut Genome Initiative:

Crop management & productivity
- Dr. John Burke, USDA ARS, Lubbock TX
- Dr. David Jordan, North Carolina State University
- Dr. Marshall Lamb, USDA ARS, Dawson GA

Crop product quality and safety
- Dr. Ed Cleveland, USDA ARS, New Orleans LA
- Dr. Tim Sanders, USDA ARS, Raleigh NC
- Dr. Art Weissinger, North Carolina State University

Disease and pest management
- Dr. Albert Culbreath, University of Georgia, Tifton GA
- Dr. Pat Phipps, Virginia Tech University, Blacksburg VA
- Dr. Yang-Rong Zhang, Guandong Academy Agricultural Sciences, Guangzhou CHINA

Gene discovery and genome analysis
- Dr. Steven Knapp, University of Georgia, Athens GA
- Dr. Rajeev Varshney, ICRISAT, Andhra Pardesh, INDIA
- Dr. David Bertioli, Catholic University & EMBRAPA, Brazilia BRAZIL

Genetics and germplasm enhancement
- Dr. Corley Holbrook, USDA ARS, Tifton GA
- Dr. Mark Burow, Texas Agricultural Experiment Station, Lubbock TX
- Dr. Boshou Liao, Chinese Academy of Agricultural Science, Wuhan CHINA

Plant transformation technology
- Dr. Beth Grabau, Virginia Tech University, Blacksburg VA
- Dr. Maria Gallo, University of Florida, Gainesville FL
- Dr. Juliette Chu, University of Georgia, Tifton GA

The product of their work, presented herein, addresses the following USDA research priorities:

Structural Comparison and Analysis of Crop Genomes where improved knowledge of legume genome architecture will facilitate the identification of a wide range of markers, genes, and genotypes influencing important traits such as disease and pest resistance, environmental stresses, and functional and nutritional quality will accelerate crop improvement.

Genetic Analyses and Mapping of Important Traits, where improved theory and the development of publicly available mapping populations will accelerate research by the legume research community. Newly identified genes and alleles controlling key traits will enable marker-assisted breeding and transgenic strategies for crop improvement

Germplasm Enhancement/Release of Improved Genetic Resources and Varieties, where enhanced varieties and germplasm will result in improved peanut productivity and quality to meet the needs of agriculture.

Applying Genomics to Crop Improvement, where gene discovery will provide new sources of enhanced traits for incorporation into breeding programs. New genetic strategies resulting from expanded genomic knowledge will accelerate the enhancement of crop productivity and the exploitation of nutritional and healthful properties of foods; and,

Improving and Assessing Genetic Engineering Technology, where accomplishments will establish a basis for interpreting variability in global gene and protein expression, as well as shifts in metabolism associated with biotechnologically derived traits. New procedures will expand the scope and reduce the cost of genetic engineering, making it useful for peanut.

This plan serves as the foundation for the International Peanut Genome Initiative, a collaborative effort that strives to develop a better understanding of the peanut genome structure, and to apply advanced genetic tools and strategies to enhance peanut productivity, increase crop protection from diseases and pests, and improve peanut product safety and quality. This plan represents a corner stone of a process for ensuring the relevance of peanut research, and the following list of milestones provides a timeline for the delivery of anticipated products which will facilitate assessment of program progress toward the accomplishment of the PGI mission.

An executive committee was selected to establish and administer governance for the Initiative; the elected members are:

Drs. Fuhe Luo, Jin-Rong Zheng, and Xuan Qiang Liang (China)
Drs. Farid Waliyar, David Hoisington, and Farid Varshney (India)
Drs. David Betioli (Brazil) and Guillermo Seijo (Argentina)
Drs. Liezel Herselman (South Africa), Ousmane Ndoye (Senegal), and Sansui Mohammed (Nigeria)
Drs. H.T. Stalker, Howard Valentine, Victor Nwosu and Richard F. Wilson (U.S.)
Dr. Robert Henry (Australia)

Coordination among international peanut research communities, plus interaction with other legume genomics communities such as *Glycine max* and *Medicago truncatula*, should effect better utilization of limited resources available for research. Establishing collaborative working relations across geographic areas also will enable timely solutions that are needed to ensure an adequate future supply of safe and nutritious peanuts for food, feed and fuel applications.

The strategic plan is posted on the website http://www.peanutbioscience.com.

Milestones for Peanut Research

PM	Performance Measure	Target in 2008	Target in 2010	Target in 2012
1.1	Optimize the use of elite genetic stocks and cultural practices to improve productivity	States employ USDA extension programs on peanuts to provide information to producers	Establish, maintain and augment adequate seed testing programs	Improve seed testing programs and storage
1.2	Develop strategies for management of soil fertility, irrigation and post-harvest handling	Field studies evaluate the role of soil and climatic variables on peanut productivity, flavor, maturation and shelf life	Implement improved practices for curing harvested peanuts, seasonal timing of crop irrigation, and use of early maturing varieties to preserve flavor quality	Commercial adoption of recommended practices
		Survey genotypic variation in flavor attributes. Develop improved curing protocol. Associate off-flavors with immature peanuts	Establish capacity to test and refine methods for analyzing flavor attributes of peanut and peanut products. Develop early maturing productive germplasm	Release agronomic conventional and GM varieties with enhanced sensory properties and uniformity for food product applications
		Sofware programs designed to assist farmers in developing whole farm plans, managing peanut irrigation, determining optimal harvest date and managing peanut curing	Develop expert decision aid software for managing non-irrigated peanut production	Integrate decision tools for other crops in rotation with peanut production
1.3	Optimize cultural management practices to limit disease/pest induced crop losses	Assess impact of IPM strategies for disease and pest control in peanut production	Determine the interaction of multiple variables on the effectiveness of cultural management plans for limiting disease severity	Develop customized comprehensive cultural practice recommendations for individual production conditions to limit disease severity and yield loss
		No herbicide resistant peanuts. Emergence of volunteer herbicide tolerant crops in peanut production	Develop IPM systems and strategies for controlling and removing secondary pests, including weeds and insect vectors of peanut pathogens	Enhance IPM strategies for implementation of identity preserved systems. Implementation of organic peanut production systems
1.4	Determine the basis for genotypic differences in water-use and drought/temperature tolerance	Characterize water use efficency of commercial peanut varieties. Discover protease involvment in temperature stress tolerance	Use crop coefficients for water-use under various cultural management systems to improve irrigation scheduling. Molecular genotyping and characterization of peanut germplasm collections	Establish relations between abiotic and biotic stresses and physiological processes among different peanut genotypes
2.1	Eliminate pre-harvest aflatoxin contamination in peanut	Identify genetic resistance, and evaluate germplasm lines for PAC resistance	Develop DNA markers for PAC resistance, use in MAS breeding program. Develop GM peanuts with anti-fungal resistance. Determine influence of GxE, drought and disease/pest interactions on PAC	Release conventional varieties with PAC resistance. Incorporate effective GM traits for anti-fungal resistance in breeding programs
		Biocontrol products such as AflaGuard available for reduction of preharvest aflatoxin contamination	Evaluate new nontoxigenic strains of Aspergillis species and implement commercial use of agents such as AflaGuard	Validate new products and implement commercial use
		Efforts focus on different *Aspergillis* species for DNA sequencing and phylogenetic analyses to identify informative DNA sequences. Pathogen microarrays developed for *Aspergillis* species. No transformation system for *Aspergillis* species. RNAi technology developed for *Aspergillis* species	Develop DNA markers to distinguish strain/isolates of *Aspergillis* species. Establish molecular population studies. Develop fungal/plant gene multispecies microrrays to identify pathogen candidate genes. Develop transformation systems for *Aspergillis* species. Perform proteomic studies to identify mechanisms of resistance	Develop models to predict the rate of *Aspregillis* species invasion and biocompetition in peanut. Incorporate pathogen sequence data into peanut microarrays. Use microarrays and other gene expression techniques to identify genes responding during peanut pathogen interactions. Improve transformation rates for Aspergillis fungi. Develop annotated proteomic maps of *Aspergillus* species, link with transcriptomic/metabolomic data to define defense pathways
2.2	Manage immunological, nutritional and digestibility properties of peanut protein	Identify peanut proteins with allergenic potential. Initate investigation of the nature of biological regulation of storage protein synthesis in peanut	Develop GM germplasm with stable mutations in gene families that encode proteins with allergenic potential. Develop quantitative model in swine for evaluating the impact of direct and compensatory changes in protein composition on digestibility and nutritional value	Develop and evaluate modified germplasm for enhanced protein nutritional value, desired functional properties, and reduced immunological response. Maintain swine model as a resourse for studying human allergy
		Support the Food Allergy & Anaphylaxis Network (FAAN). Clinical studies reveal molecular indicators of anaphylactic response in sensitized individuals	Develop educational programs on peanut allergy. Determine digestibility and kinetics of peanut protein absorption into the blood stream. Define regulatory mechanisms for immunological response to peanut protein	Expand coverage of educational programs on peanut allergy. Evaluate nutritional value of peanuts and peanut products with modified protein composition
		Determine effect of roasting and frying on the allergenic potential of peanut proteins	Determine the impact of processing treatmens on the physical chemistry of protein structure and function	Design processing methods to reduce sensitization and severity of reaction to potential peanut allergens

Milestones for Peanut Research

PM	Performance Measure	Target in 2008	Target in 2010	Target in 2012
2.3	Enhance levels of peanut constituents associated with health benefits	Identify potential health benefits of various nutraceutical and antioxidant compounds in peanut. Initiate evaluation of secondary metabolite composition among peanut germplasm	Determine the biological and genetic regulation of folate and other antioxidants. Initate genetic studies on trait inheritance. Establish state-of-art analytical facilities for peanut constituents	Use selected conventional and GM germplasm for clinical evaluations of enhanced health benefits
2.4	Enhance peanut for bioenergy applications	Incorporate a trait for high oleic acid concentration in peanut oil into new cultivars. Identify genes that mediate the trait	Develop CAPS markers for genes and alleles that encode w-6 desaturates in peanut. Deploy CAPS markers in MAS breeding programs	Release conventional and GM germplasm for production of low-trans fat ingredients for dietary foods
		Screen germplasm collections for genetic variation in oil concentration. Identify biological mechanisms and putative genes that mediate the trait	Develop CAPS markers for genes and alleles that encode DGAT and relevant glycerolipid synthetic enzymes in peanut. Deploy CAPS markers in MAS breeding programs	Release conventional and GM germplasm for increased vegetable production for food and industrial applications
3.1	Optimize fungicide & pesticide application to maximize profitabilty of peanut production	Chemical control of diseases and pests is efficacious but expensive; Peanut cultivars often lack resistance; Chemical control products are available and performance and technical attributes are well known. Define and evaluate labeled products	Determine the efficacy of labeled products for disease/pest management. Evaluate product application scenarios including rates, residual activity and comparisons of sequential applications and mixtures of fungicides, insecticides, and herbicides. Develop educational programs on chemical control strategies in peanut production	Monitor pathogen/pest populations for resistance to chemical control programs. Evaluate new products. Develop a regionally based disease management strategy. Update educational materials on chemical control agents use and information on disease severity and yield losses
3.2	Define decision criteria for disease and pest management	Quantify disease, environmental, and economic thresholds; translate model outputs into decision criteria using data from literature on disease, environmental, and economic thresholds	Establish databases for predictive models for various management scenarios. Validate predictive models. Improve translation of model output into decision criteria using quantitative data on disease, environmental, and economic thresholds	Deliver tested Web-based decision support system to stakeholderx, integrating data from climatological, atmospheric transport, epidemiology, and socio-economic models with decision criteria for disease management to predict risk/benefit over multiple temporal and spatial scales
3.3	Improve understanding of peanut pathogen epidemiology	Evaluate greenhouse and field experiments for the effect of temperature and relative humidity conditions on diseases such as TSWV, *Sclerotinina* spp and pests such as root knot nematode. Develop understanding of spatial distribution of epidemics in fields	Elucidate the impact of interrupted leaf wetness periods on the onset of S. sclerotiorum epidemics. Conduct field experiments to elucidate the impact of rain, temperature, inoculum density and solar radiation on disease development. Develop preliminary models with predictive capability that could be used in a disease warning system	Continue development of models with predictive capability that could be used in a disease warning system. Incorporate other cultural practices as factors in the predictive model. Continue evaluation of the association between weather variables and epiphytotics of S. sclerotiorum. Start field validation of models
4.1	Develop DNA sequence resources for characterization of peanut genome structure	Submit 150,000 ESTs plus chromatograms to GenBank	Generate EST derived microarrays to identify gene families for specific traits, and evaluate compensatory effects of gene silencing on peanut productivity, protection or quality	Evaluate expression of genes and gene families in germplasm with multiple directed mutations for selected traits. Develop specific DNA markers for desired traits
		Establish standard genotypes that represent A, B, and AB genomes. Determine A-genome space. Characterize Florunner BAC library for gene-rich BACs	Sequence whole A-genome. Define B-genome gene space. Define AB-genome gene space. Anchor BACs to genetic map	Sequence whole B-genome. Sequence AB genome. Develop genetically, cytogentically and physically integrated BAC-based maps
4.2	Determine genetic diversity and DNA polymorphism in peanut genomes	2,000 SSR markers mapped in reference genomes. High-Quality reference A-genome reference map	3,000 additional mapped SSR markers; 800 mapped SSR markers in cultivated peanut	Develop comparative genetic analyses with other legumes and wild peanut species
		Discover and validate 3,072 SNPs in cultivated peanut	Discover and validate 9,216 SNPs in Cultivated Peanut	Minimum 10,000-16,000 validated and genetically mapped SNPs
		Prepare for use of SNP markers in MAS	1,000-2,000 high-throughtput SNP markers for applications in breeding programs	1,000-2,000 additional high-throughtput SNP markers for applications in breeding programs
4.3	Develop translational tools and technologies for characterizing gene function	High-density proteome map of mature peanut seed with peptides that represent abundant seed proteins. Characterize allergen protein isoforms	Proteome and transcript atlas for multiple tissues and development stages exposed to biotic and abiotic stresses	Develop resources for candidate gene discovery and association with metabolic function
		Initate TILLInG and reverse-genetic resources for random mutations in genes	Establish facility for high-throughput TILLInG resource in peanut	Maintain TILLInG resourse
		Employ RNAi protocol for targeted gene mutations and characterize gene function	Use of Zinc finger nucleases and other gene targeting techniques to determine function of expressed gene sequences	VIGS-mediated protocol for producing stable mutations in genes and non-transgenic gene insertion

Milestones for Peanut Research

PM	Performance Measure	Target in 2008	Target in 2010	Target in 2012
4.4	Establish bioinformatic resources and comparative genome analysis tools for peanut	Map linkage & physical data in LIS. Port metadata into CMAP for comparison of genetic maps between peanut and other legumes. Establish automated linkage of sequence-based markers to EST & genomic data in LIS	All public sequences (EST and genomic), expression array and protein information from peanuts migrated to LIS and fully supported with comparative analysis tools in a web-enabled environment	Evolution of the Legume Information Network and the Virtual Plant Information Network
		Initiate enhancement of peanut transformation protocols	Migration of peanut transformation and regeneration protocols, sequences, results and educational materials in LIS	Migration of transformation vectors, GM plant material and biorisk databases for public access in LIS
5.1	Enhance understanding of genetic diversity and genomic variation for important traits in Arachis	Deploy genomic tools for identification of gaps in the collections, and phylogenetic study of accessions and species	Initial SSR characterization of subsets of collections, and cytogenetic characterization of section Arachis	BAC-FISH correlation of genetic and physical maps, characterization of Procumbentes and Erectoides
		Deploy genomic tools for determining population structure LD and association mapping.	Develop capacity for genotypic and phenotpic analysis of breeding populations for specific and general traits	Develop capacity for genotypic and phenotpic analysis of germplasm collections for specific and general traits
		Genetic analysis (diallels, Generation means, etc.) for traits of interest	Determine complexity of trait inheritance, influence of environment on trait expression, and germplasm release	Field increase and evaluation of selected germplasm for enhanced variety release
5.2	Improve methods to develop genetic resources with useful traits	Develop bioinformatics tools to identify genes and germplasm possessing specific genes	Identify trait-specific genetically-diverse germplasm	Utilization of marker trait association in germplasm following association mapping
		Enhance pre-breeding activities to introduce genes into cultivated peanuts	Identification of hybrids with desirable gene combinations for breeding programs, and polyploidization	Introgression of desired traits in cultivated peanut
5.3	Improve selection efficiency through use of genomic resources	Initiate mapping populations for selected qualitative and quatitative traits	Validation of markers and utilization in enhancing QTL discovery	Implementation of marker-assisted-selection for agronomic and quality traits
6.1	Optimize peanut transformation and regeneration protocol	Develop biolistic and *Agrobacterium* methods for peanut	Standardized, semi-high throughput transformation adopted	Target highest priority traits: aflatoxin, drought resistance, disease resistance
6.2	Develop improved and useful transformation vectors	Investigate possibilities	Identify tissue specific promoters, optimize vectors for functional genomics studies	Adopt a validated common set of transformation vectors with IP free sequences for expressiion
6.3	Develop transgenic breeding lines with useful and stable traits	GM peanut germplasm expressing an ox-ox gene, a chitinase, a phamaceutical protein and others	Field tests of GM peanut lines with antifungal properties	Field tests of GM peanuts with various protection and quality traits
6.4	Develop biotech risk assessment and regulatory compliance strategies	Prepare transgenic peanuts for field evaluation	Submit at least one US and one international petition for regulatory approval. Develop the capacity for international exchange of transgenic materials	Streamline the process for international exchange of transgenic peanut materials, navigating the deregulation process

Introduction

Cultivated peanut (*Arachis hypogaea* L.) is grown on 21 million hectares world-wide, predominantly in Asia, Africa and the Americas. The estimated current economic value of world peanut production is about $35 billion. Peanut is distinguished with high oil content, more than 50% of seed dry mass, and a large percentage of oleic acid which confers superior oxidative stability and enables use in food products without processing steps involving hydrogenation. These traits give manufacturers greater flexibility in formulating nutritious foods with lower levels of *trans*-isomers. Peanut oil also is low in saturated fat content, another trait that has been shown to lower serum LDL-cholesterol levels. In addition, endogenous nutriceuticals, such as resveratrol, also may improve cardiovascular health.

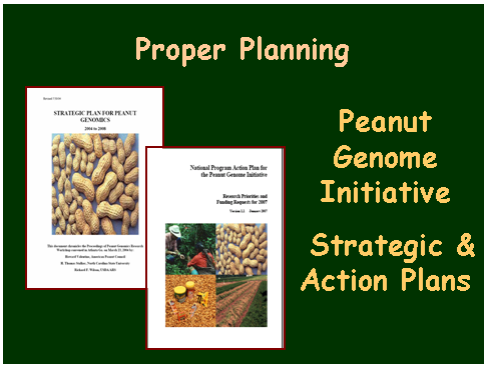
Although peanut is important economically and as a source of nutrition, it has not been studied extensively at the genomic level. Peanut has a large 2,800 Mb genome, comparable in size to the human genome. Peanut is a polyploid species, where duplicate sets of chromosomes make genetic analysis difficult. Slow progress in overcoming these factors has impeded the development of genomic tools to help decrease production costs and improve health concerns related to product consumption. Among the highest priorities is developing large numbers of user-friendly genetic mapping tools, sequencing DNA from populations of diverse tissues and genotypes, assembling a genetically-anchored physical map, aligning this map to related legume crops, and characterizing gene-rich regions to discover genes that influence peanut quality, nutritional value and productivity.

Investment in peanut genomics continues to lag other crop species, but efforts in related cool and warm season legumes are expanding both in the U.S. and abroad. The importance of coalitions among legume communities is defined by a 2001 meeting in Huntsville, MD where scientists first developed a strategy to advance genomics across legume species. The product of that conference, the *U.S. Legume Crops Genomics White Paper*, outlined six research needs: (i) genome sequencing of strategic legume species, (ii) physical map development and refinement, (iii) functional analysis, (iv) development of DNA markers for comparative mapping and breeding, (v) characterization and utilization of legume biodiversity, and (vi) development of legume data resources. Another meeting was convened in Santa Fe, NM to refine objectives and to develop a more definitive white paper for legume research entitled, *Legumes as a Model Plant Family: Genomics for Food and Feed*. This plan focused genomic resources toward crop protection and crop product quality; and served as the impetus for the development of genome initiatives for peanut and each of the other legume crops.

Development of the Peanut Genome Initiative. The market competitiveness of peanuts is threatened by losses in productivity and quality attributed to diseases, pests, environmental stresses and allergy or food safety issues. The germplasm repositories and gene banks maintained by the USDA-ARS National Plant Germplasm System (NPGS) and the Consultative Group on International Agricultural Research (CGIAR) typically provide the first line of long-term defense against those problems. In the United States, 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 and biotechnological 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.

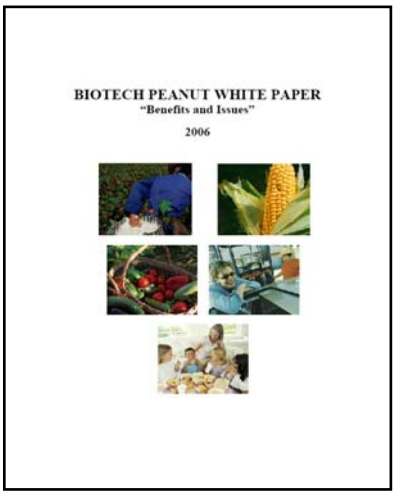
However, to realize such ability, it was necessary to establish an infrastructure for genomic research with a coordinated research approach to guide the effective development of peanut germplasm, genetic tools and bioinformation. On 22-23 March 2004, 26-scientists with expert knowledge of critical fields 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 (LCGI). In affiliation with LCGI and other stakeholders, the Peanut Genome Initiative (PGI) was launched at this workshop. An advisory committee, representing the broad interests of industry and the peanut research community, was selected to guide the growth of the PGI. A *Strategic Plan for the Peanut Genome Initiative 2004-2008 (v2.4)* was developed that outlined research goals objectives, performance measures and significant near-term milestones representing 'quantum leaps' in the advancement of this emerging science.



With stakeholder input, research priorities were identified and aligned with the goals or components of the PGI Strategic Plan. On June 28, 2004, the advisory committee charged individuals to initiate team building toward achieving all performance measures for each Component, and tasked a writing team to develop an Action Plan that defines those performance measures of the Strategic Plan that addressed initial high priority research program needs. The *National Action Plan for the Peanut Genome Initiative: Application of Plant Genomics to Mitigate Peanut Allergy* was adopted by the peanut research community at the American Peanut Research & Educational Society (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-2.4, March 2006).

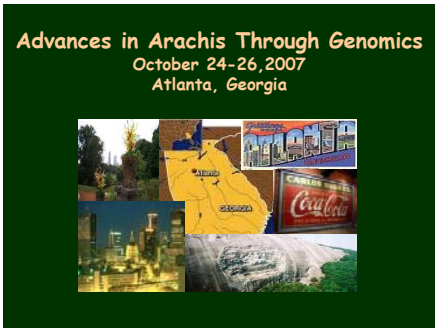
Recently, the US peanut industry agreed that all available methods should be used to reduce cost of production and improve peanut quality. For example, promising experimental bioengineered peanuts have been available since 1989, but none have been advanced to commercial production. This technology was re-evaluated by the U.S. peanut industry and their assessment was presented in the "*Biotech Peanut White Paper: Benefits and Issues*" at the winter meeting of the American Peanut Council, December 8, 2006 in Atlanta GA. During the discussion, it was recognized that this technology could lead to significant improvements in the cost of peanut production, nutrition and overall product quality. Biotechnology also could provide the industry with greater flexibility to bring new traits to the market more rapidly, and to resolve seemingly intractable problems such as peanut allergy. Considering these and other factors, the industry reached consensus that with proper funding bioengineered peanuts could be commercially available in the U.S. in 5-7 years. Accepting this challenge, PGI elaborated its Action Plan with emphasis on initial steps that will enable the timely development and delivery of bioengineered peanuts to the U.S. industry with the document, *Research Priorities & Funding Request for 2007 (v 1.3 July 2007).*



In 2006, the PGI sought to expand its mission through outreach to the international peanut research community. The foundation for this effort was established in November 2006 in Guangzhou, PRC at the "International Conference on Aflatoxin Management and Genomics" when delegates from nine nations voted to maintain an open dialog to explore opportunities for cooperative research, and to take steps toward achieving that goal with annual meetings. A proposal was accepted to host the second conference of the international peanut research community on October 24-26, 2007 in Atlanta GA. That meeting, *Advances in Arachis through Genomic &*

Biotechnology: An International Strategic Planning Workshop, was another committed step toward bringing elite members of the international peanut community together in a manner that fosters research collaboration on high priority issues.

The *International Strategic Plan for the Peanut Genome Initiative 2008-2012: Improving Crop Productivity & Protection, Product Safety & Quality*, a major product of the Workshop in Atlanta, addresses six Goals, developed with stakeholder input, that define the rationale and scope of the research strategy to enhance peanut productivity; increase protection against diseases, pests and stresses; and to improve crop product safety and quality. The performance measures (research objectives) under each Goal state the problems that will be addressed, and anticipated products suggest what will be done to meet the objective. In addition, periodic milestones set for each performance measure constitute a 'yardstick' or performance plan by which research progress may be measured. Annual accomplishments by U.S. and international collaborators will be reported with reference to the performance measures addressed.



This strategic plan establishes a basis for project and grant proposal development, and implementation of an internationally coordinated peanut research program for the next 5-years. Coordination among international peanut research communities, plus interaction with other legume genomics communities such as *Glycine max* and *Medicago truncatula*, should effect better utilization of limited resources available for research. Establishing collaborative working relations across geographic areas also will enable timely solutions that are needed to ensure an adequate future supply of safe and nutritious peanuts for food, feed and fuel applications.

Information on the Peanut Genome Initiative may be accessed at:

http://www.peanutbioscience.com

Strategic Research Goals & Performance Measures for the Peanut Genome Initiative

Crop Management & Productivity

Peanut is fourth in world oilseed production at about 32 million metric tons (MMT) on about 21 million hectares (Mha). China leads global regions in peanut production followed by India, African nations, SE Asia, the U.S.A., and South America. Average peanut yields range from 2.7 to 3.3 MT/ha in the U.S.A, China and S. America to 0.9 to 1.4 MT/ha in India and African nations. Average farm prices for U.S. peanuts averaged about $390/MT in 2006/07. However, operating and allocated overhead costs often exceed the total gross value of production. Increased productivity is needed to ensure adequate supplies to meet greater global demand for peanuts in food, feed and fuel applications; and to lower costs to help sustain profitable peanut production.

Goal 1: Develop strategies to lower production costs of high quality peanuts.

Performance Measures:

1.1 Optimize the use of elite genetic stocks and cultural practices to improve productivity. Large-scale field demonstrations in multiple environments under conventional and experimental systems help establish and implement best management practices for peanut production. Seed testing helps improve germination and seedling vigor. Yield testing helps evaluate the respective abilities of elite genotypes in commercial production environments.

Anticipated Products

- Effective testing programs for seed quality and variety performance
- Best management practices for land preparation, seed placement, stand establishment and crop rotation

1.2 Develop strategies for management of soil fertility, irrigation and post-harvest handling. A majority of global peanut production is non-irrigated. Poor yields often result from inadequate moisture, soil erosion, and poor soil fertility. Peanut flavor quality often is reduced by inadequate post harvest handling and curing of immature kernels. Adequate analytical facilities for flavor attributes and chemical constituents are essential for improvement of management practices for peanut production. Management decision aids are essential for successful implementation of best practices.

Anticipated Products

- Expert decision-aids for managing non-irrigated peanut production
- Best management practices for improved soil fertility, irrigation, soil erosion control and post-harvest handling of peanuts
- Agronomic performance data for early-maturing varieties in all market-types

1.3. Optimize cultural management practices to limit pest induced crop losses. Nematodes, weeds and insect vectors of pathogens often facilitate the spread of disease epidemics, such the role of thrips in disseminating TSWV. Weed control is impeded by the emergence of herbicide-resistant biotypes. IPM strategies are needed to mitigate these biotic stresses without compromising product quality in conventional, no-till and organic peanut production systems.

Anticipated Products:

- IPM strategies for controlling weeds and insect vectors of peanut pathogens
- Improved tillage systems with reduced chemical control agent inputs
- Organic peanut production systems with superior product quality

1.4. Determine the biological basis for genotypic differences in water-use and drought/temperature tolerance.

Inadequate precipitation and high-temperature stresses not only reduce crop productivity but also mediate increased incidence of infection by pathogens and toxigenic organisms. Genetic variation for physiological differences in water-use and heat tolerance has been observed among peanut genotypes. Knowledge of the biological mechanisms that effect these traits is needed to develop elite cultivars for dry-land peanut production..

Anticipated Products:

- Decision aids for irrigated and dry-land production customized for specific varieties
- Molecular genotype maps of germplasm collections for abiotic stress tolerance genes
- Knowledge of principle elements of the molecular mechanisms regulating the response of peanuts to temperature stress

Potential Benefits: Improved management practices will restore depleted soil nutrients and enable sustainable production systems. Use of elite germplasm and proper cultural practices will significantly improve product quality by reducing potential contamination by excessive pesticide applications, improving use of available water resources, lowering the incidence of biotoxins, and protecting peanut flavor attributes during post-harvest handling.

Product Quality & Safety

The competitiveness of peanut producers in global markets is threatened by losses in product quality that are attributed to food safety and human health issues. In addition, increased demand for vegetable oil in industrial and bioenergy applications threaten adequate peanut supply for food products. The infrastructure for future advances in peanut research to resolve each of these important issues should encumber all aspects of relevant practical, basic, and clinical research in an integrated approach.

Goal 2: Integrated research strategies for major issues that impact global marketing and consumer preferences for peanuts and peanut products

Performance Measures:

2.1 Eliminate pre-harvest aflatoxin contamination in peanut. The presence of mycotoxins such as aflatoxin in peanut products threatens the competitiveness of the peanut industry in the world export market because of stringent threshold limits of acceptability. Impeding the infection of pre-harvested peanuts by *Aspergillis* species is an important step in reducing aflatoxin contamination. Integrated research efforts are needed to achieve that objective. Rapid and affordable chemical toxin identification and quantitation are the basis of both industry and regulatory food safety assurance activities. Understanding of fungal/crop/environment interactions during both fungal and plant growth and maturation is necessary to develop effective pre- and post-harvest crop management practices including use of rotation crops. Both genomic and proteomic tools and resources are needed to guide traditional breeding, marker assisted selection and/or genetic engineering to develop aflatoxin-resistant varieties. Biocontrol technologies that use competitive exclusion to prevent aflatoxin in peanuts are needed to augment genetic resistance and chemical control measures for long-term suppression of aflatoxin contamination by *Aspergillis* species.

Anticipated Products

- PCR based tests including microarrays to rapidly identify mycotoxigenic fungi in contaminated peanut and peanut products
- Cultural crop production and handling practices that can assist in the reduction of pre-harvest aflatoxin contamination (PAC)
- Decision aids to provide useful predictions for mycotoxin occurrence
- PCR based tests (including microarrays) to determine biological and physiological function of unique fungal genes
- DNA markers for marker assisted selection of PAC resistant peanut germplasm
- Atoxigenic or modified biocontrol organisms that do not produce aflatoxin
- PAC resistant peanut germplasm and varieties

2.2 Manage immunological, nutritional and digestibility properties of peanut protein. Peanut allergies are reported by more than 4 million Americans and are becoming an increasingly serious public health and food safety issue, especially for affected children. Fatal reaction may occur in severely allergic individuals. There is no cure for peanut allergy, and it is difficult to avoid foods with peanut-ingredients. Poor digestibility and immunological attributes of certain seed proteins are suggested to be causal factors of peanut allergy. Integrated research and educational efforts are needed to mitigate the incidence and severity of peanut allergy, and to improve the nutritional value of peanut meal. Development of agronomic peanut varieties with modified protein composition may provide a solution to this problem. Genomic tools and technologies are needed to elucidate expression of gene families that govern composition and concentration of peanut proteins, and to provide useful DNA markers for MAS breeding programs. 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. Clinical studies are needed to determine immunological threshold levels of absorption of natural and genetically modified proteins or peptides into blood serum, study mechanisms of sensitization and develop potential vaccines. Educational efforts are needed to maintain transparency and provide consumers with credible decision making information.

Anticipated Products:

- Tools for modern molecular immunological and physiological measurements of peanut allergy response in pigs
- Molecular strategies to identify peanut genes with large effects on the allergic response in sensitized humans
- Immunoassays for improved detection of compensatory changes in protein composition in genetically modified peanut 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 randomly induced and targeted mutations in potential allergen genes
- Estimates of the threshold doses for peanut sensitized individuals
- Germplasm with meal exhibiting enhanced digestibility and nutritional value
- Vaccines and therapeutic remedies for immunological response to peanuts
- Advanced media networks for consumer education

2.3 Enhance levels of peanut constituents associated with health benefits. In addition to a high level of monounsaturated fatty acids in peanut oil, peanuts feature an array of other nutrients that have been shown to promote heart health. Peanuts are good sources of vitamin E, niacin, folate, protein and manganese. Peanuts also are a source of *resveratrol*, the phenolic antioxidant found in red grapes and wine. An integrated research effort is needed to enhance levels of these nutraceuticals and antioxidants in peanut. Analytical facilities are needed for characterization of genetic variation in bionutrient levels among peanut germplasm. Breeding studies of trait inheritance are needed to guide investigation of genes regulating relevant metabolic pathways. Clinical studies are needed to establish the impact of these compounds in reducing reduced risk of cardiovascular disease and other human health maladies.

Anticipated Products:

- Quantitative databases for bionutrient levels among peanut germplasm
- Agronomic peanut varieties with optimal levels of bionutrients
- Clinical verification of the health benefits of bionutrients in peanut

2.4 Enhance peanut composition for bioenergy applications. Foreign Agricultural Service estimates current global use of peanut oil consumes 99+% of global supply. Because of the emerging market for biodiesel, a deficit in supply of all vegetable oils is projected by 2020. 2020. Greater use of oilseeds for industrial and bioenergy applications threatens ability to meet oilseed demand for food products. Peanut can play an important role in mitigating this situation, in two ways, by genetic modification of oil concentration and oil composition. An integrated research effort is needed to develop agronomic varieties with increased oil concentration to achieve over one ton of peanut oil production per acre, and varieties with increased oleic acid concentration for low-saturated trans-fat free foods, and biodiesel with improved ignition properties and lower-NOx emmisions.

Anticipated Products:

- Quantitative characterization of germplasm collections for genetic variation in oil and fatty acid composition
- Molecular markers for genes and alleles that govern fatty acid and glycerolipid synthesis in peanut

- Germplasm and varieties with enhanced oil quality traits

Potential Benefits:

Varieties with PAC resistance will help ensure the safety and marketability of peanuts and peanut products. Quantitative model systems for evaluating the human response to peanut proteins will provide clinical researchers with resources 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. Enhanced bionutrient levels could enable health claims in the marketing of peanut products. Enhanced oil quality traits could help capitalize on the increased economic value of peanuts in an energy driven environment.

Disease & Pest Management

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, peanut rust, white mold and leaf spot. Development of elite cultivars and implementation of profitable production systems requires establishment of improved crop management practices and knowledge of disease/pest/host/environmental interactions. In lieu of genetic resistance, fungicides and chemical control measures are a first line of crop protection and account for a substantial of operating costs in commercial peanut production. Useful criteria are essential for the development of decision aids for economically sound management of pests and pathogens in commercial production.

Goal 3: Develop multi-tactical & economical disease & pest management strategies

Performance Measures:

3.1 Optimize fungicide & pesticide application schedules in peanut production. Fungicides and pesticides are available and their performance in disease epidemics is documented. However, there is little guidance on how these chemical control agents may be used to achieve the greatest economic returns. Fungicide and pesticide application scenarios are needed to evaluate and determine optimal timing of application, residual activity and curative properties of the agents, as well as the interactions of the fungicides with adjuvants, herbicides, insecticides and other fungicides in the presence or absence of pathogens.

Anticipated Products:

- Educational programs for chemical control of peanut pathogens and pests
- Evaluation of new labeled products
- Documentation of production losses attributed to diseases and pests
- Monitoring of pathogen/pest populations for resistance to control agents

3.2 Define decision criteria for disease & pest management. Current guidelines for effective use of fungicides and pesticides are not entirely based on environmental or economic thresholds. Databases are needed to support and validate predictive models for various management scenarios. Decision aids should facilitate proper timing of applications and avoidance of excessive applications that may be inefficient, unneeded and costly.

Anticipated Products:

- Decision aids for chemical control of peanut pathogens and pests
- Risk-benefit decision models for specific geographic regions
- Environmentally sound practices that reduce pesticide residues in peanuts

3.3 Improve understanding of the epidemiology of peanut pathogens. Environmental conditions play an important role in establishing pathogens such as *Sclerotinia* spp. in disease nurseries. Information on effects of specific temperatures on urediniospore germination, germ tube growth, penetration and early pathogen establishment during nighttime dew periods should be determined for specific isolates. Information on the effects of temperature, moisture, and light on pathogen longevity, over seasoning, sporulation, inoculum dissemination, and urediniospore transport also are needed to make informed decisions on disease control strategies. Comparison of old and new isolates of pathogens may provide useful information for the development of scientifically valid prediction models.

Anticipated Products:

- Effective pathogen inoculation methods for field and greenhouse experiments
- GPS-based disease and pest warning system

Potential Benefits: Economic and environmentally sound chemical control strategies for peanut pathogens and pests. Improved methods for determining weaknesses in pathogen and pest life cycles. Predictive models for an early-warning protection network.

Gene Discovery & Genome Analysis

The nuclear genome of cultivated peanut contains approximately 3 billion base pairs, and is similar to the size of the human genome. The peanut genome may contain about 50,000 genes. Analysis of gene-rich genomic regions should lead to genomic maps, gene markers, expressed gene microarrays and other technologies that help capitalize on the full genetic potential of peanut as a healthful and profitable crop for food, feed and fuel applications.

Goal 4: Genomic tools and technologies to identify genes that mediate the biological regulation of productivity, protection and quality traits.

Performance Measures:

4.1 Develop DNA sequence resources for characterization of peanut genome structure. Sequencing cDNA transcribed from expressed sequence tags (EST) is an efficient approach for gaining information on genome structure in peanut. EST derived microarrays can be used to identify alleles within and among members of gene families for genetic traits. Genome sequence analysis of diploid progenitors may accelerate progress toward a complete picture of the tetraploid genome. BAC libraries for diploids *A. duranensis* and *A. ipaensis* will facilitate construction and proper alignment of physical maps with genetic, cytogentic and transcript maps from standard peanut genotypes.

Anticipated Products

- Useful EST libraries from specific peanut organs exposed to various environmental/ experimental conditions during various stages of plant development
- Microarrys representing a full complement of unigenes for characterization of candidate genes governing quality and agronomic traits
- Bacterial Artificial Chromosome (BAC) and BIBAC libraries enriched in genes
- Physical maps of sequenced and aligned gene-rich regions of A, B and AB genomes

4.2: Determine genetic diversity and DNA polymorphism in peanut genomes. 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.

Anticipated Products:

- Genetic maps of peanut genomes saturated with SSR and SNP markers
- High-throughput systems for genotyping breeding populations and germplasm collections
- Useful DNA markers for MAS breeding programs

4.3 Development of transcriptional tools and technologies for characterizing gene function. 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 various reverse-genetic technologies, such as: TILLInG (Targeting Induced Local Lesions in Genomes), RNA interference (RNAi) and VIGS (Viral Induced Gene Silencing). Proteomics is the extensive characterization of proteins in biological organs that may help define candidate gene function in these reverse-genetic approaches.

Anticipated Products:

- Annotated high-density proteomic maps of developing & mature peanut seed of cultivars and germplasm exposed to various biotic and abiotic stresses

- Gene silencing technologies that help identify gene function and develop stable mutations in genes governing biological processes and traits
- A reference proteomic map from peanut leaf tissue
- Knowledge of the genetic and metabolic regulation of biological processes in peanut
- Genetic resources exhibiting unique gene insertions or deletions that influence peanut productivity and quality
- TILLInG resources for peanut
- Germplasm and breeding lines with beneficial mutations in genes that govern the expression of allergens and other agronomic traits.

4.4 Establish bioinformatic resources and comparative genome analysis tools for peanut. Bioinformatics involves management and interpretation of data from DNA sequences, forms of gene expression, protein interactions and the relationships of these data with genetic traits. A distinct resource would facilitate the storage of bioinformation for peanut and enhanced comparative genomics approaches within the genus *Arachis* and among other legume genomes.

Anticipated Products

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

Potential Benefits: Physical maps of gene rich regions of peanut genomes and other DNA resources will facilitate gene discovery and development of useful DNA markers for selectable traits. Use of markers on microarray chips will help characterize biological effects of reverse genetic technology and expression of desired gene combinations from segregating populations. Advances in functional genomics and proteomics will provide assurance that targeted gene expression is arrested and will facilitate the assessment of possible collateral alteration of other expressed genes. Plant bioinformation systems will enable comparative genomics among species to identify candidate genes, unique genes, and evolutionary relationships among genes for crop improvement.

Genetics & Germplasm Enhancement

Many of the most difficult traits to improve in a selection program for peanut are multi-genic. Gene families govern the expression of many seed traits. Genes that protect plants against pathogens often exhibit multiple components of resistance. Molecular markers are necessary to exploit untapped sources of resistance, and enable accelerate genotyping segregating populations and accessions of germplasm collections for specific crop improvement traits. MAS should provide a more efficient method for combining desirable genes in agronomic cultivars.

Goal 5: Ensure an adequate supply of agronomic and high-quality peanut cultivars for commercial production.

Performance Measures:

5.1 Enhance understanding of genetic diversity and genomic variation for important traits in *Arachis*. The cultivated peanut collection contains more than 8,000 accessions in the U.S. and 14,000 at the International Crops Research Institute for the Semi-Arid Tropics. Evaluation of this large group of materials on a timely basis is not possible. A peanut core collection has been used to identify areas where additional plant collections may be warranted to increase genetic variation, to identify accessions for resistance to leaf spots, nematodes, aflatoxin and several other diseases of peanut, and to identify genetic variation for oil content and fatty acid composition. Preserving DNA from core accessions will allow more efficient use of time and facilities to answer pertinent questions in molecular biology.

Anticipated Products:

- Estimates of linkage disequilibrium among wild and cultivated species
- Expanded descriptors for chemical constituents of peanut in GRIN
- Useful core-collections and genetic populations for phenotype association with specific genotypes in genetic populations
- Knowledge of genetic variation among wild species

5.2 Improve methods to develop genetic resources with useful traits. High levels of variation within and among closely related *Arachis* species leads to potential use for gene identification, marker assisted selection, and introgression to the cultivated species. Homologies between the genomes of *A. hypogaea* and related species have been estimated. Genes from *A. cardenasii* (an A-genome species) have been introgressed into 10 linkage groups of *A. hypogaea*. Hybrids from these crosses have been used to identify RAPDs and sequence characterized amplified regions (SCARs) to map genes conferring resistance to the peanut root-knot nematode. RAPDs have also been linked to several components of leaf spot resistance, to Clindrocladium black rot resistance, and to several insect pests. AFLP markers in other hybrids have been linked to tomato spotted wilt virus resistance. Although linkages of resistance genes to different molecular markers may prove useful for selecting breeding lines with desirable traits, there have been limited successes in peanut for utilizing these materials for cultivar development.

Anticipated Products:

- Peanut germplasm and hybrids with beneficial exotic traits
- Cultivated peanut varieties with beneficial exotic traits

5.3 Improve selection efficiency through use of genomic resources. 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.

Anticipated Products:

- Useful genetic populations and methods for accurately mapping and positioning gene markers on genetic maps of the peanut genome.
- Knowledge of trait inheritance
- Germplasm and varieties enhanced for quality traits, flavor, reduced pre-harvest aflatoxin contamination, disease & pest resistance, drought tolerance, and greater productivity.

Potential Benefits: Exotic germplasm will provide a valuable gene source for disease and pest resistance. 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

High-throughput protocols for peanut transformation/regeneration using techniques such as: microprojectile bombardment, viral-mediated insertion, and *Agrobacterium*-mediated gene transfer may provide valuable genetic resources that exhibit beneficial changes in genome structure and gene expression that complement genetic enhancement of peanut.

Goal 6: Improved peanut transformation technology for manipulation of genetic traits in agronomic germplasm and functional analyses of the peanut genome

Performance Measures

6.1 Optimize peanut transformation and regeneration protocol. 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. Improvements will be made in methods that help ensure greater efficiency and effectiveness of peanut transformation to facilitate production of cultivars with multiple transgenic events, gene discovery and determination of gene sequence function.

Anticipated Products

- Non-genotype specific peanut transformation protocol
- Transformation methods that target specific genes or regions of chromosomes
- Enhanced stable transformation frequency and reduced plant regeneration time

6.2 Develop improved and useful transformation vectors. Peanut transformation capacity is limited by a lack of high-throughput transformation vectors capable of delivering numerous simultaneous transformations. New elements of transgenic constructs will be developed to expedite delivery of multiple gene sequences and genetic material into peanut cells and tissues without regard for stage of plant development. Regulatory approvals and appropriate agreements among collaborators will be established to ensure efficient transfer of putative transgenic materials among collaborating institutions.

Anticipated Products

- Ability to generate and evaluate transgenic plants expressing multiple constructs
- Effective gene promoters, selectable markers, vectors and terminators in public domain

6.3 Develop transgenic breeding lines with useful and stable traits. Enhancement of many agronomic and quality traits in peanut is impeded by a low level of genetic variation in relevant gene systems in cultivated peanut. Transgenic approaches will be used to provide an expanded arsenal of germplasm resources with genetic modifications that mediate major constraints in peanut productivity, protection or product quality.

Anticipated Products

- Germplasm with transgenic enhancement of product quality and safety
- Germplasm with transgenic resistance to diseases and pests
- Germplasm with transgenic tolerance or resistance to abiotic stresses

6.4 Develop biotech risk assessment and mitigation strategies. Many of the gene sequences and tools required for producing transgenic plants are subject to patent protection. Transgenic peanuts will be evaluated and approved by governmental agencies charged with oversight of the safety of agricultural products for agriculture, humans, and the environment. License agreements will be obtained for the traits and processes that are protected by patents and necessary for the development of improved peanut cultivars.

Anticipated Products

- Operative agreements on the use of technology for gene insertion, selectable marker, promoter/terminator, and gene sequences into peanut
- Regulatory approval for field testing of transgenic material
- Protocol for managing gene-flow and volunteer transgenic plants in commercial production systems
- Protocol for monitoring changes in ecosystems that may be attributed to transgenics

Potential Benefits: 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. These capabilities will facilitate discovery of gene sequence function and the development of cultivars with novel genetic attributes that mitigate major concerns and problems in peanut production and marketing.

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