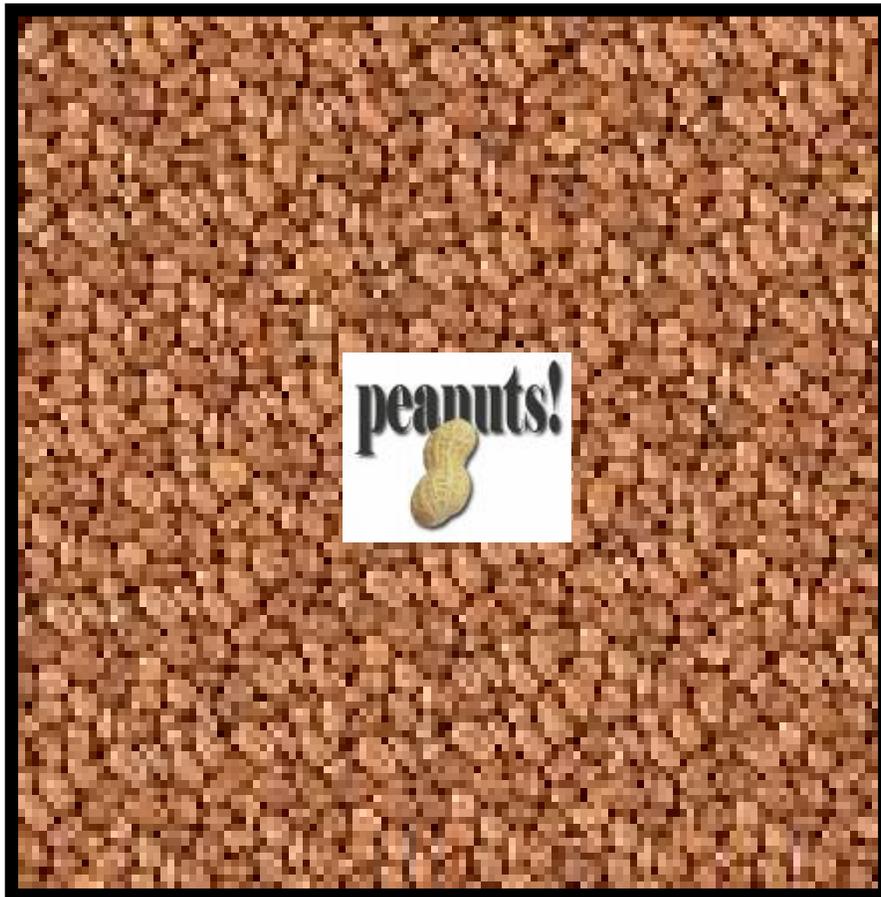


# **International Peanut Genome Initiative**

## **Accomplishments Report 2008**

**Improving Crop Productivity & Protection,  
Product Safety & Quality**

**Version 1.0 December 2008**



## **2008 Research Accomplishments Report for the Peanut Genome Initiative**

This report outlines peanut research accomplishments during 2008 that address the Performance Measures of the International Strategic Plan for the Peanut Genome Initiative 2008-2012, v1.2 March 08. The abridged description of accomplishments is based on presentations made at the 2008 Spring APC meeting in Alexandria VA, the 2008 APRES annual meeting in Oklahoma City OK, and various contributions as provided by members of the research community. This report will be amended to include the accomplishments of international peanut researchers that were presented during AAGB-2008 in Hyderabad, India. Each item is referenced to an Abstract, peer reviewed publication, or an official report. More detailed information on each achievement may be obtained upon request from Richard F. Wilson at [rfwilson@mindspring.com](mailto:rfwilson@mindspring.com).

### **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

#### **Accomplishments**

- 1 GxE performance of runner cvs in VA-NC (Stokes, VA)
- 2 Tillage, CV, Row patterns on yield and TSWV (Beasley, GA)
- 3 Reduced tillage practices for OK (Damicone, OK)
- 4 BMPs in NC (Jordan, NC)
- 5 BMPs in Mozambique (Jordan, NC)
- 6 Germination optimum between 19 to 35C (Beasley, GA)
- 7 Cover crops have no effect on yield (Tubbs, GA)
- 8 Strip till raised beds improve digging efficiency (Jackson, GA)

## **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

### **Accomplishments**

- 1 Reduced irrigation on profitability in TX (Burow, TX)
- 2 Host pathogen pest interactions in dry land production systems (Hagan, AL)
- 3 Medium maturity cvs with multiple disease resistance GA (Holbrook, ARS)
- 4 Selenium fertilization (Sorensen, ARS)
- 5 Maturity estimates for digging based on RTK (Balkcom, AL)
- 6 Moisture meters (Kandala, ARS)
- 7 Variety response to harvest date GA (Beasley, GA)
- 8 Evaluating early maturing cvs for TX (Burow, TX)
- 9 Estimating maturity from yield trials (Isleib, NC)
- 10 Cercosporidium resistance in medium maturing cvs GA (Culbreath, GA)
- 11 N fertilization of Virginia-type cvs (Estienne, VA)
- 12 Ca requirements GA (Von Waldner, GA)
- 13 Breeding for early maturity in NC (Isleib, NC)
- 14 Decision model for irrigation scheduling (Suleiman, GA)

## **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

### **Accomplishments**

- 1 Seed treatments for organic production (Shaw, NC)
- 2 Digging efficiency of strip-till (Jackson, GA)
- 3 Economics of organic production (Keiser, GA)
- 4 Weed management in organic production (Jordan, GA)
- 5 Cultivar differences in economics of tillage and row pattern (Ziehl, GA)
- 6 Cultivar tolerance to KIH485 herbicide (Prastko, GA)
- 7 Cultivar response to paraquat, metolachlor (Dotry, TX)
- 8 Cv response to glyphosate (Grey, GA)
- 9 Cultivation for weed control in organic production (Johnson, ARS)

- 10 Row spacing and weed control (Brecke, FL)
- 11 Weed response to herb-fungicide combos (Grichar, TX)
- 12 Management options for insect damage control (Herbert, VA)
- 13 Water capture tillage practices reduce PAC, reduces fuel costs (Nutti, ARS)

#### **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

##### **Accomplishments**

- 1 Soil conservation equipment (Nutti, ARS)
- 2 Transcriptional response to temp and water stress in core collection (Payton, ARS)
- 3 Proteomic analysis of abiotic stress in 2 accessions (Puppala, NM)
- 4 GE drought tolerance concept (Blumwald, CA)
- 5 Varieties may acclimate to drought stress (Boote, FL)
- 6 Narrow/strip till & twin row spacing conserves soil moisture (Arriaga, ARS)
- 7 Leafspot resistant cvs have reduced canopy photosynthesis (Boote, FL)

## **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 *Aspergillus* 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 *Aspergillus* 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
- Atoxicogenic or modified biocontrol organisms that do not produce aflatoxin
- PAC resistant peanut germplasm and varieties

#### **Accomplishments**

- 1 Characterized mitigating effects of drought tolerance on PAC (Holbrook, ARS)
- 2 DNA markers for PAC resistance (Isleib, NC)
- 3 Beneficial impact of winter cover on PAC (Hagen, AL)
- 4 Water capture tillage practices reduce PAC (Nutti, ARS)
- 5 Released C79-16 with PAC resistance (Holbrook, ARS)
- 6 RKN resistance reduces PAC (Holbrook, ARS)
- 7 Remote sensing for drought symptoms used to reduce PAC (Sullivan)
- 8 PeanutAGE Oligo-microarray with 8400 markers from ESTs for PAC, late/early leaf spots, oleic, TSWV, white mold (Guo, ARS)
- 9 Initiated marker screen of wild species for PAC resistance and MAS for cv development (Isleib, NC)
- 10 GM Georgia Green and NCV11 with Mod1 insert for PAC and Sclerotinia resistance (Weissinger, NC)
- 11 New formulation to enhance efficiency of nontoxicogenic strains (Zabotowicz, ARS)
- 12 Clay absorbs aflatoxin B1 (Jaynes, TX)

## **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

#### **Accomplishments**

- 1 Phenolic compounds inhibit IgE binding to peanut allergens (Chung, ARS)
- 2 RNAi silencing of ara-h2 (Ozias-Akins, GA)
- 3 International breeding program for altered protein composition in India (Sheikh, FL)
- 4 Demonstrated reduced IgE binding in RNAi knock-outs of ara h2 (Ozias-Akins, GA)
- 5 Thresholds for food allergens (Taylor, NE)
- 6 FAAN educational resources (Weiss)
- 7 Characterized 6 IgM epitopes on ara-h1 (Maleki, ARS)
- 8 Demonstrated high homology & epitope cross reactivity between ara-h6 & ara-h2 (Koppleman)
- 9 Discovered 3 SNPs in ara h2 genes via TILLInG (Ozias-Akins, GA).
- 10 Discovered GT-C9 and GT-C20 germplasm lacking isoforms of ara-h3 (Guo, GA)

**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

#### **Accomplishments**

- 1 Demonstrated GxE effects on physical properties of snack peanuts (Smyth, Kraft)
- 2 Characterized antioxidant strength in peanut flour (Davis, ARS)
- 3 Uniform tests of breeding lines for sensory quality (Pattee, NC)
- 4 High moisture reduces roasted peanut flavor (Dean, ARS)

- 5 Demonstrated feed value of in-shell peanuts for beef production (Myer, FL)
- 6 Sonication and coatings reduce rancid flavors in peanut oil (Yang, AL)
- 7 Determined amino acid (lys, met, thr, arg), fatty acids, tocopherols, folate (Sanders, ARS)
- 8 Breeding to improve grade in disease resistant lines (Baring, TX)

**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. 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 emissions.

**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

**Accomplishments**

- 1 Development of high oleic peanut cvs for OK (Chenault, ARS)
- 2 Economic benefits of peanut for biofuel vs other vegetable oils (Faircloth, ARS)
- 3 Demonstrated in-field processing for biodiesel (Butts, ARS)
- 4 Peanut collection has 42 to 54% oil (Wang, ARS)
- 5 QTL markers for Pod and Kernel traits Oil content (Burow, TX)

## **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

**Accomplishments**

- 1 Demonstrated improved control of fungicide applications at night (Culbreath, GA)
- 2 Fungicide efficacy among cvs with partial resistance to leaf spot (Tillman, FL)
- 3 Documented cultivar response to disease and insects under dry land conditions (Hagan, AL)
- 4 Suppression of *Cylindrocladium* black rot with Provost and Proline fungicides (Phipps, VA; Hagan, AL)
- 5 Evaluation of host resistance vs fungicide for late leaf spot in NC (Isleib, NC)
- 5 Response of runner peanuts to verticillium wilt (Woodward, TX)
- 6 Field tests for *Sclerotinia* and TSWV resistance (Branch, GA)
- 7 Seed treatments for *Lasiodiplodia theobromae* (Brenneman, GA)
- 8 Efficacy of aldicarb & phorate on TSWV and yield (McGriff, GA; Rowland, ARS)
- 9 Efficacy of fungicide combinations and foliar spray products (Wigley, GA; Baughman, TX)
- 10 Efficacy of fungicides and phosphorus in Ghana (Boote, FL)

**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

**Accomplishments**

- 1 Validated prescription fungicide programs (Culbreath, GA)
- 2 Effective extension programs (Jordan, NC)
- 3 Incidence of TSWV in peanut (Brown, GA)
- 4 Decision support system for IPM in V-C diseases, insects, nematodes (Jordan, NC)
- 5 TSWV response correlated with planting date (Tillman, FL)

**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

**Accomplishments**

- 1 Evaluation of peanut inocula effectiveness (Jordan, NC)
- 2 Weather based warning system for leaf spot in OK (Damicone, OK)
- 3 Projected effect of climate change on PAC in Australia (Wright, PCA)
- 4 Remote sensing for drought symptoms used to reduce PAC (Sullivan)

## 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, cytogenetic 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
- Microarrays 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

**Accomplishments**

- 1 Oligonucleotide microarray for gene profiling from EST libraries (Payton, ARS)
- 2 Leaf and Seed EST library yields 19000 genes including major peanut proteins (Guo, ARS)
- 3 26800 total unigenes identified from 34K (B), 1034K (A) ESTs (Knapp, GA)
- 4 Cloned and characterized a MADS-box gene (He, AL)
- 5 PeanutAGE Oligo-microarray with 8400 markers from ESTs for PAC, late/early leaf spots, oleic, TSWV, white mold (Guo, ARS)
- 6 Developed 2600 EST-SSR markers (Knapp, GA)
- 7 3000 SNPs in A-Genome (Knapp, GA)
- 8 BAC library tetraploid with 3785 clones, 2% genome. 60% hybridized to RGH primers (Cook, CA)
- 9 Discovered 380 genes with SNPs in diploid genomes (Cook, CA)

**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

**Accomplishments**

- 1 Association of allelic variation in RGH and COS sequences with resistance genes (He, AL)
- 2 SSR characterization of allelic diversity in all commercial Virginia-type cvs (Isleib, NC)
- 3 Discovery of TSWV resistance genes and markers (Guo, ARS)
- 4 QTL markers for Pod and Kernel traits Oil content (Burow, TX)
- 5 SNP markers for RGH genes (Cook, CA)
- 6 Mapped 2000 SNP in A-Genome
- 7 Initiated SNP map of tetraploid peanut

**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.

**Accomplishments**

- 1 Discovered 3 SNPs in ara h2 genes via TILLInG (Ozias-Akins, GA).

**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.

**Accomplishments**

- 1 Developed transcript assemblies & EST database for gene and SNP discovery (Knapp, GA)

**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

**Accomplishments**

- 1 Inverse relation of seed size and distance from main stem (Williams, TX)
- 2 Evaluation of oil content in wild species (Burow, TX)
- 3 UT documented increase in seed and pod size in runner-type cvs (Isleib, NC)
- 4 Marker characterization of diversity in wild species (Tallury, NC)

**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

**Accomplishments**

- 1 New sources of high oleic germplasm from Thailand (Puppala, NM)
- 2 Introgression of multiple disease resistance genes from wild species (Holbrook, ARS)
- 3 Use of a bridge-species for development of interspecific hybrids (Simpson, TX)
- 4 New breeding lines for disease resistance in TX (Baring, TX)
- 5 3 new varieties for OK (Chenault, ARS)
- 6 New interspecific germplasm with leaf spot resistance (Burow, TX)
- 7 New Virginia-type germplasm with Late leaf spot, Stem rot and TSWV resistance (Shokes, VA)
- A/U New and pending cvs: Tifrunner, Tifguard (high resistance to RKN, TSWV), Brantley (hiOL), N99103ol, N00098ol, N03081T (Bailey), N01090T (Sugg), Olin, TamrunOL01, TamrunOL02, TamrunOL06, TamrunOL07, NM02565, NM02322, Georgianic (leafspot TSWV), TifGP-1, Georgia-07W (TSWV, Sclerotinia)
- 9 Breeding for early maturity in NC (Isleib, NC)
- 10 Released C79-16 with PAC resistance (Holbrook, ARS)
- 11 Backup collection with 16 wild species (Simpson, TX)

**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.

**Accomplishments**

- 1 Evaluation of annual peanut for forage (Tillman, Myer, FL)
- 2 Digestibility of forage peanut for horses (Sanders, ARS)
- 3 Heritability estimates for drought tolerance (Chen, ARS)
- .4 Initiated marker screen of wild species for PAC resistance and MAS for cv development (Isleib, NC)

## **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

#### **Accomplishments**

- None

**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

#### **Accomplishments**

- 1 Demonstrated RNAi knock-out of ara h2 (Ozias-Akins, GA)

**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

#### **Accomplishments**

- 1 GM cvs with resistance to Sclerotinia blight (Grabau, VA)
- 2 GM cvs with resistance to stem rot (Holbrook, ARS)
- 3 GM concept for PGR mediated drought tolerance (Blumwald, CA)
- 4 GM concept for modification of oil and protein composition (Schmidt, MO)
- 5 GM Georgia Green and NCV11 with Mod1 insert for PAC and Sclerotinia resistance (Weissinger, NC)
- 6 GM cvs with elevated folate expression (Grabau, VA)

**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

**Accomplishments**

- 1 Low gene flow, outcrossing in GM peanut (Grabau, VA)
- 2 Progress toward regulatory approval for GM peanut (Grabau, VA)
- 3 Positive industry assessment (Smuckers, MARS, Kraft)