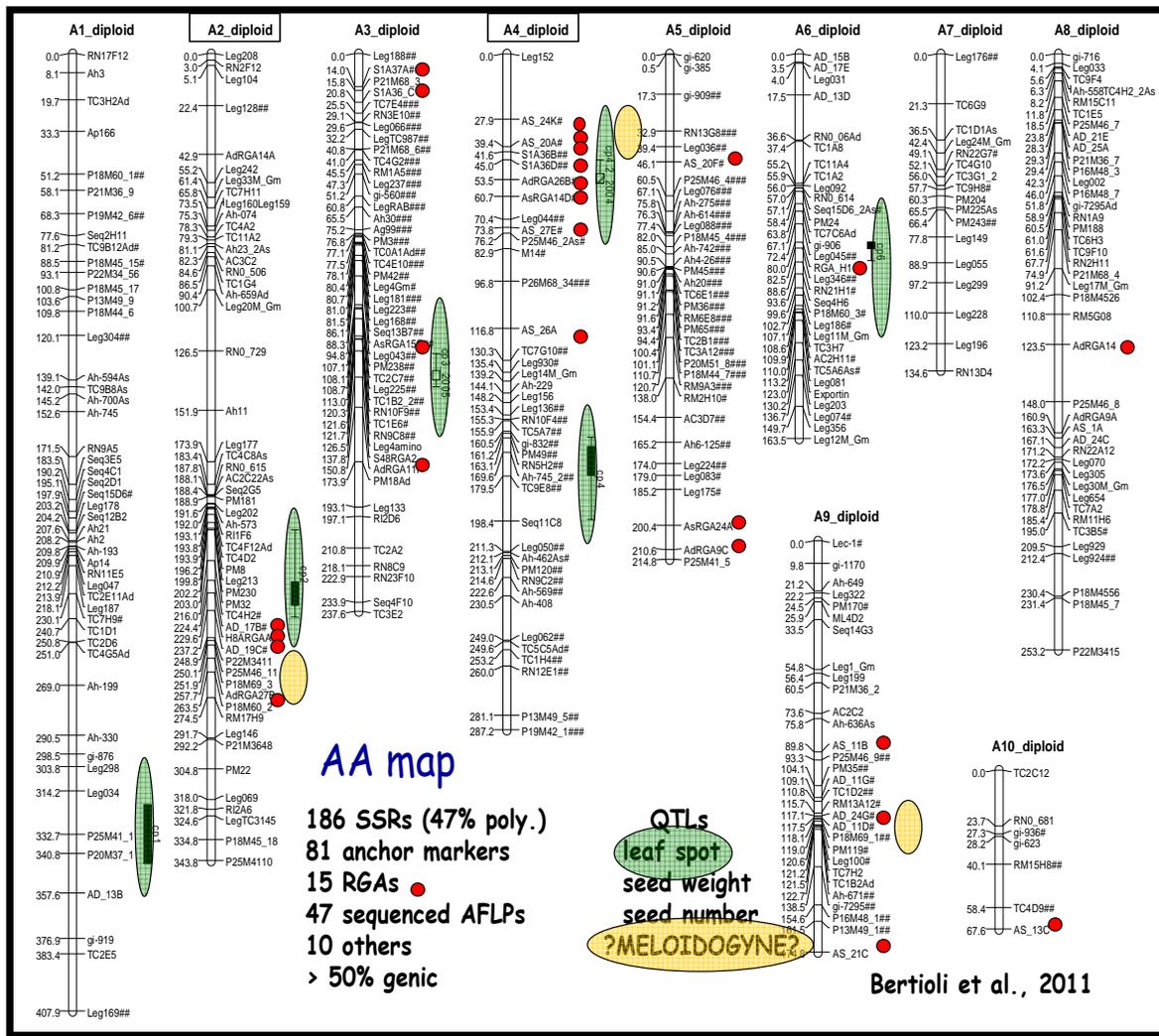


# International Peanut Genome Initiative

## Most Significant Accomplishments 2009

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

Version 3.0 June 2010



## **Executive Summary**

This report outlines the most significant peanut research accomplishments during 2009 that address the Performance Measures of the International Strategic Plan for the Peanut Genome Initiative 2008-2012, v1.2 March 08. These accomplishments are based on presentations made at the 2010 Spring APC meeting in Washington DC, the 2009 APRES annual meeting in Raleigh NC, publications in peer reviewed scientific journals and various contributions as provided by members of the research community. This report also includes major accomplishments of international peanut researchers that were presented during AAGB-2009 in Bamako, Mali. More detailed information on each achievement may be obtained upon request from Richard F. Wilson at [rfwilson@mindspring.com](mailto:rfwilson@mindspring.com)

<b>Most Significant Research Accomplishment &amp; Milestone Summary for the Peanut Genome Initiative</b>					
	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>	<b>2012</b>
<b>Crop Management &amp; Productivity</b>					
<b>1.1 Elite production practices</b>	Strip till raised beds improve digging efficiency	Rotation with perennial grasses increased yield			
<b>1.2 Soil fertility, irrigation, crop handling</b>	Decision model for irrigation scheduling	Early hi-O/L variety developed			
<b>1.3 IPM</b>	Management options for insect damage control	Grade increased w/ imazethapyr & chlorimuron			
<b>1.4. Drought tolerance; Maturity</b>	GM growth regulator approach to drought tolerance	Synthetic polyploids w/ genes for drought tolerance			
<b>Product Quality &amp; Safety</b>					
<b>2.1 Pre-harvest aflatoxin contamination</b>	DNA markers for PAC resistance, Released C79-16 w/ PAC resistance	Process for removing aflatoxin from meal			
<b>2.2 Peanut Proteins</b>	Gene silencing for ara-h2; Thresholds for allergens; 3 SNPs for ara-h2 alleles	Discovered 32 genes govern storage protein composition			
<b>2.3 Nutrition &amp; health benefits</b>	Characterized sensory traits in UPPT lines	Determined antioxidant levels in Core of Core			
<b>2.4 Bioenergy applications</b>	42% to 54% oil in germplasm collection; 5 QTL for oil%	Reference gene for detecting marker for oil concentration			
<b>Disease &amp; Pest Management</b>					
<b>3.1 Fungicide &amp; pesticide application</b>	Efficacy of fungicides w/ foliar spray products	Leaf spot control w/ prothiconazole > tebuconazole, chlorothalonil			
<b>3.2 Disease &amp; pest management practices</b>	Decision support system for IPM in V-C diseases, insects, nematodes	N2 fixation impeded by co application w/ fungicides			
<b>3.3 Pathogen epidemiology</b>	Weather based warning systems for leaf spots in OK	Improved method for phenotyping CBR resistance			
<b>Gene Discovery &amp; Genome Analysis</b>					
<b>4.1 Characterize peanut genome structure</b>	Oligo array for profiling EST libraries; EST library w/ 19000 genes including seed proteins; PeanutAGE array w/ 8400 markers for PAC, leaf spot, O/L, TSWV, white mold	Physical map of A-genome			
<b>4.2: Genetic diversity &amp; genomic differences</b>	Initiated tetraploid SNP map; Associated RGH & COS sequences with alleles	4135 new validated SSR, SNP, SSCP, Indel markers			
<b>4.3 Breeders tool box</b>	Discovered 3 SNPs in ara-h2 genes via TILLING	Perfect markers for 2 alleles each of ara-h1, ara-h2, AhFad3			
<b>4.4 Databases &amp; Analytical software</b>	Transcript assemblies & EST dbase for SNP discovery	EST dbase with 2.5 billion EST sequences			
<b>Genetics &amp; Germplasm Enhancement</b>					
<b>5.1 Utility of the germplasm collection</b>	Genetic marker characterization of genetic diversity in wild species	Evidence for genetic bottleneck during evolution of peanut			
<b>5.2 Germplasm, variety enhancement</b>	Interspecific germplasm w/ leaf spot resistance; VA germplasm w/ TSWV & leaf spot genes; 18 new cvs	Synthetic polyploids facilitate gene transfer from wild species to cultivated			
<b>5.3 Modern breeding methods</b>	Heritability estimates for drought tolerance; Marker screen for PAC in wild species; MAS for PAC resistance	Tifguard Hi-O/L on schedule, Marker for TSWV resistance			
<b>Plant Transformation Technology</b>					
<b>6.1 Transformation methods</b>	No progress	Transformation efficiency boosted to 15%			
<b>6.2 Transformed Traits</b>	RNAi knock-out of ara-he gene	Agrobacterium transformation deployed successfully			
<b>6.3 GM peanuts</b>	GM cvs w/ resistance to Sclerotinia blight, stem rot; oil and protein composition; high folate	Blight Blocker cv ready for release; High Folate under construction			
<b>6.4 Regulatory approval</b>	Low outcrossing in GM peanut	APHIS approval pending			

# Peanut Genome Initiative Major Accomplishments: 2009

## Crop Management & Productivity

**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

Peanut (*Arachis hypogaea* L.) response to inoculation with *Bradyrhizobium* can vary depending on edaphic and environmental conditions and cropping history. Determining if response is associated with the number years between peanut plantings may increase understanding of when to expect a positive response to inoculation of peanut. Four experiments were conducted in North Carolina to determine peanut response to in-furrow inoculation with *Bradyrhizobium* when a range of years and typical crops grown in North Carolina often separating peanut plantings. Rotations varied from continuous peanut in some experiments to as many as five years of a non-peanut crop separating peanut plantings. The interaction of crop rotation by inoculation treatment (no inoculation versus in-furrow application of *Bradyrhizobium*) was not significant for visually estimated peanut canopy color or pod yield in any of the experiments. However, the main effect of rotation was significant in three of four experiments while the main effect of inoculation was significant in two of four experiments. Increasing the number of years a non-peanut crop was planted between peanut plantings increased yield in three of four experiments. Results from these experiments suggest that using the number of non-peanut crops included between peanut plantings is not a good indicator of determining when peanut will respond positively to inoculation with *Bradyrhizobium*.

Rotation of row crops with perennial grasses has been shown to improve soil quality characteristics, decrease pest incidence, and increase crop yield. Scientists at IFAS conducted experiments in Florida at Marianna and Quincy to determine effects of termination date and subsequent tillage of established bahiagrass (*Paspalum notatum* L.) on peanut (*Arachis hypogaea* L.) yield and market grade characteristics when rotating from bahiagrass to a row crop. Treatments included two bahiagrass termination dates (fall vs. spring) and six tillage methods [strip-till (in-row subsoiling), disk plus moldboard plow, disk plus chisel, disk plus paratill plus strip-till, disk, and strip-till with 45 kg N/ha]. Bahiagrass termination timing did not affect soil mechanical resistance, peanut yield or market grade characteristics, including percentages of sound mature kernels, split kernels, other kernels, and hulls. Although significant differences in soil mechanical resistance were detected among tillage treatments, peanut yield did not differ among the tillage methods except for the disk alone in 2007 at Quincy. Strip-till peanut yield did not respond to N application. When pooled over termination timing, peanut yields in strip-till, disk plus moldboard plow, disk plus chisel, disk plus paratill plus strip-till, disk, and strip-till plus 45 N were 4830, 5000, 4810, 4810, 4770, and 4620 kg/ha, respectively. These results indicate that when using perennial grasses in sod-based rotations, farmers have a wide window from fall to spring to terminate bahiagrass for optimum peanut production regardless of tillage system.

## **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**

Senescence and abscission of mature peanut pods is controlled by the ethylene cascade. Reducing senescence and abscission could involve inhibiting the ethylene cascade and allow greater harvest flexibility in peanut. Application of 1-methylcyclopropene (1-MCP), the ethylene binding inhibitor, may reduce senescence and abscission of mature peanut pods. Scientists at NCSU determined the effects of 1-MCP on pod yield and percentages of sound mature kernels (%SMK), sound splits (%SS), total sound mature kernels (%TSMK), other kernels (%OK), extra large kernels (%ELK), fancy pods (%FP), and pod retention. Treatments of 1-MCP were applied at 26 g ai/ha plus a crop oil concentrate at 7, 10, or 14 d prior to digging peanut at the projected optimum digging date. Peanut was dug at the projected optimum digging date or at 7 or 20 d after projected optimum digging date. The cultivars NC-V 11, Phillips, and Perry were evaluated in separate experiments. Pod yield, %SMK, %TSMK, %SS, %OK, %ELK, and %FP were not affected by 1-MCP regardless of application timing when NC-V 11 and Phillips were evaluated. Only %SMK and %TSMK were affected by 1-MCP when applied to the cultivar Perry. Digging date affected pod yield and market grade characteristics. When digging of Phillips and Perry was delayed by 7 or 20 d past the optimum digging date, %SMK and %TSMK increased. Pod retention, determined by comparing the number and mass of pods/plant following digging, was affected by digging date and location but not 1-MCP treatment. These data suggest that 1-MCP will have little activity on peanut pod yield, market grade characteristics, or pod retention

Scientists at NCSU developed populations derived from runner and Virginia parents x Spanish and Valencia parents, and incorporated highland Bolivian germplasm for early maturity. They also introgressed black-pod and high O/L traits. Lines were tested in NC and TX. Results produced an early maturing version of the Virginia cv Wilson, and an array of elite lines with high O/L. NCSU has amassed the world's largest collection of black-podded peanuts. In addition, scientists at ACI generated new populations segregating for early maturity, disease resistance and high O/L for west Texas. Advanced field trials over 4 years at 3 locations distinguished WT05-0219, WT04-0121, M04-0149, WT05-0372, and FR458 with yields between 6000 to 6400 pounds per acre.

## **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

Scientists at Texas Tech University conducted field studies in south Texas and the southern High Plains of Texas to determine peanut response to POST applications of chlorimuron at 9 g ha<sup>-1</sup>. Treatments included chlorimuron alone, imazethapyr applied 21 days after planting (DAP) followed by chlorimuron applied POST, and chlorimuron plus either 2,4-DB or chlorothalonil in combination applied POST. Postemergence herbicide applications were made 60, 74, and 88 DAP at the southern High Plains location or 67, 81, or 95 DAP at the south Texas location. No difference in peanut stunting was observed with any chlorimuron treatments at the south Texas location. At the High Plains location, chlorimuron alone, imazethapyr followed by chlorimuron, and chlorimuron in combination with 2,4-DB stunting was greater than chlorimuron in combination with chlorothalonil in one of two years. Imazethapyr followed by chlorimuron reduced peanut yield in one year in south Texas. No peanut grade (sound mature kernels plus sound splits) differences between chlorimuron treatments were noted at the south Texas location, but for the southern High Plains location, peanut grade was greater when peanut was treated with imazethapyr followed by chlorimuron compared to the other chlorimuron treatments.

Strip tillage with various crop covers in peanut (*Arachis hypogaea*, L.) production has not shown a clear yield advantage over conventional tillage, but has been found to reduce yield losses from some diseases. Scientists at USDA-ARS Dawson GA and UGA-Tifton GA determined pod yield and disease incidence between two tillage practices, five winter cover crops, three peanut cultivars, and three fungicide programs. Conventional and strip tillage treatments were implemented on a Greenville sandy loam (fine, kaolinitic, thermic Rhodic Kandiudults) near Shellman, GA. Five winter cereal grain cover crops (strip tillage) and a no-cover crop treatment were sprayed at recommended (1R), half recommended (0.5R) or untreated (0R) fungicide programs. Within peanut cultivars, leaf spot (*Cercospora arachidicola* Hori) intensity decreased as the number of fungicide applications increased; however, stem rot (*Sclerotium rolfsii*) incidence was the same for the 1R and 0.5R fungicide programs but increased 0R program. Conventional tilled peanuts developed more leaf spot compared with strip tillage. There was no difference in leaf spot ratings among winter crop covers. There was no difference in stem rot incidence with tillage or winter cover crop. There was no yield difference with peanut cultivar. Pod yield was the same for the 1R and 0.5R fungicide program (3867 kg/ha) but decreased at the 0R fungicide program (2740 kg/ha). Pod yield was greater with conventional tillage and strip tillage with black oats (*Avena sativa* L.) (3706 kg/ha) compared with strip tillage of other winter crop cover treatments (3358 kg/ha). Conventional tillage had more leaf spot, equal incidence of stem rot, and higher yield compared with strip tillage. The 0.5R fungicide program had the same yield compared with the 1R fungicide program implying a possible 50% savings on fungicide applications on well rotated fields with lower disease risk.

#### 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**

Scientists at EMBRAPA demonstrated genotypic differences in transpiration efficiency between the B-genome wild species *A. magna* KG 30097 and cultivated peanut. *A. magna* exhibited a slower loss of water under drought stress. Similar results were observed between the A-genome *A. duranensis* K7988 and cultivated peanut. Synthetic polyploids were created that were cross compatible with cultivated peanut from the mating: [*A. stenosperma* x *A. duranensis*] X [*A. magna* x *A. ipanensis*]. A subtractive EST library was created to identify expressed genes associated with drought stress.

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

### **Accomplishments**

USDA ARS scientists at Raleigh NC developed a processing technique for removal of aflatoxin from peanut meal. Application of 2% AB20A absorbent during processing of defatted peanut meal produced feed grade fractions with 0.9 ppb aflatoxin, and food grade fractions with zero aflatoxin.

## **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**

Scientists at NCSU generated a cDNA (EST) profile from midmaturation peanut to establish a baseline for determining the impact of genetic modifications on peanut composition. This library enabled the first detailed inventory of peanut storage genes and proteins, and the first proof that specific genes in cultivated peanut came from A or B-genome progenitors. 454 FLX-Titanium sequence analysis of a non-normalized cDNA library from Tifrunner was carried out to establish a message profile. This step yielded raw sequences with an average length of 351 bp. Seqclean software was used to eliminate vector and primer sequences, as well as poly A/T tails. BLASTN analysis was performed against the trimmed sequences to eliminate sequences with homology to rRNA. 886,994 “Clean” Sequences were assembled into 32,000 contigs with an average length of 730 bp and 47,000 singletons with an average length of 173 bp. Sequence identification and annotation was accomplished by BLASTX analysis. An analysis of 2S, 7S and 11S seed storage protein (SSP) contigs revealed that current peanut protein nomenclature is misleading. For example, Ara-h2, h6, h7 are clinical groups that are regulated by 8 genes in 3 subgroups of 2S proteins. Likewise, Ara-h1 is regulated by 4 genes in 3 subgroups of 7S proteins; and Ara-h3, h4 are regulated by 20 genes in 5 subgroups of 11S proteins. In addition, sequence association analysis determined that 3 of 8 2S genes

resided in the A-genome; 1 of 4 7S genes resided in the A-genome; and 6 (A-genome), 5 (B-genome), 9 genes remain unassigned for 11S proteins.

**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**

USDA-ARS scientists at Raleigh grew 108 accessions of the “Core of the Core” a subset of the USDA peanut germplasm collection at one location in the same year. These samples were analyzed for total and individual amino acid content, fatty acid content, tocopherols, and folic acid content. These data provided a starting point for establishing nutrient composition within these accessions and provide an early indication of currently important characteristics in these lines which might be suited for use in random breeding initiatives.

Scientists at NCSU, IFAS-Marianna, USDA-ARS Raleigh cooperated to evaluate environmental and genetic interaction on seed composition using data from the multi-state Uniform Peanut Performance Tests (UPPT). Data were subjected to restricted maximum likelihood estimation of variance components reflecting the main effects of year, production region, location within region, genotype (cultivar or breeding line), and kernel grade (“seed size”) within genotype, and the interactions among these main effects. Genetic variation in oil content was low (9% of total variation); however, fatty acid composition of the oil was highly influenced by genotype (34–77%) with the exception of lignoceric acid (1%). Genetic influence on tocopherols was generally less than that of fatty acids. Environmental variation of tocopherols was greater than the variation attributable to genotype-by-environment interaction. The lowest genetic variation was observed in sugar content; however, environmental variation was high (68%). The magnitude of genetic influence on oil content and fatty acid concentrations suggests that these traits are amenable to improvement through breeding.

**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**

Scientists determined the range of oil concentration among cultivars grown across the state of Texas.

Scientists in Israel used real-time qPCR the most sensitive technique available for the detection of low-level mRNA expression, to develop more reliable and precise gene expression analyses. Real-time PCR data for a sequence of interest was normalized against a control gene, which is uniformly expressed in various tissues and during different phases of development. Expression of 10 frequently used housekeeping genes, specifically *ubq10*, *gapdh*, *heli*, *yls8*, *14-3-3*, *60s*, *ubc*, *ef-1a*, *act7*, and *adh3* was evaluated using GeNorm and NormFinder programs. The gene with the most stable expression across all of the examined tissues and both programs was *adh3*, followed by *60s* and *yls8*, which had minimal estimated intra- and inter-tissue variation. The effect of the use of one or more reference genes on the observed relative expression levels of an important seed oil metabolism gene, diacylglycerol acyltransferase 1 (*Dgat1*), during kernel development was demonstrated. The *adh3*, or a combination of this gene with *60s* and *yls8* should be considered for use in quantitative mRNA expression analyses in *Arachis*, particularly in studies involving increased oil concentration.

## **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**

Scientists at UGA-Tifton conducted field experiments in Tifton and Plains, GA to determine the efficacy of prothioconazole on early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Cercosporidium personatum*) of peanut (*Arachis hypogaea*). In five of six experiments, application of one or both rates (0.18 and 0.20 kg ai/ha) of prothioconazole in sprays 3–6 (chlorothalonil at 1.26 kg

ai/ha in sprays 1, 2, and 7) provided leaf spot control superior to tebuconazole (0.23 kg ai/ha) in a similar regime, and superior to chlorothalonil at 1.26 kg ai/ha applied full season (seven times) in four of six experiments. In a similar series of six experiments, application of 0.085 kg ai/ha of prothioconazole + 0.17 kg ai/ha of tebuconazole provided better leaf spot control than tebuconazole (0.23 kg ai/ha) applied in regimes similar to those described above. Leaf spot control with prothioconazole + tebuconazole was similar to chlorothalonil applied at 1.26 kg ai/ha full season in five of eight experiments, but was less effective in the remaining three experiments. Fungicide effects on yield were inconsistent, but in all experiments, yield response with either rate of prothioconazole was similar to or greater than that obtained with 0.23 kg ai/ha tebuconazole on the same schedule. In a third series of four experiments, full-season (seven sprays) application of mixtures of prothioconazole at 0.063 kg ai/ha with trifloxystrobin at 0.063 kg ai/ha gave similar or better leaf spot control than chlorothalonil full season.

Recurrent problems with peanut seed germination and stand establishment have raised concerns about the potential effects of herbicides on peanut seed quality. Scientists at USDA-ARS Dawson GA and UGA-Tifton conducted field trials in south Georgia to evaluate the effects of imazapic and 2,4-DB on peanut yield, grade, incidence of tomato spotted wilt virus (TSWV), and seed germination. Imazapic at 71 g ai ha<sup>-1</sup>, applied at 30 and 40 days after planting (DAP), and 2,4-DB at 270 g ai ha<sup>-1</sup>, applied at 75 or 90 DAP, had no effect on peanut yield, grade, incidence of TSWV, and seed germination when averaged over two locations (Dawson and Tifton) and three cultivars (Georgia Green, Georgia-01R, and C-99R). Normal cultivar yield variations were observed; however, Georgia-01R had reduced standard (25 C) and cold (15 C) germination when compared to the other cultivars.

Peanut (*Arachis hypogaea* L.) is an important component of cropping systems in West Africa. Identifying production constraints in farmers' fields and evaluating possible management strategies are of prime importance to improve peanut productivity and adoption of new technologies. Scientists at Kansas State University, University of Florida and Ghana studied the influence of fungicides and phosphorus application on severity of leaf spot, dry matter production and pod yield of peanut crops grown in on-station and farmer participatory tests (on-farm conditions) in Northern Ghana. On-station tests to evaluate yield benefits of fungicide sprays and applications of phosphorus were conducted at Wa. On-station tests included two fungicide treatments (no-spray versus fungicide spray) at four P fertilizer levels (0, 30, 60 and 90 kg P ha<sup>-1</sup>). On-farm tests were conducted in three villages Nakor, Piisi and Janguasi with participation of 6–11 farmers per village. On-farm tests included three treatments: (i) farmers' practice of no-fungicide and no-fertilizer (control), (ii) only fungicide, and (iii) combination of fungicide and phosphorus. The commonly grown Spanish type cultivar Chinese (90-d duration) was selected. Both leaf spot and lack of phosphorus nutrition were yield-limiting factors in on-farm tests. Applications of fungicide were effective in controlling leaf spot and improved peanut pod yield on average by 49% in the three tested field sites in on-farm tests and by 40% in on-station tests. Application of phosphorus to fungicide-treated plots further increased pod yield by 32% when compared to fungicide alone in on-farm tests. Combination of both fungicide and P fertilizer improved peanut pod yield by 95% (ranged from 75 to 120%), when compared to farmers' practice of no-fungicide and no-fertilizer.

**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**

*Bradyrhizobia* is often applied in the seed furrow when peanut is planted to ensure nodulation and subsequent biological nitrogen fixation (BNF). Several fungicides, insecticides, and fertilizer solutions are registered for in-furrow application in peanut while others or currently being evaluated for possible use. The effect of these products on efficacy of *Bradyrhizobia* inoculant has not been thoroughly investigated. Research was conducted in North Carolina and Virginia to determine peanut response to in-furrow application of *Bradyrhizobia* inoculant alone or with the fungicides azoxystrobin, boscalid, pyraclostrobin, propiconazole plus trifloxystrobin, and tebuconazole; the insecticide imidacloprid; and the commercial fertilizer Asset® RTU. Peanut yield did not differ in three experiments when inoculant was applied alone or with the fungicides azoxystrobin, boscalid, pyraclostrobin, propiconazole plus trifloxystrobin, or tebuconazole. In experiments, pod yield was lower when inoculant was applied with azoxystrobin, pyraclostrobin, tebuconazole, and fertilizer in three of 11, six of 11, three of 8, and three of 11 experiments, respectively, when compared with inoculant alone. Imidacloprid did not affect peanut yield. Pod yield was not improved by any of the fungicide, insecticide, or fertilizer treatments when compared with inoculant alone. These experiments were conducted in fields without previous peanut plantings or where rotations were long enough to minimize disease incidence. Although benefits of disease control were not defined in these experiments, these data suggest that adverse effects on inoculant can occur when co-applied with azoxystrobin, pyraclostrobin, tebuconazole, and fertilizer.

**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**

Identification and utilization of peanut cultivars with resistance to *Cylindrocladium* black rot (CBR) is a desirable approach to manage this disease. Scientists at UGA-Tifton and USDA-ARS Tifton GA cooperated to improve greenhouse and field screening techniques for resistance to CBR, and to evaluate the reaction of selected runner-type peanut genotypes. Georgia-02C (moderately resistant to CBR) and C-99R (CBR-susceptible) were used in comparing the effectiveness of different inoculation methods in the greenhouse. Disease development was affected by both size and density of microsclerotia in soil. Use of microsclerotia at a size of 150 to <250  $\mu\text{m}$  and a density of 1 to 5 microsclerotia/g soil provided the best separation the CBR-resistant cultivar Georgia-02C and the susceptible C-99R based on root rot severity. Genotypes with varying resistance to CBR were evaluated by growth in a naturally infested field, and by inoculating plants in the field and greenhouse. Disease incidence and severity at harvest were the most effective parameters for evaluating CBR resistance in the field and greenhouse, respectively. The cultivars Georgia-02C and Georganic had the lowest disease incidence, whereas C-99R and DP-1 had the highest disease incidence in a naturally infested field in 2005 and 2006. Incidence of CBR was moderate for Georgia-01R in both years, but was inconsistent for C34-24-85. Georgia-02C and Georganic also showed partial resistance to CBR in greenhouse tests. Inoculated plants in the field had similar reaction with Georgia-02C and Georganic showing higher CBR resistance than C-99R and DP-1 in both 2006 and 2007. The root rot severities for genotypes Georgia-02C and Georganic were lower than those for C-99R and DP-1. Incidence of CBR in the naturally infested field was significantly correlated with CBR incidence in the inoculated plants in the field ( $r = 0.84$ ,  $P = 0.01$ ), but neither was correlated with

disease ratings for greenhouse experiments. Peanut genotypes are most reliably screened by inoculating plants in the field or using uniformly infested fields.

## 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

Scientists at UGA have generated an extensive EST database consisting of:

- 140,000 Sanger ESTs Produced by Sequencing Normalized and Non-Normalized cDNA Libraries Developed from Diverse Diploid and Tetraploid mRNA Sources
- 869,200 454 ESTs Produced by Sequencing Normalized cDNAs Isolated from Developing Seeds and Roots of Two *A. duranensis* Ecotypes (DUR2 and DUR25)
- A-Genome Sanger/454 EST Database—Developed for Ecotype-Specific SNP Discovery and High-Density Genetic Mapping in *A. duranensis*
- 312,000 454 ESTs Produced by Sequencing Normalized cDNAs Isolated from NC12C and New Mexico Valencia A—Developed for ‘Common’ SNP Discovery and Mapping Study
- 900,000 454 ESTs Produced by Sequencing Normalized cDNAs Isolated From Tifrunner—Developed for ‘Common’ SNP Discovery and Mapping Study

Scientists at EMBRAPA, ICRISAT, UGA, Tuskegee and UC-Davis collaborated to develop BAC (Hind III) libraries containing 80,000 clones for 6.5X coverage each from *A. duranensis* (A-genome) and *A. ipaensis* (B-genome). 40,000 BAC-end sequences using EcoRI increased coverage to 12X. BAC hybridization and fingerprinting generated over-goes for alignment of a physical map of the A-genome. SSR, EST and other markers were used to anchor the physical map to the genetic map of the A-genome. High sequence similarity was discovered over extensive regions of the A and B genomes. Differences were associated with SNPs (single nucleotide polymorphisms). These findings enable strategies for sequencing the tetraploid genome.

**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

Scientists at UGA reported the status for marker development and validation:

Source	Target	Type	Number Developed	Number Validated	Number Mapped
Genomic DNA	Random	SSR	612	556	37
Genomic DNA	Hypomethylated DNA	SSR	97	97	48
EST Database	Repeat Length Filtered	SSR	2,134	1,830	886
EST + Genomic DNA	NBS-LRR	SSCP/INDEL	248	248	33
EST + Genomic DNA	Other Resistance Genes	SSCP/INDEL	128	128	40
EST	Transcription Factors	SSCP/INDEL	143	143	143
EST + Genomic DNA	Seed Storage Proteins	SSCP/INDEL	5	5	5
EST	A-Genome Ecotype-Specific	SNP	1,536	1,127	1,127
EST	Elite Line Common	SNP	TBD	TBD	TBD

Scientists at UGA reported the status for SSR/INDEL/SSCP marker dbases:

- Primer Sequence and Polymorphism Databases for 612 Previously and 97 Newly Developed Genomic Survey Sequence SSR Markers
- Early Sanger-454 Transcript Assembly and Database of 7,000+ SSRs Identified by Mining a Tetraploid Peanut Transcript Assembly
- Primer Sequence and Polymorphism Databases for 2,134 EST-SSR and 524 SSCP/INDEL Markers
- Fluorescently End-Labeled Primers for 2,847 SSR Markers
- Unlabeled Primers for 524 SSCP/INDEL Markers

Scientists at Texas Tech, Texas A&M and USDA-ARS-Lubbock used association mapping to discover SSR markers for drought tolerance. 90 genotypes were selected from the 108 available accessions of the U.S. peanut minicore collection. The unified mixed linear model (MLM) in TASSEL software was used to perform association analysis. The MLM model controls for spurious associations incorporating population structure (Q) as well as the relatedness (K) between the individuals in the population. The Q matrix was generated using the program STRUCTURE and the K matrix was obtained using the “kinship” option employed in the software package TASSEL. Reliable markers were identified for five of the six drought tolerant-related traits, SPAD chlorophyll meter readings (SCMR), paraheliotropism, flowering, plant height and plant width. No reliable marker was identified for canopy temperature which is not a good measure of drought-tolerance. Comparatively higher number of markers associated with paraheliotropism and flowering traits showed correlation over the two years as compared to other traits. One marker was same as identified as a drought QTL by Varshney

#### 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**

Scientists at UGA-Tifton and USDA-ARS-Tifton screened 3772 M2 plants in TILLING populations finding 27 confirmed mutations; and developed CAPS (perfect) markers for the following genes:  
 Ara h 1 – Seed storage protein (Vicilin) = Ara h 1 $\beta$  (A genome) and Ara h 1 $\alpha$  (B genome)  
 Ara h 2 – Seed storage protein (Conglutin) = Ara h 2.01 (A genome) and Ara h 2.02 (B genome)  
 Ah FAD2 – Oleic acid desaturase = Ah FAD2A (A genome) and Ah FAD2B (B genome)

#### **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**

Scientists at UGA, NCSU and Texas A&M have constructed extensive transcript assemblies and have created an EST database (<http://www.peanut.uga.edu>) consisting of the following:

- *A. hypogaea* (multiple cultivars & tissues)
  - Long sequences..... 149,803
  - Short sequences..... 1,275,000 (101,132 unigenes assembled)
- *A. duranensis* (A genome)
  - Long sequences..... 35,291
  - Short sequences..... 1,000,000
- *A. ipaensis* (B genome)
  - Long sequences..... 32,787

## 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

Scientists at Texas Tech, Texas A&M and USDA-ARS-Lubbock determined Linkage Disequilibrium estimates in cultivated peanut using 92 accessions of the U.S. peanut minicore collection, Diploid progenitors *Arachis duranensis* (AA) and *Arachis ipaensis* (BB), Synthetic amphidiploid TxAG-6, and a subline of the US variety Florunner, UF 439-16-10-3-2. These lines were genotyped using a 3-primer system in Beckman CEQ-8000. The LD study was conducted with 150 SSR markers and generated a total of 11,175 pairwise comparisons. Of these, 8.9%, 4.50% and 2.86% of SSR marker pairs were found to be in LD at  $p \leq 0.05$ ,  $p \leq 0.01$  and  $p \leq 0.005$ , respectively. 12.60%, 4.85% and 1.37% of SSR marker pairs had significant LD at  $r^2 \geq 0.05$ ,  $r^2 \geq 0.1$  and  $r^2 \geq 0.2$ , respectively. These data suggest a significant genetic bottleneck during the domestication of cultivated peanut.

Scientists from Oklahoma State University, USDA-ARS Tifton GA & Stillwater OK planted entries from the peanut core collection, a subset of the USDA peanut germplasm collection, non-replicated plots in a field with a history of Sclerotinia blight caused by *Sclerotinia minor*. Variability existed among entries for reaction to Sclerotinia blight. Of the 744 entries evaluated, 11% had no disease, nearly 30% had <10% disease incidence, and only 21% had 50% disease incidence or more. Most of the resistant entries had an upright growth habit and were in early and mid-maturity groups. Many of the early maturing entries were susceptible to the foliar disease pepper spot which occurred throughout the study. Entries were selected for further evaluation in replicated plots based on a nil to low (<10%) incidence of Sclerotinia blight, adaptation and/or vigor, and other desirable characteristics such as an intermediate to prostrate growth habit and pepper spot resistance. Selected entries were retested ( $n = 62$ ) and compared to resistant (Tamspan 90), moderately resistant (Tamrun 96), and susceptible (Okrun) reference cultivars. Most entries had disease incidence less than Tamrun 96 and similar to Tamspan 90. Entries 208, 128, 804, 582, and 273 had resistance to Sclerotinia blight, pepper spot, and web

blotch. Entry 103 had good Sclerotinia blight resistance and yield, but an upright growth habit. Entry 92 had an upright growth habit and low yield, but good Sclerotinia blight resistance. Entries 92 and 103 had upright growth habits but were among the best entries for resistant to pepper spot and web blotch. Entries 426, 184, and 562 were upright and susceptible to pepper spot, but had resistance to web blotch and the best resistance to Sclerotinia blight. These entries appear to be useful sources of resistance to Sclerotinia blight for breeding programs and for increasing the probability of finding additional sources of resistance in clusters of germplasm identified within the entire USDA collection.

*Arachis pintoii* Krapov. & W.C. Gregory is a herbaceous, perennial legume, exclusively native to Brazil. It is considered a multiple use legume, being grown for forage; ground cover in fruits orchards, forest, and low tillage systems; erosion control; and ornamental purposes. Accessions of the *A. pintoii* USA germplasm collection of the National Plant Germplasm System (NPGS) were evaluated by scientists at UF-Gainesville and EMBRAPA to characterize its reaction to *Meloidogyne arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood, and *M. incognita* (Kofoid and White) Chitwood. *Arachis pintoii* germplasm presented great variability and high levels of resistance to *M. arenaria*, *M. javanica*, and *M. incognita*.

Many important disease and insect resistance genes are found in the wild peanut species, but transfer of these genes is very difficult due to ploidy and genomic differences between the species and cultivated peanut. Marker assisted selection will greatly facilitate utilizing the wild *Arachis* species, but first markers need to be associated with disease and insect resistances and the polymorphism between the wild and cultivated peanut must be documented. Scientists at NCSU developed the first database of wild peanut species polymorphism with sufficient numbers of markers to be useful in a plant breeding program. During the past two years there have been more than 2700 Simple Sequence Repeat (SSR) markers identified, of which about 20% are polymorphic among cultivated lines. In this project, multiple accessions of 40 peanut species will be evaluated with a minimum of 100 SSR markers. This research will establish a database of species/accession polymorphism which will enable more effective association of markers with agronomic traits, identify hybrids, and assure that the basic germplasm collection remains pure.

**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**

Cultivated peanut is an allotetraploid which has its origin in the hybridization of two wild diploid species followed by a spontaneous duplication of chromosomes. Although wild species are highly genetically diverse, cultivated peanut suffers from an apparent genetic bottleneck which led to very low genetic diversity and limited variability for certain traits of agronomic interest. As a result, conventional breeding progress is slower; and would benefit from the development of genetic tools necessary for modern breeding. Wild diploid *Arachis* species have a wide range resistance to diseases, but introgression of new allelic diversity from these species

is complicated by infertility barriers. Scientists at EMBRAPA and NCSU created “synthetic” polyploids from wild *Arachis* species that have the same ploidy level as *A. hypogaea* and are sexually compatible with cultivated peanut. These resources will provide plant breeders with useful germplasm with segments of the wild species genome. First traits of interest will be leaf spot resistance and seed quality.

Susceptibility to viral and fungal diseases is a major factor limiting profit in the production of virginia-type peanuts (*Arachis hypogaea* L.) in the South Carolina coastal plain. Scientists at Clemson, NCSU, Va Tech, UGA-Tifton, and UF-Mariana conducted a three-year test (2006–08) to evaluate the disease resistance of 47 experimental virginia-type breeding lines and eight cultivars. Relative to commercially available standards, cultivar Bailey (recently released by N. C. State Univ.), three sister lines (N03088T, N03089T, and N03090T), and N03091T were found to have consistently less susceptibility to tomato spotted wilt tospovirus; late leaf spot, *Cercosporidium personatum* (Berk. and Curt.) Deighton; and stem rot, *Sclerotium rolfsii* Sacc. The level of field resistance measured for these three diseases was comparable to that of a resistant runner-type cultivar, Georgia-03L. Yield was highly correlated with multiple disease resistance, and yield performance of some resistant lines exceeded the best commercial standard cultivars under reduced fungicide programs. Potential negative attributes of Bailey, its sister lines, and N03091T were a greater susceptibility to leafhopper injury, *Empoasca fabae* (Harris), and a relatively larger plant size at maturity, without well defined rows to facilitate digging. Other lines that demonstrated reduced susceptibility to both tomato spotted wilt and stem rot were N03005J and N02009. Although only evaluated in the last test year, five Univ. of Florida lines (FLMR7, FLMR9, FLMR12, FLMR14, and FLMR15) and Georgia-08V (recently released by the Univ. of Georgia) also showed some reduction in stem rot susceptibility relative to the standard (cultivar NC-V 11). Equally important, many experimental lines were identified with significantly greater disease susceptibility than current commercial cultivars. Under South Carolina production conditions, these lines would be poor candidates for advancement. Deployment of the multiple disease resistance found in these experimental cultivars offers several potential benefits beyond direct yield improvement: reduction of fungicide input costs for both foliar and soil disease control, prolonging the utility of currently available fungicides, and reduction of weather related harvest risk by allowing earlier initial planting dates.

Diagnosis of *Tomato spotted wilt virus* (TSWV) in peanut can be accomplished by enzyme-linked immunosorbent assay (ELISA) or reverse transcription polymerase chain reaction (RT-PCR) but there has been no report of a direct comparison of the success of the two assays in evaluating infection rates of field-grown peanut. Scientists at USDA-ARS, Dawson GA collected peanut root samples from field-grown plants and tested these samples by both ELISA and RT-PCR assays for the presence of TSWV. Out of 124 samples, 50 (40.3%) and 57 (46.0%) were positive for TSWV by ELISA and RT-PCR respectively. In 13.7% of these samples, ELISA and RT-PCR differed in their results. However, Chi square analysis showed no significant difference between the results for these two assays. This result supports the conclusion that ELISA and RT-PCR are comparable for detecting TSWV infection rates in field-grown peanuts.

In the Virginia-Carolina (V-C) production area, use of resistant cultivars to reduce leaf spots would be a cost-effective and environmentally safe alternative to chemical applications. Scientists at NCSU evaluated 26 interspecific hybrid derived breeding lines from 5 *Arachis* species, six resistant *A. hypogaea* checks and 11 susceptible cultivars for leaf spot resistance in field tests at the Peanut Belt Research Station in Lewiston, NC without leaf spot fungicides. Defoliation was rated on a 1–9 proportional scale with 1 = no defoliation (resistant) and a 9 = complete defoliation (susceptible). The mean defoliation score of the cultivars was  $6.8 \pm 0.1$  (range = 6.4 to 7.4), compared to  $5.3 \pm 0.1$  (range = 4.4 to 6.3) for the interspecific hybrid derived breeding lines. Some of the interspecific hybrid derived breeding lines showed levels of leaf spot resistance similar to the resistant *A. hypogaea* checks (mean =  $4.3 \pm 0.2$ ), suggesting that these breeding lines contain genes conditioning resistance to the leaf spots. The combined mean yield of the cultivars was  $2709 \pm 103$  kg/ha (range = 2296 kg/ha to 3070 kg/ha), whereas that of the interspecific hybrid derived breeding lines was  $3169 \pm 119$  kg/ha (range = 2467 kg/ha to 3767

kg/ha). Evaluation of selected grade characteristics showed that several interspecific hybrid derived breeding lines have grade similar to those of the commercial cultivars. Sixteen of the 26 interspecific hybrid derived breeding lines with five different diploid species in their pedigrees and NC 7, the commercial flavor standard for the V-C area, were also evaluated for sensory quality. No significant variation among test entries was found for the roasted peanut, sweet, or bitter sensory attributes. This suggests that high levels of leaf spot resistance can be combined with superior yield, grade and other quality factors and that some of these lines may become useful for commercial production in the V-C area.

The 2009/2010 PRWN occupied 2299 plots on 5.1 acres. All plots have been harvested, processed and returned for planting on the mainland. Primary users plus percent total nursery area were: ACI, Moore (1.8%); NCSU Isleib (9.8%); NCSU, Tallury (6.0%); NMSU, Puppala (2.9%); UF, Tillman (0.0%); TAMU, Burow (13%); USDA, Guo 26.2 %P; USDA, Holbrook (40.3%). The size of the Puerto Rican winter nursery has increased 3-fold since 2005/2006.

**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**

Scientists at USDA-ARS-Tifton and UGA Tifton GA are on schedule to demonstrate an accelerated backcrossing program to develop Tifguard High O/L. The last backcross was completed, and those seed were planted in the greenhouse. Seed from those plants will be harvested in the next 10-14 days (25 months after we began this process). Those seed will be planted in the greenhouse and screened using marker assisted selection. Individual that are homozygous for nematode resistance, and high O/L will be identified for the initial seed increase of Tifguard High O/L.

Scientists at USDA-ARS-Tifton and NCSU have coordinated the CAP winter nursery. The 2009/10 nursery consisted of 3201 hill plots representing 8 of 16 crosses from the CAP factorial mating. The crosses represented were of Tifrunner and Florida-07 with high-oleic virginia-type line N08082oIJCT, C76-16, CBR-resistant NC 3033, and species-derived disease resistant runner-type line SPT 06-06. The goal is to achieve 400 F2-derived families from each cross. Additional crosses of Tifrunner and Florida-07 with Valencia-type New Mexico Valencia A, high-oleic Spanish type OLin, TSWV-resistant hirsuta-type PI 576638 (SSD 6), and Florunner are a year behind the first set of eight. Other traits include: Early & Late leaf spots, white mold and Sclerotinia blight. Corley Holbrook and Tom Isleib harvest, shell and package seed, and plant the first eight RIL development populations twice each year; Mark Burow and Barry Tillman handle the other eight. The plan for these populations is as follows:

Summer 1: Crosses made (this occurred in the summers of 2008 and 2009)

Winter 1: F1 seeds sent to breeders' Puerto Rico Winter Nursery

**Summer 2: F2 populations on mainland, plants harvested individually (current for Group 2)**

Winter 2: F2:3 progenies grown in Genomics PRWN

**Summer 3: F3:4 progenies grown on the mainland (current for Group 1)**

Winter 3: F4:5 progenies grown in Genomics PRWN

Summer 4: F5:6 progenies grown on the mainland

Winter 4: F6:7 progenies grown in Genomics PRWN followed by F7:8 progenies on mainland  
OR

Summer 5: F6:7 progenies grown on mainland

With F6:7 or F7:8 progenies will enable phenotypic evaluations in replicated trials for QTL discovery and mapping. These CAP populations must also be maintained to provide larger amounts of seed for evaluation of traits appropriate to the specific population.

Spotted wilt of peanuts is caused by the tomato spotted wilt tospovirus (TSWV) and is currently one of the major limiting factors to peanut production in the U.S. Cultivar selection remains the most important component for reducing the risk of spotted wilt. Therefore, breeding for resistant cultivars appears to have the most potential for combating spotted wilt. Although field resistance has been selected in cultivated peanut, plants of all *A. hypogaea* lines die when they are artificially inoculated with TSWV. However, one accession of *Arachis diogeni* (a diploid wild relative of peanut) is immune to TSWV and represents the only true resistance known in species that are cross-compatible with the cultivated peanut. Transfer of resistance into breeding lines has been extremely difficult because hybrids are sterile and the low seed numbers produced has resulted in loss of the desired gene. Scientists at NCSU have initiated work to identify the gene that conditions TSWV resistance in *A. diogeni* pursuant to development of a useful molecular marker.

Scientists at USDA-ARS-Tifton used 709 (556) public available SSRs, 2136 (1768) EST-SSRs, and 1450 BAC-end sequence SSRs to screen two populations: Tifrunner x GTC20 and SunOleic 97R x NC94022 for TSWV, Early & Late Leaf Spot, White Mold, PAC and high O/L QTL. At least one DNA marker was identified for a QTL that may harbor a TSWV resistance gene in cultivated peanut. When confirmed this may be the first marker for TSWV resistance.

In peanut hybridization, distinguishing inadvertent selfs from the true hybrids may be difficult. Scientists at Texas Tech, Texas A&M-Stephenville, USDA-ARS Lubbock, NMSU, Ghana and India collaborated to differentiate between selfs and hybrids, DNA was extracted from leaf tissue of F1 or F2 plants, and SSR markers were amplified and bands separated by a novel submarine horizontal polyacrylamide gel electrophoresis (H-PAGE). By comparing the resulting banding patterns to those of the parents, 70% of the putative hybrids were shown to be true hybrids on the basis of possessing a marker allele from the male parent. The H-PAGE gels gave better band separation and differentiation of selfed progenies than agarose gels, and were compatible with the common horizontal agarose gel units. This method provides a quick assay to distinguish hybrids from inadvertent selfs, and should result in greater efficiency and more effective use of resources in peanut breeding programs.

Multiple QTL mapping populations are under construction at NMSU, NCSU, Texas A&M, Tuskegee, and USDA-ARS Tifton for: drought tolerance, RKN, maturity, Sclerotinia blight, SCR, potato leafhopper, CEW, early & late leaf spots, TSWV, O/L, CBR, and white mold.

## 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**

Tissue culture is a necessary tool in the genetic modification of peanut for the improvement of its agronomic and nutritional attributes. Since genotype can affect tissue culture responses, the main objective of this research was to determine the optimum concentration of auxins and cytokinins in the basal media needed for organogenesis from *Arachis hypogaea* L. cv. Florman INTA. Scientists from UGA-Tifton and Argentina dissected the first two leaves (2–5 mm in length) from aseptically germinated seeds and cultivated on Murashige and Skoog (MS) medium supplemented with 16 combinations of  $\alpha$ -naphthaleneacetic acid (NAA) (0.01 and 1 mg/l) and benzyladenine (BAP) or kinetin (KIN) (1 to 10 mg/l) during the initiation stage. Bud regeneration occurred in all growth regulator combinations, but the maximum number of buds per explant (1.2) was regenerated at 1 mg/l NAA with 3 mg/l BAP. Development of buds into shoots was readily achieved by transferring regenerated buds onto fresh medium containing 0.01 mg/l NAA (without BAP). Roots were induced to grow when shoots were transferred to medium containing 3 mg/l of NAA. The vigorous root system allowed for a high survival rate of the plantlets after transplanting. The overall efficiency of the system was 15%. Plants transplanted into soil were completely normal and capable of producing seeds.

**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**

Scientists at the Donald Danforth Plant Science Center have developed effective vectors for a number of traits in *Agrobacterium* mediated transformation of cultivated peanut. However, the nature of these experiments may not be disclosed at this time.

**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**

Scientists from UGA-Tifton, USDA ARS, New Orleans, Texas Tech, Cornell, UGA-Athens, Japan and Argentina introduces a nonheme chloroperoxidase gene (*cpo-p*) from *Pseudomonas pyrocinia*, a growth inhibitor of mycotoxin-producing fungi, into peanut via particle bombardment. The

expression of the *cpo-p* gene is predicted to increase pathogen defense in peanut. Embryogenic peanut tissues were bombarded with gold particles coated with plasmid pRT66 carrying the *cpo-p* and hygromycin phosphotransferase (*hph*) genes, under the control of a double CaMV 35S and a single CaMV 35S promoter, respectively. Selection for hygromycin-resistant somatic embryos was performed on a liquid medium containing 10–20 mg/L hygromycin 3–4 days after bombardment. The integration and expression of the *cpo-p* gene was confirmed by Southern, Northern and Western blot analyses. In vitro bioassay using crude protein extracts from transgenic T0, T1, and T4 plants showed inhibition of *Aspergillus flavus* hyphal growth, which could translate to a reduction in aflatoxin contamination

Scientists at Virginia Tech are preparing to release, pending regulatory approval, Blight Blocker peanuts with engineered resistance to Sclerotinia blight. In addition, GM fortification of folate levels is proceeding toward transformation of the cvs Bailey, VA98R and VT950683-3.

**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**

Scientists at Virginia Tech have provided APHIS with substantial data demonstrating low risk of gene flow from GM peanuts to other plant species. Regulatory approval is expected, however requirements from EPA are not known. This work will lead to the first regulatory approval for GM peanuts.