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Research, Education & Economics

Agricultural Research Service

Office of National Programs

International Peanut Genomic Research Initiative

Strategic Plan for 2012 to 2016

Characterization of the Peanut Genome

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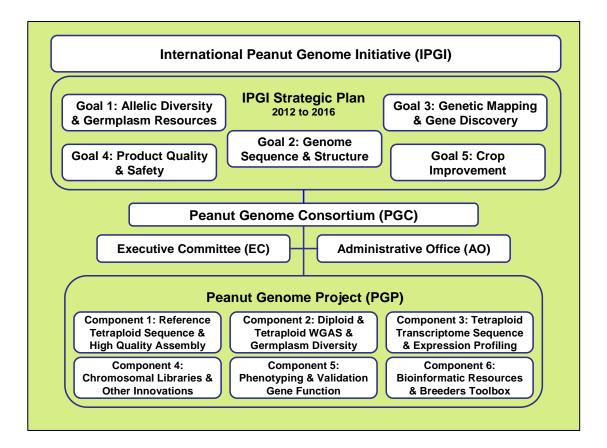


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Executive Summary

Vision Statement: An international workforce will produce a high quality reference genome sequence of cultivated peanut that is anchored to chromosomal linkage groups. This genomic resource will establish a knowledge platform for enhanced quality trait technologies, disease management systems, agronomic varieties that ensure the economic competitiveness of peanuts and the creation of a better future through global food security.

Process & Development of the Strategic Plan: The first *International Strategic Plan for the Peanut Genome Initiative 2008-2012* was developed during the second International Peanut Genome Initiative (IPGI) meeting, *Advances in Arachis Through Genomics and Biotechnology: An International Strategic Planning Workshop* in Atlanta, GA in October, 2007. The conference was organized by the leaders of the Peanut Genomics Initiative in association with the Peanut Foundation, the International Crops Research Institute for the Semi-arid Tropics (ICRISAT), and representatives of three institutes in China (Shandong Academy of Agricultural Sciences). Seventy-three participants with expertise in genomics, transformation technologies, genetics, plant pathology, food science, agronomy, entomology, and plant germplasm preservation represented South America (Brazil, Argentina), Asia (China and India), and Africa (Benin, Mali, Nigeria, Kenya, and South Africa). Financial support was received from: Bayer CropScience Inc., the Georgia Peanut Commission, MARS Inc., J.M. Smucker Inc., the National Peanut Board, North Carolina State University, the Peanut Company of Australia, the Peanut Foundation, USAID Peanut Collaborative Support Program, USDA Agricultural Research Service (ARS), and USDA Cooperative State Research, Education and Extension Service.

An internal review of progress toward research priorities in the Strategic Plan for 2008-2012 was documented in 2010 and published online in the *International Peanut Genome Initiative: Most Significant Accomplishments for Improving crop productivity & Protection, Product Safety & Quality.* Based on that report, the first IPGI meeting was convened on genome sequencing in Atlanta GA in December 2010. Participants included: Pat Donahue (Kraft), Jim Elder (Smuckers), Howard Shapiro and Victor Nwosu (MARS), Joe Bodiford and Tim Burch (Georgia Peanut Commission), Max Grice (Birdsong Peanut Company), Alan Orloff (Clint Williams Company), Greg May (NCGR), Roy Scott (USDA, ARS, ONP), Richard Michelmore, Scott Jackson, Mark Burow, Peggy Ozias-Akins, Baozhu Guo, Corley Holbrook, and Tom Stalker. Consensus was reached on the following committed steps toward launching a project to generate a high-quality chromosomal scale map of the peanut genome.

Plans also were initiated to update IPGI strategic goals and research priorities to accommodate the genome sequencing project. A strategic planning workshop was included in the technical program of the 5th meeting of the IPGI, *Advances in Arachis through Genomics & Biotechnology* (AAGB-2011) in June 2011 in Brasilia, Brazil. 93 registered participants represented 10 countries (Brazil, Argentina, China, Japan, Australia, France, Senegal, Mexico, United Kingdom, and U.S.) The technical program featured plenary and breakout discussion sessions where facilitators captured stakeholder input that helped define research goals, performance measures and anticipated products for the following research areas:

<u>Allelic Diversity & Germplasm</u> Resources (Tom Stalker, NC State University; Jose Valls, EMBRAPA) Goal: Characterize genetic diversity and transfer useful genes into new sources of germplasm for crop improvement. Objectives: 1) Conservation of A. hypogaea and wild peanut species collections so that pure lines are available for analyses; 2) Evaluation of A. hypogaea and Arachis species for important agronomic traits; 3) Improvement of access to genetic diversity through germplasm resources that facilitate transfer of useful traits to cultivated peanut.

<u>Genome Sequencing & Structural Characterization</u> (Scott Jackson, University of Georgia; David Bertioli, University of Brasilia) Goal: An ordered, anchored, annotated and accessible genome sequence to facilitate peanut improvement. Objectives: 1) Production of a reference genome sequence for cultivated tetraploid peanut; 2) Comparative structural analyses of A and B-diploid genomes, 3) Annotation of the genome sequence; 4) A 'breeder-friendly' database to house and curate the primary and annotated genome sequences; 5) Evaluation of emerging technologies for genome sequencing and characterization

<u>Genetic Trait Mapping & Gene Discovery</u> (Peggy Ozias-Akins, University of Georgia; Rajeev Varshney, ICRISAT). Goal: Enhancing crop improvement using genetic and genomic tools. Objectives: 1) Molecular marker resources in peanut; 2) Genetic and consensus maps for diploid and tetraploid peanut; 3) Phenotypic validation of gene predictions; 4) Genome mapping and allelic analysis through Genome-Wide-Association-Studies; 5) Catalog of expressed genes and profile gene expression in cultivated peanut

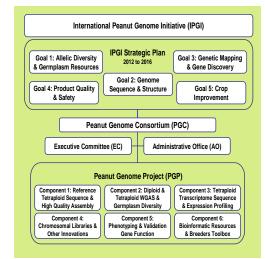
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<u>Crop Improvement</u> (Corley Holbrook, USDA-ARS; Ignacio Godoy, EMBRAPA, Mark Burow, Texas A&M University). Goal: Ensure an adequate supply of agronomic and high-quality peanut cultivars for commercial production. Objectives: 1) Enhanced understanding of genetic diversity and genomic variation for important traits in Arachis; 2) Improved methods to develop genetic resources with useful traits; 3) Improved selection efficiency through use of genomic resources; 4) Optimized fungicide & pesticide application schedules in peanut production; 5) Improved understanding of the epidemiology of peanut pathogens.

The product of their work, presented herein, serves as the foundation for the IPGI for 2012 to 2016. This plan ensures that research conducted by the IPGI is relevant to the needs of the global peanut industry, provides a basis for project implementation & assessment of program performance toward accomplishment of IPGI goals

<u>Creating a Better Future through Global Food Security:</u> The central thrust of the IPGI Strategic Plan will be implemented by the Peanut Genome Consortium (PGC), a coalition of international scientists and stakeholders engaged in the Peanut Genome Project (PGP). PGC is governed by published *Policies & Procedures*.

Contributing members are: Howard Valentine, The Peanut Foundation (*Administrator*); Howard Shapiro & Victor Nwosu, MARS, Inc; Richard Michelmore, University California-Davis (*Co-Chairperson*); Lutz Froenicke, University California-Davis; Scott Jackson, University Georgia (*Chairperson*); Peggy Ozias-Akins, University Georgia (*Co-Chairperson*); Baozhu Guo (*liaison to China*), Corley Holbrook & Brian Scheffler, USDA ARS; Greg May, National Center Genome Resources; David Bertioli, University Brasilia (*liaison to South America*); Soraya Bertioli, EMBRAPA; Rajeev Varshney, ICRISAT (*liaison to India*); Xingyou Zhang, Henan Academy of Agricultural Sciences; Xun Xu, The Beijing Genome Institute; Xingjun Wang, Shandong Academy of Agricultural Sciences; Mark Burow, Texas A&M University; Farid Waliyar, ICRISAT



(*liaison to W. Africa*); Graeme Wright, Peanut Corporation of Australia (*liaison to Australia*); Sachiko Isobe, Kazusa DNA Research Institute (*liaison to Japan*); Ran Hovav, Agricultural Research Organization of the Volcani Center (*liaison to Israel*); Tom Stalker, North Carolina State University; and Richard Wilson, Oilseeds & Biosciences Consulting. **Ex Officio** members are: Roy Scott, USDA, ARS, ONP; Pedro Arraes, President, EMBRAPA; Jean-Marcel Ribaut, Director, The GENERATION Challenge Grant Program; Chairman Luo Fuhe, Vice-Chairman CPPCC and Executive Vice-Chairman CAPD Central Committee, Beijing; and David Hoisington, Deputy Director General for Research, ICRISAT

The PGP is different from other genome projects in that it includes: 1) genome distinguishing gene expression studies that will generate a transcriptome based atlas of expressed protection & quality gene profiles; 2) characterization of genetic diversity through identification of genotypic haplotypes among accessions of the USDA, Chinese & ICRISAT germplasm collections; 3) Genome Wide Association

Studies that will identify useful gene markers for targeted traits in cultivated & wild populations; 4) a high density consensus genetic map that anchors DNA markers to chromosomes of cultivated and wild peanut genomes; and 5) a high resolution assembly of BACs from a reference population of recombinant inbred lines.

Specific PGP goals include: 1) development of a high quality chromosome scale draft of a tetraploid (cultivated species) reference genome sequence, 2) high throughput genome and transcriptome characterization of tetraploid, amphidiploid and diploid (progenitor species) germplasm, 3) phenotypic trait association with mapped genetic markers, and 4) interactive bioinformatic resources for data curation and analysis. These efforts will enable molecular breeding approaches for enhancing peanut yielding ability, optimizing resistance to diseases and insects, tolerance to environmental stresses, and improved quality traits. This technology will promote peanut crop competitiveness and enhance grower's profitability in an environmentally sustainable manner.

Summation: Peanut varietal development is totally a function of the public research sector worldwide. Genome sequence assisted breeding methods is essential for timely increases in crop productivity and quality to help ensure global food security. However, the cultivated peanut poses one of the most difficult challenges that has been attempted to date for crop genome sequencing. The size of the peanut genome is nearly equal to the human genome, and is distinguished by duplicate sets of 10 chromosomes from two different Arachis species. An integrated international effort is needed to accrue the availability of resources that are needed to meet this challenge. The PGC, by virtue of the latest advances in genomic technology, is well positioned to accomplish IPGI priorities and successfully deliver a high quality reference genome sequence of cultivated peanut within 2 to 3 years because of multilateral multi-disciplinary cooperative research relations with world class scientists, organizations and institutions in the U.S., China, Brazil, India, Japan, W. Africa, Australia, and Israel.

This strategic plan establishes a foundation for PGP and other relevant projects under an internationally coordinated peanut research program for the next 5-years. Coordination among international peanut research communities, plus interaction with other legume genomics communities such as *Glycine max* and *Medicago truncatula*, should effect better utilization of limited resources available for research. Establishing collaborative working relations across continents also will enable timely solutions that are needed to ensure a better future through global food security.

Information on the International Peanut Genome Initiative, the Peanut Genome Consortium, and the Peanut Genome Project may be accessed at:

http://www.peanutbioscience.com

Introduction

Cultivated peanut (*Arachis hypogaea* L.) is grown on 21 million hectares world-wide, and is a principle source of human nutrition predominantly in Asia and Africa. Peanut is distinguished with high oil content with a large percentage of oleic acid which confers superior oxidative stability and certain health benefits. Peanut oil also is low in saturated fat content, another trait that has been shown to lower serum LDL-cholesterol levels. In addition, endogenous nutraceuticals, such as resveratrol, also may improve cardiovascular health.

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

Investment in peanut genomics continues to lag other crop species, but efforts in related cool and warm season legumes are expanding both in the U.S. and abroad. The importance of coalitions among legume

communities was defined by a 2001 meeting in Huntsville, MD where scientists first developed a strategy to advance genomics across legume species. The product of that conference, the U.S. Legume Crops Genomics White Paper, outlined six research needs: (i) genome sequencing of strategic legume species, (ii) physical map development and refinement, (iii) functional analysis, (iv) development of DNA markers for comparative mapping and breeding, (v) characterization and utilization of legume biodiversity, and (vi) development of legume data



resources. Another meeting was convened in Santa Fe, NM to refine objectives and to develop a more definitive white paper for legume research entitled, *Legumes as a Model Plant Family: Genomics for Food and Feed*. This plan focused genomic resources toward crop protection and crop product quality; and served as the impetus for the development of genome initiatives for peanut and each of the other legume crops.

Development of the Peanut Genome Initiative. The market competitiveness of peanuts is threatened by losses in productivity and quality attributed to diseases, pests, environmental stresses and allergy or food safety issues. The germplasm repositories and gene banks maintained by the USDA-ARS National Plant Germplasm System (NPGS) and the Consultative Group on International Agricultural Research (CGIAR) typically provide the first line of long-term defense against those problems. In the United States, the Peanut Germplasm Collection at Griffin, GA contains ca. 9900 accessions of 72 species from 106 countries. Natural genetic diversity among wild relatives and accessions of cultivated peanut provides the primary means to attain durable resistance or tolerance to major constraints such as peanut root-knot nematode, tomato spotted wilt virus, drought, and pre-harvest aflatoxin contamination. Even so, new technology is needed to facilitate more rapid discovery of genes that confer a remedy to these constraints and the incorporation of those genes into elite germplasm by conventional and biotechnological breeding methods in a timely manner. Genomic, proteomic and bioinformatic research can provide the genetic tools to effectively mine useful genes from the wealth of natural genetic diversity that exists in peanut.

However, to realize such ability, it was necessary to establish an infrastructure for genomic research with a coordinated research approach to guide the effective development of peanut germplasm, genetic tools and bioinformation. In March 2004, 26-scientists with expert knowledge of critical fields in genetics and plant molecular biology participated in a workshop hosted by The Peanut Foundation/ American Peanut

Council in Atlanta, GA. These scientists reviewed the status of peanut genomic research, which was documented in the book, *Legume Crop Genomics* published by AOCS Press under the auspices of the U.S. Legume Crop Genome Initiative (LCGI). In affiliation with LCGI and other stakeholders, the Peanut Genome Initiative (PGI) was launched at the Atlanta workshop. An advisory committee, representing the broad interests of industry and the peanut research community, was selected to guide the growth of the PGI. A *Strategic Plan for the Peanut Genome Initiative 2004-2008* was developed that outlined research goals objectives, performance measures and significant near-term milestones representing 'quantum leaps' in the advancement of this emerging science.

With stakeholder input, research priorities were identified and aligned with the goals or components of the PGI Strategic Plan. In June 2004, the advisory committee charged individuals to initiate team building toward achieving all performance measures for each Component, and tasked a writing team to develop an Action Plan that defines those performance measures of the Strategic Plan that addressed initial high priority research program needs. The *National Action Plan for the Peanut Genome Initiative: Application of Plant Genomics to Mitigate Peanut Allergy* was adopted by the peanut research community at the American Peanut Research & Educational Society (APRES) meetings in San Antonio, Texas in July 2004. At the APRES meeting in Portsmouth, Virginia in July 2005, the PGI amended the Action Plan to include ancillary performance measures specific to the immunology of peanut proteins in model systems (PGI Action Plan v-2.4, March 2006).

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



In 2006, the PGI sought to expand its mission through outreach to the international peanut research community. The foundation for this effort was established in November 2006 in Guangzhou, PRC at the "International Conference on Aflatoxin Management and Genomics" when delegates from nine nations voted to maintain an open dialog to explore opportunities for cooperative research, and took steps toward achieving that goal with annual meetings. The 2nd conference of the international peanut research community was held in October 2007 in Atlanta GA. *Advances in Arachis through*

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

The International Strategic Plan for the Peanut Genome Initiative 2008-2012: Improving Crop Productivity & Protection, Product Safety & Quality, was a major product of the Workshop in Atlanta. Developed with stakeholder input that plan defined the rationale and scope of the research strategy to enhance peanut productivity; increase protection against diseases, pests and stresses; and to improve crop product safety and quality. The performance measures (research objectives) under each Goal stated the



problems that were addressed, and anticipated products that world meet the objectives. The periodic milestones for each performance measure constituted a 'yardstick' by which research progress was measured. Annual accomplishments by U.S. and international collaborators were reported at subsequent AAGB meetings in Hyderabad, India (2008), Bamako, Mail (2009), and Brasilia, Brazil (2011). The 6th AAGB meeting is scheduled to be held in Zhengzhou, China (2013).



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Plans also were initiated to update IPGI strategic goals and research priorities to accommodate the genome sequencing project. A strategic planning workshop was included in the technical program of AAGB-2011 in Brasilia Brazil. 93 registered participants represented 10 countries (Brazil, Argentina, China, Japan, Australia, France, Senegal, Mexico, United Kingdom, U.S.) The technical program featured plenary and breakout discussion sessions where facilitators captured stakeholder input on research goals, performance measures and anticipated products for the following research areas:

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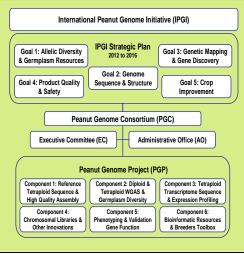
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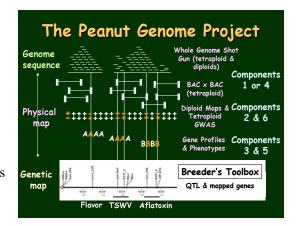
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Specific PGP goals include: 1) a high quality chromosome scale draft of a tetraploid (cultivated species) as the reference genome sequence, plus high density maps of both progenitor and synthetic amphidiploid genomes; 2) high throughput transcriptome characterization of the reference tetraploid cultivar; 3) characterization of gene space in amphidiploid and diploid (progenitor species) germplasm, 4) phenotypic association with mapped genetic markers, and 5) interactive bioinformatic resources for data curation and application in a breeder's toolbox to enable molecular breeding approaches for enhancing peanut yielding ability, optimizing resistance to diseases and insects, tolerance to environmental



stresses, and improved quality traits. This technology will promote peanut crop competitiveness and enhance grower's profitability in an environmentally sustainable manner.

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Strategic Research Goals for Peanut Genomics Research

Allelic Diversity & Germplasm Resources

Crop improvement depends upon utilizing genetic resources with unique and useful traits for disease, insect, and quality characters. Properly maintained germplasm collections serve as the foundation of genetic resources for plant breeding. Understanding the genotypic and phenotypic variation within the collections is facilitates discovery of useful DNA markers for genome assembly and comparative analysis. Associating molecular markers with phenotypic traits in a wide range of genotypes will enable marker assisted pre-breeding to enhance the efficiency of introgressing multiple traits into an elite cultivar.

Goal 1: Characterize genetic diversity and transfer useful genes into new sources of germplasm for crop improvement

Performance Measures

1.1 Conservation of *A. hypogaea* and wild peanut species collections so that pure lines are available for analyses. The cultivated peanut collection contains approximately 14,000 accessions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 10,000 in the U.S., and other large collections in China and South America. In addition, approximately 1000 accessions of *Arachis* species are being preserved in nurseries in Brazil, Argentina, India, and U.S. collections. It is critical that all accessions are backed-up at multiple locations to preserve these valuable genetic resources.

Milestones and Deliverables:

- An updated catalog of wild species accessions with origins of collection and nursery site for each germplasm line that is being preserved in cultivation.
- Identification of *A. hypogaea* accessions in the U.S., ICRISAT, China, EMBRAPA and other germplasm collections that must be replaced with viable seed.
- Freedom to exchange germplasm in a systematic manner among institutions & nations.
- International adoption of on-line comprehensive uniform standards for hebarium and photo-documented peanut phenotypic descriptors.
- Sound, long-term infrastructure for preserving peanut genetic resources.
- **1.2 Evaluation of** *A. hypogaea* accessions for important agronomic traits. Although large numbers of accessions of both cultivated and wild species are being maintained in germplasm collections relatively little evaluation work has been completed other than for peanut rust and late leaf spot where more than 10,000 entries of each have been screened. Core and mini-core collections have been developed in the U.S., ICRISAT, and China to evaluate segments of the larger collections. Work has been initiated to evaluate these core collections for genetic variation in pre-harvest aflatoxin resistance, certain diseases and quality traits. However, the intrinsic diversity resident in thousands of accessions remains unknown and therefore untapped.

Milestones and Deliverables:

• Identification of QTL for segregating traits among recombinant inbred lines from mapping populations created for the U.S. 'CAP' research program.

- Discovery of sources of resistance for leaf spot resistance and other crop protection traits where high levels of resistance have not been found in cultivated peanut.
- A diversity panel of accessions representing the genetic and phenotypic diversity in the peanut germplasm collections held in India, China, and the U.S.
- Customized core and mini core-collections that facilitate genotypic and phenotypic characterization of a) foliar diseases, b) soil born diseases and insect pests, c) quality traits, and d) parents commonly used in breeding programs.

1.3 Improvement of access to genetic diversity through germplasm resources that facilitate transfer of useful traits to cultivated peanut.

A. hypogaea has a very narrow genetic base, leading to lack of variability in important traits, limited availability of allelic combinations and consequently to genetic restrictions in productivity. In contrast, wild diploid Arachis species are genetically very diverse and have been selected during evolution by a range of abiotic and biotic stresses, providing a rich source of variation in agronomic traits; but sterility barriers hamper the use of wild species in breeding. However, synthetic amphidiploids of wild Arachis species that incorporate different genomes may produce fertile hybrids with peanut, thus providing a route for exploiting the genetic diversity of wild species. High levels of genetic variation within and among closely related *Arachis* species leads to potential use for gene identification, marker assisted selection, and introgression to the cultivated species.

Milestones and Deliverables:

- Standard procedures for screening accessions of section *Arachis* for new sources of disease resistances and molecular variation.
- Diploid RIL mapping populations for both genomes of the progenitor species (AA: *A. duranensis x A. stenosperma*; BB: *A. ipaensis x A. magna*).
- Synthetic amphidiploid RIL mapping populations that exhibit substantial allelic variation from progenitor diploid species
- A tetraploid mapping population derived from *A. hypogaea* x a synthetic amphidiploid that presents a polymorphism-rich model for allelic variation between diploid species and cultivated peanut.
- Generation of backcross progeny that demonstrate the utility of amphidiploids in the introgression of agronomic traits into cultivated peanut.

Genome Sequencing & Structural Characterization

The origin of *A. hypogaea* was via hybridization of two diploid wild species followed by spontaneous duplication of chromosomes. The resultant allotetraploid plant would have had hybrid vigor, but was reproductively isolated from its wild relatives. Therefore, all peanut land races are probably derived from a very narrow genetic base, which may explain the relatively low diversity for agronomic traits. 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. A chromosomal scale draft reference sequence of a tetraploid peanut genome would help overcome the apparent genetic 'bottleneck' by facilitating discovery of gene-rich genomic regions and the development of genomic maps, gene markers, and other technologies that will help capitalize on the genetic potential of peanut as a healthful and profitable crop for food, feed and fuel applications.

Goal 2: An ordered, anchored, annotated and accessible genome sequence to facilitate peanut improvement

Performance Measures

2.1 Production of a reference genome sequence for cultivated tetraploid peanut.

A reference genome sequence of peanut (*Arachis hypogaea L. Fabaceae*) will be produced with genomic DNA from *Arachis hypogea* cv. Tifrunner, cv. GT-C20 and 100 lines of a RIL population developed from Tifrunner x GT-C20. One parent will be deep sequenced using a combination of whole genome shotgun and BAC-by-BAC approaches. Sequence information from the other parent, RILs, and a diploid A-genome progenitor species will facilitate assembly of the reference sequence for tetraploid peanut.

Milestones & Deliverables

- BAC x BAC assembly of chromosomes of the cv Tifrunner
- A high-density genetic map based on whole genome shotgun sequencing and resequencing of the cv Tifrunner, GT-C20 & hybrid RILs
- A high-resolution genome assembly of each haplotype present in the 2 genomes of the allotetraploid.
- Raw data sufficient to allow genome assembly
- Physical and genetic coordinates of the scaffolds and contigs in chromosomal linkage groups based on analysis of the segregation data from the RILs.
- Data on genome assembly quality (euchromatic & gene region coverage with sequencing depth)
- Genome annotation results (repeat analysis; protein-coding genes including gene structure prediction and gene function annotation; non-coding microRNA, tRNA, rRNA and other ncRNA; transposons and tandem repeats)
- Comparative genomics and evolution analysis (chromosome structure variation detection of specific genome regions; fast evolutionary gene detection; synteny blocks; and gene family analysis)
- Validated genome assembly with a linear order of the contigs in chromosomal linkage groups.

2.2 Comparative structural analysis of A and B diploid genomes

Preliminary investigations suggest that the cultivated peanut genome may lack structural complexity. Significant portions of the tetraploid genome may consist of almost identical DNA sequences that are duplicated or repeated multiple times. Such a situation increases the probability of erroneous reassembly of DNA sequences in the proper order. Given the high probability of this complication, various steps may be taken to tailor the genome sequencing strategy to minimize errant sequence assembly. Possible solutions may include using longer reads, diverse scaled paired end reads or BAC x BAC approaches on the tetraploid genome. However, the use of a high-quality diploid sequence as a template is an additional measure that may be taken to ensure correct de novo assembly of the tetraploid genome. In-silico experiments using homeologous A and B BAC sequences suggested that the A and B sequences assemble satisfactorily using simulated Hi-Seq paired-ends at 60x coverage. Therefore, comparative association of BAC sequences from A and B genomes would provide useful information as to the properties of the tetraploid genome.

Milestones & Deliverables

- BAC libraries for wild species representing the component genomes of A. hypogaea
- A set of characterized probes for cytogenetic analysis of Arachis species
- Assembly simulations using 10 homologous A and B-genome BAC sequences
- A genotyping system with about 10 markers per chromosome
- Markers linked to genes/QTL based on SSR, intron, SNP, or BAC-end sequence markers from diploid mapping populations
- Anchor marker sequences based on GWAS of RILs from A, B, and amphidiploid populations
- Assignment of gene expression profile origin among A and B genomes
 - Validated A and B genome assembly with a linear order of the contigs in chromosomal linkage groups.

2.3 Annotation of the genome sequence

Annotation of any genome is complicated when repetitive DNA sequences comprise a majority of the DNA in a genome. Careful annotation of the repetitive sequences will aid in the useful annotation of genes for application to peanut improvement. Therefore, annotation of the reference genome relies upon good gene transcript information. Large-scale transcript sequencing in various plant organs, stages of development, and exposure to environmental variables will facilitate the annotation of gene/protein coding regions of the reference peanut genome. This platform will guide future efforts to provide annotation for specific traits (e.g. resistance genes). A long-term funding program should be established to ensure that the needs of the peanut industry, consumers and stakeholders are met in a timely manner.

Milestones & Deliverables

- An International Annotation Group to standardize peanut genome annotation and the establishment of a controlled vocabulary nomenclature.
- Conversion of linkage groups to internationally accepted numbered chromosomes Community standards for expression, protein and metabolite profiling platforms and data.
- Confirmation of predicted expressed genes in A, B and tetraploid transcriptomes
- Overlay of transcriptome, genetic and physical maps from A, B, amphidiploid and whole tetraploid genome sequences

- 15
- Databases that connect DNA sequences to linkage groups, chromosomes, QTL, candidate genes, polymorphisms, and eventually phenotypic traits
- Tools for the identification of candidate genes underlying QTLs.
- Integration of phenotypic descriptors (e.g., digital image or measurement data) with genetic maps and other genetic data derived from accessions of germplasm collections.
- Integration of mutagenesis, knockout, expression data for gene function annotation.
- Resources for annotation of transposons, repeats, small RNAs, and conserved non-coding elements.
- Integration of annotation data with orthologous genes, expressed genes and regulatory networks in a comparative context with other plant genome resources.
- Correlation of expression profiles with QTL to measure both copy number and allelic variation.

2.4 A 'breeder-friendly' database to house and curate the primary and annotated genome sequences

Bioinformatic resources provide access to interactive programs for sequence analysis Genome user-guidelines for genomic data submission and application also are needed to establish the best means for implementing a breeder-friendly database. The database will include markers and genetic maps, QTL, phenotypes, diversity, breeding information and population information, and will be expanded to meet the needs of the user community. Curation of the primary sequence as part of the public database will require ongoing expert quality control and updating to incorporate additional information that improves the utility of the sequence in various applications.

Milestones & Deliverables

- A PGP Informatics Steering Committee to address current and future informatics needs.
- A peanut genomic database that facilitates navigation from maps to genes to traits
- An integrated database including available genetic stocks, mutants and germplasm collections
- A HapMap browser that connects the sequence to polymorphisms for traits of interest
- Ability to map RNA-seq and Sanger reads from expression data onto QTL data
- Integration of genome sequence with physical, genetics and transcriptome maps
- Molecular tools for the identification of candidate genes underlying QTLs.
- Integration of plant trait and phenotypic data with genetic maps and other genetic data.
- Workshops/jamborees for community-driven annotation updates at the gene family level
- Integrated mutagenesis, knockout, and expression data for gene function annotation.
- Annotation resources for transposons, repeats, sRNAs, and conserved non-coding elements
- A plan for long-term curation of the peanut genome sequence, updates on annotation, correction of assembly errors and incorporation of other relevant data

2.5 Evaluation of emerging technologies for genome sequencing and characterization

Technological advances in genome sequencing and characterization will be considered as they become available. Two potential opportunities that may be considered are: 1) direct sequencing using the Pacific Biosciences platform for single molecule real-time analysis; and 2) analysis of chromosome specific libraries. Direct sequencing technology is potentially a very powerful complement to the short reads generated by Illumina methods. Strobe sequencing in particular could be useful for scaffolding contigs and assigning haplotypes in heterozygous and tetraploid

genomes. If individual peanut chromosomes can be separated by microfluidic techniques, the DNA should be suitable for whole genome amplification and small insert paired-end library sequencing. Analysis of chromosome specific libraries would complement the BAC-based, whole-genome sequencing approach and should strengthen the assignment of homeologous sequences.

Milestones & Deliverables:

- Evaluation of the utility of the Pacific Biosciences platform by UC-Davis.
- Pending collaboration with Stanford University, UC-Davis will evaluate microfluidic methods that separate and amplify individual chromosomes from single peanut cells of the cv.Tifrunner.

Genetic Trait Mapping & Gene Discovery

Marker-assisted selection (MAS) is now a well-established and powerful technology in plant genetics for indirect selection of traits at the seedling stage, thus speeding up the process of conventional plant breeding. To use this powerful technology in peanut improvement, molecular markers (SSRs, DArT, SNPs), genetic maps (diploid, tetraploid) and precise phenotyping data are needed to identify genomic regions that control economically important traits. A variety of genetic maps (diploid, tetraploid) are being developed for identification of quantitative trait loci (QTLs) for agronomic traits of interest to peanut breeders. A catalog of all useful markers for assignment of important trait loci to specific chromosomes on a consensus map for peanut is needed to develop comparative maps between diploid and tetraploid species, and compile information on known QTLs for economically important traits in peanut. The outcome of these efforts will enable effective and precise molecular breeding for peanut improvement.

Goal 3: Enhancing crop improvement using genetic and genomic tools

Performance Measures:

3.1. Useful molecular marker resources in peanut

More than 5000 SSRs, DArT arrays (15,360 features) and thousands of SNPs have been developed in peanut. Genomic screening with DArT and SNP markers has indicated low levels of polymorphism in cultivated genotypes. Therefore, SSR markers are still the marker of choice for peanut genetics and breeding applications. Given a reference genome sequence for peanut, the utility of DArT and SNP markers will empower advances in genotyping by sequencing (GBS) methods. The peanut genomics community will be moving toward GBS which will reveal thousands of new SSR and SNP markers. Future advances in molecular marker technology will be achieved with the development of allele specific markers and focused efforts to make them useful in modern breeding programs.

Milestones & Deliverables:

- Centralized accessible database for molecular genetic marker information
- Identification of a core set of informative markers for deployment in genotyping systems suitable for use in breeding programs
- DNA markers that contribute to the assembly and annotation of the peanut genome
- Allele specific DNA markers that can be used in pre-breeding for disease and pest resistance including TSWV, Early & Late Leaf Spot, CBR, nematodes, PAC, drought tolerance

- Allele specific DNA markers that can be used in pre-breeding for quality traits including seed fatty acid composition, flavor quality, nutritional benefits, and other seed composition traits
- Allele specific DNA markers for peanut yielding ability and other agronomic traits

3.2 Genetic and consensus maps for diploid and tetraploid peanut

Several genetic maps for diploid and tetraploid genomes have been constructed and are being developed by several research groups in the U.S., China, Japan, France, Brazil and India. Genotyping data for these mapping populations should be made available in the public domain for the benefit of the community. These data can be used to compile a consensus map, which will be useful in genome assembly as well as for aligning the physical maps of AA and BB genomes. Prototype genetic maps of the diploid AA and diploid BB genome are based on the crosses of: A. duranensis x A. stenosperma (A genome) and A. ipaënsis x A. magna (B-genome). The parents of each of these crosses contain the most likely ancestral species of the cultivated peanut. Levels of polymorphism within these diploid populations are favorable for marker and linkage map development. For the parental genotypes of the AA population, about 50% of the SSR markers are polymorphic, and intron regions have on average 1 SNP per 88 bp and 1 indel per 1640 bp (unpublished results, University of Aarhus). About 40% of the SSR markers show polymorphism for the BB population. Genic markers will be used to saturate the AA and BB diploid maps. The same markers will be used to develop a tetraploid map using the F2 progeny from a cross between A. hypogaea and a selected synthetic amphidiploid. Sets of selected markers will be used for the creation of a high throughput genotyping system for multiple loci. In addition, legume anchor markers are being placed on the genetic maps of diverse legumes, bean, lupin, and *in-silico* on the genome of L. *japonicus* (through other funded projects), therefore, comparison of the map positions will allow the determination of synteny within legumes.

Milestones & Deliverables

- Compilation of marker genotyping data for different mapping populations
- Improved understanding of the genome affinities of wild species within the section *Arachis*.
- Characterized probes for cytogenetic analysis of Arachis
- Improved gene rich reference maps for the component AA and BB, diploid genomes
- Identification of candidate genes for induced and constitutive traits and their placement on the genetic map of Arachis.
- Integration of diploid and tetraploid maps
- A consensus genetic map for cultivated tetraploid
- Placement of candidate genes for disease resistance on the genetic map of Arachis.
- Analyses of genome synteny of Arachis with other legumes, especially the model plant L. japonicus

3.3 Phenotypic validation of gene predictions

A majority of the genetic maps developed for diploid and tetraploid peanut are being used for identification of markers linked with QTLs controlling several foliar diseases (early leaf spot (ELS), late leaf spot (LLS), rust, groundnut rosette disease (GRD), tomato spotted wilt), soil borne pests/diseases (root-knot nematode, white mold, Sclerotinia, cylindrocladium black rot), drought tolerance related traits, oil quality and other agronomic traits. Identified QTLs and linked markers will be compiled for development of a consensus QTL map. This QTL map will

help breeders to select genomic regions with the aid of linked markers for introgression of traits of interest to locally adapted germplasm.

Milestones & Deliverables:

- Compilation of QTL data and development of a consensus QTL map
- Core sets of DNA markers for QTL discovery for disease and pest resistance including TSWV, Early & Late Leaf Spot, CBR, nematodes, PAC, drought.
- Core sets of DNA markers for QTL discovery for quality traits including seed fatty acid composition, flavor quality, nutritional benefits, and other seed composition traits
- Core sets of DNA markers for QTL discovery for peanut yielding ability and other agronomic traits

3.4 Genome mapping and allelic analysis through Genome-Wide-Association-Studies. The international peanut research community has created sets of genetic resources that facilitate both the generation of high resolution genetic maps through mapping by sequencing as well as the mapping of important agricultural traits through genome wide association studies (GWAS). Genetic mapping through sequencing and analysis of diploid and amphidiploid RIL populations will capture gene space in parental lines and each related RIL. Analysis of RIL data will be used to generate an ultra-dense, gene-based genetic map for each population. Genetic mapping and GWAS through low-coverage sequencing of the diversity panel (ICRISAT) and parallel analyses of the Mini-Core collections from the U.S. and China will capture gene-space and sequence variation in cultivated peanut germplasm. Analysis of SNPs will reveal the level of linkage disequilibrium in these germplasm and help refine the genetic maps generated from Tifrunner x GT-C20 RILs and the populations described above. These analyses provide the foundation for efficient QTL mapping and the generation of a peanut haplotype map in conjunction with the reference sequence.

Milestones & Deliverables:

- High resolution genome maps of A and B genomes of the cultivated peanut ancestors and the amphidiploid synthetic hybrid of A x B genomes species.
- SNP maps correlated with the variation captured in the diversity panels and germplasm collections.
- GWAS studies of the agricultural traits phenotyped on the ICRISAT genetic diversity panel.

3.5 Catalog expressed genes and profile gene expression in cultivated peanut

Other genome projects suggest the number of protein encoding genes in crop species may exceed 40,000. Genome sequencing reveals all of the genes present within an organism, but does not reveal which of those genes are active in different metabolic pathways, tissues, or stages of development. Until recently, analysis of cDNA libraries of expressed gene sequences (ESTs) was limited to a gene-by-gene approach. New high-through-put sequencing platforms (such as RNA-seq) provide a rapid and sensitive means to survey gene expression and create a comprehensive peanut gene expression atlas that catalogs gene activity in different tissues and treatments. Such an atlas would be a valuable resource for the study of peanut gene function. RNA-Seq (whole transcriptome shotgun sequencing) deploys high-throughput sequencing technology to discern how individual alleles are expressed, detect post-translational mutations, and discover other functional aspects of gene expression that complements cDNA characterization by Sanger sequencing, SAGE and MPSS methods. RNA-seq will be used to catalog expressed genes,

validate gene predictions and profile gene expression in *Arachis hypogea* cv. Tifrunner tissues (leaf, apical meristem, stem, root, flower, gynophore, pericarp, seed) across multiple developmental stages and under challenge with various stresses. This information will add definitive context to the annotation of the reference peanut genome sequence.

Milestones & Deliverables:

- A standardized methodology for submitting data towards annotation of the whole peanut genome.
- Expression profiles of genes that mediate resistance to diseases and pests, such as: tomato spotted wilt virus (TSWV), leaf spot (early *Cercospora arachidicola*; late *Cercosporidium personatum*), rust (*Puccinia arachidis*), white mold (*Sclerotium rolfsii*), nematode (*Meloidogyne arenaria*), and pre-harvest aflatoxin contamination (*Aspergillus flavus*)
- Expression profiles of genes that mediate tolerance to abiotic stresses, such as: drought, temperature (cold, heat), and nutrient deficiency
- A peanut gene atlas which includes a comprehensive list of all expressed soybean genes, alternative splice products, the identification of co-regulated genes and gene networks.

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. Many of these conditions are attributed to the plant's inability to withstand environmental stresses. Food security also is a major problem in peanut producing nations. The infrastructure for future advances in peanut research to mitigate these important issues should encumber all aspects of relevant practical, basic, and clinical research in an integrated approach.

Goal 4: Integrated research strategies for major issues that impact global marketing and consumer preferences for peanuts and peanut products

Performance Measures:

4.1 Improved food safety by minimizing aflatoxin level in finished food products

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

Milestones & Deliverables

- Improved cost reliability and competitiveness of biocontrol strategies for Aspergillus contamination by about 75%.
- Develop elite, short season varieties with inherent resistance to Aspergillus infection

- Develop molecular markers for QTL associated with substantial reduction of pre-harvest aflatoxin contamination
- Changes in plant architecture that minimize peanut colonization by Aspergillus
- Improved peanut harvesting and handling practices that reduce foreign material by 50% on farmer stock.

4.2 Improved consistency and quality of raw peanuts

Increasing the intrinsic level of monounsaturated fatty acids in peanut oil will improve the oxidative stability and nutritional value of peanut oil. Peanuts also feature other nutrients that have been shown to promote heart health. Peanuts are good sources of vitamin E, niacin, folate, protein and manganese. In addition, peanuts are a source of protein, albeit antigenic properties of some storage proteins may elicit allergenic reactions in sensitized individuals. An integrated research effort is needed to enhance levels of these nutraceuticals and antioxidants; and develop strategies to mitigate peanut allergy. Analytical facilities are needed for characterization of genetic variation in bionutrient and seed constituent levels among peanut germplasm and breeding populations. Breeding studies of trait inheritance are needed to establish the impact of these compounds in reducing risk of cardiovascular disease and other human health maladies.

Milestones & Deliverables

- Find genetic factors that control flavor & develop useful molecular markers for specific traits
- Define chemical reactions and metabolic intermediates that control the expression of flavor components in roasted peanuts
- Raise "roasted peanut" flavor scores to a 7 as measured by trained panels
- Improve levels of folate and/or essential nutrients above the thresholds that are required to make health claims
- Genomic and biotech tools to manipulate metabolic and proteomic factors that govern the activity of biosynthetic pathways
- Develop elite high oleic acid varieties that exhibit high or low oil content for specific markets
- Commercial production of early maturing high-oleic peanut varieties
- Industry adoption of practices that minimize admixtures that reduce the O/L ratio below 20:1 in marketed peanuts
- Improve protein digestibility and completeness of protein.

Crop Improvement

Genomic tools are expected to provide the means to greatly accelerate and improve selection in varietal development. Use of this technology will help meet the increasing demands of growing world population for food security. Improved output traits such as yielding ability will help abate limitations on arable land and water, protect crops against changes in pathogen populations, and enhance food safety. Many of the most difficult traits to improve in a selection program for peanut are multigenic. Gene families govern the expression of many seed traits. Genes that protect plants against pathogens often exhibit multiple components of resistance. Tolerance to abiotic stress frequently is the product of many genes and different responses, which can be difficult to measure, and which are difficult for this reason to incorporate into improvement programs. 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. Marker-assisted-selection should provide a more efficient method for combining desirable genes in agronomic cultivars.

Goal 5: Ensure that the new genetic information can be used by plant breeders to provide an adequate supply of agronomic and high quality peanut cultivars.

Performance Measures:

5.1 Characterization of genetic diversity for phenotypic traits in reference germplasm populations. The cultivated peanut collection contains more than 8,000 accessions in the U.S., 14,000 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and 5,600 in the Chinese collection. Additional collections may exist in other countries. These collections provide valuable sources of genetic variation for crop improvement. Core accessions that represent the range of diversity in these collections have been selected to exploit the untapped genetic potential of these resources with greater efficiency. Evaluation of subsets of the reference collections by association analysis will provide an initial set of markers for further verification in germplasm collections representing a substantial part of the allelic diversity of the cultivated species.

Milestones and deliverables:

- Libraries of herbarium samples with photo documented descriptors of phenotypic variation among accessions of the reference peanut germplasm collections
- Publication of an internationally accepted CODEX for phenotypic descriptors in Section Arachis
- Phenotypic characterization of reference sets of germplasm accessions that represent genetic diversity for productivity, protection and/or quality traits.

5.2 Support policies and initiatives for unrestricted international exchange of peanut germplasm and breeding populations

Historically, advances in crop research and public plant breeding in particular have been achieved through free-exchange of data and materials. However, in 1980 the Bayh–Dole Act or Patent and Trademark Law Amendments Act (35 U.S.C. § 200-212) ushered an era focused on rights to intellectual property arising from federal government-funded research. An unintended consequence of that legislation was a negative influence on data sharing and use. This situation has given rise to a counter movement within the public scientific communities for policies that recognize the value of sharing resources, including germplasm and genetic data. The PGC is committed to promoting the use of the data held within the PGP archive for research, education and other public-benefit purposes. The benefits of genomics will be greatest to the overall peanut community if genetic resources, especially populations, are shared among researchers, allowing

evaluation in multiple environments, and utilization of populations for varietal development. There are various institutional and legal barriers to germplasm exchange, and it is the goal to find ways to enhance germplasm exchange within the limits that exist, and to find new ways to minimize barriers to germplasm exchange.

Milestones and deliverables:

- Develop a catalog of genotypic/phenotypic traits of germplasm and breeding populations to select candidates for exchange among cooperative research efforts
- Suggest protocol and procedures to help facilitate exchange of germplasm with as few IP restrictions as possible, taking into account institutional and grant constraints.

5.3 Develop and provide user-friendly, affordable genomic services and tools to all peanut breeders. To be useful, the tools need to be easily incorporated into breeding programs, and sufficiently inexpensive to be used. Key needs are reliable and timely provision of marker data to breeding laboratories, as well as computational tools to merge genomic, phenotypic, and pedigree information. In addition, genomics tools needs to be made available to breeders without IP restrictions; otherwise tools will be unavailable to many programs, and the time and effort required for setting up legal agreements will delay or make incorporation of genomics tools prohibitive.

Milestones and deliverables:

- Establishment of regional or local laboratories to provide marker data.
- Provision of bioinformatics tools to breeders to use for marker analysis.
- Internet-accessible database with comprehensive lists of populations, pedigrees, available markers, and DNA sequences.
- Marker technology that allows freedom-to-operate without IP restrictions

5.4 Improved methods for phenotyping. As genomics becomes more efficient, limitations in phenotyping capacity will remain a limiting factor in genomic analysis and breeding. Innovative approaches are needed to develop accurate high-throughput phenotyping methods that are adopted internationally for comparison of data among breeding programs.

Milestones and deliverables:

- Guidelines and protocol for standardizing phenotypic description of mapping populations
- Develop or modify existing phenotyping methods for greater efficiency in terms of reduced time required for scoring and greater ease of conversion into electronic format.

5.5 Provide multipurpose breeder training. Coordination in employment of phenotyping methods among breeders in various countries will be important to the success of the genomics work in crop improvement. For this reason, training in phenotyping methods is needed, both in person, and by development of training materials such as internet-accessible protocols or videos.

Milestones and deliverables:

- Compilation of a list of venues for training.
- Training in phenotyping at next AAGB meeting.
- Further development of training materials.

5.6 Develop Recombinant Inbred Line (RIL) and Near-Isogenic Line (NIL) populations for discovery of useful molecular markers and gene discovery. A marker-assisted selection system 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. Several populations are available currently, and have been developed specifically for identification of economically-important traits, and having relatively high polymorphism needed for efficient marker analysis. These populations (derived from crosses among cultivated materials, wild species-derived and introgression populations) provide a set of ready-to-go lines chosen for segregation for important traits. These genetic resources also will be used to verify gene markers from association analysis of reference populations. Evaluation in multiple environments is needed to identify useful alleles common to multiple environments, as well as those that are useful to specific environments. The following populations are available:

A. duranensis (PI475887 x A. duranensis (Grif 15036)	PI158839 (554CC) x Tifguard
Gregory x Tifguard	SunOleic-97R x NC94022
Tifrunner x GT-C20	BSS56 x Tamrun OL01
BSS56 x Tx994415	TxAG-6 x Florunner
A. cardenasii x A. Duranensis	ICGS-76 x Tamrun OL02 Fn
Tamrun OL01 x TxL017746	OLin x New Mexico Valencia C
TAG24 x ICGV 86031	TAG24 x GPBD4
TG26 x GPBD4	ICGS44 x ICGS-76
ICGS-76 x CSMG84-1	TG49 x GPBD49
A. duranensis x A stenosperma	A. batizocoi x A. magna
(A. duranensis x A. batizocoi)4x x IAC Runner	[(A. correntina x A. cardenasii) x A. batizocoi] x A. hypogaea
at of 16 posted association populations, involving Florida	07 and Tifrunner as one set of perents and

A set of 16 nested association populations, involving Florida-07 and Tifrunner as one set of parents, and SSD6, C76-16, NC3033, SPT06-06, Bailey High O/L, Olin, New Mexico Valencia C, and Florunner as the other parents.

Milestones and deliverables:

- Financial support for phenotyping efforts
- Initial phenotyping on "T" populations, and other populations in appropriate environments for traits to score chosen by individual projects for their relevance
- Phenotypic characterization of appropriate mapping populations for genetic variation in <u>Stresses:</u> Early leafspot, Late leafspot, Rust, Tomato spotted wilt virus, Sclerotinia blight, White mold, Aflatoxin production, Drought; <u>Agronomic traits</u>: yielding ability, Nitrogen fixation, Oil content, Fresh seed dormancy, maturity

5.7 Generating new populations for varietal development. Breeding is a dynamic process. In many instances, QTL analysis in current mapping populations has identified new parents or contributed to development of new populations for crop improvement. In this objective, we will move beyond the current populations to develop new populations for future evaluations. It is expected that with enhanced genomic tools, future evaluation of new populations will be more rapid, inexpensive, and powerful than using current methods.

Milestones and deliverables:

- Develop highly sustainable peanut varieties with improved water use efficiency
- Develop early maturing varieties to help improve flavor characteristics of roasted peanuts.
- Develop elite cultivars with genetic resistance to major weed problems.

Collaborators

AAGB-2010 Participants

AAGD-2010 I al ticipants		
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Collaborative Research Projects

IPGI Goal	PGP Goal	Collaborative Research Projects
		Employing SNP Markers to Track Mutations & Evaluate Genetic Diversity in the USDA, Chinese & ICRISAT Arachis Germplasm
1	2	Collections, NA Barkley1, ML Wang1, HT Stalker, 1USDA ARS Plant Genetic Resources Conservation Unit, Griffin, GA; 2Department of Crop Science, North Carolina State University, Raleigh, NC; 3 Boshou Liao, Chinese Academy Ag. Sci., Oil
1	2	Crop Institute, Wuhan, China; 4 Rajeev Varshney, ICRISAT, Hyderabad, India Advances on the scientific knowledge and use of peanut wild relatives in Brazil, JFM Valls, Embrapa Genetic Resources and Biotechnology, Brasília, Brazil
1	2	Capturing genetic diversity from peanut wild relatives: Advanced Backcross QTLs analysis and CSLL construction, D Foncéka1*, T Hodo-Abalo2, R Rivallan1, H Vignes1, I Faye2, O. Ndoye2, B Courtois1 & JF. Rami1. 1 CIRAD, Montpellier, France. 2l
		SRA/CERAAS, Thiès Escale, Senegal. Broadening the genetic base of peanut: Introgression of wild Arachis resistance genes using the Tetraploid Route with the aid of
1	2	molecular and cytogenetic markers. SCM Leal-Bertioli1*, MC Moretzsohn1, PM Guimarães1, SP Santos2,3, S Nielen1, ACG Araujo1, ACM Brasileiro1, CV Morgante1,4, DJ Bertioli2,3. Embrapa Genetic Resources and Biotechnology, Brasília, Brazil. 2University of Brasília, Campus Darcy Ribeiro, Brazil. 3Catholic University of Brasília, Brasília, Brazil. 4Embrapa Semi-Arid, Petrolina, Brazil.
1	2	Potential of new Arachis amphidiploids as sources of resistance to leafspots and rust. AP Fávero1*, MD Michelotto2, JF Santos3, ALM Martins2 & IJ Godoy3. 1Embrapa Pecuária Sudeste, São Carlos-SP, Brazil, 2APTA, Pólo Regional Centro-Norte, Pindorama-SP, Brazil 3Instituto Agronômico de Campinas, Campinas-SP, Brazil.
1	2	The haplotypes of Arachis correntina and A. villosa reflects an associated history with the alluvial fan of the Parana River during the Upper Quaternary. M Grabiele, LMI Chalup, G Robledo & G Seijo*. Instituto de Botánica del Nordeste y FaCENA, Universidad Nacional del Nordeste, Argentina.
1	2	Unlocking the genetic diversity of peanut's wild relatives with genomic and genetic tools. J. Valls, EMBRAPA, D. Bertioli, UCB, Brazil, S. Braconnier, CERAAS, Senegal; J. Crouch ICRISAT Kenya; P. Piffanelli, CIRAD, France; G. Seijo, IBONE, Argentina; J. Stougaard, UAarhus, Denmark; V Valdez, ICRISAT, India
1	5	<u>Developing Introgression Pathways for Gene Transfer to Arachis hypogaea. L.,</u> CE Simpson1*, MD Burow2 & JL Starr3, 1Texas AgriLife Research, Texas A&M System, Stephenville, TX
1	5	<u>Carbon isotope discrimination of representative Brazilian cultivars under drought stress.</u> GG Brito & TMF Suassuna* 1Embrapa Cotton / Advanced Savannah Nucleus, Santo Antônio de Goiás, GO, Brazil.
1	6	Some biogeographic, genomic and speciation considerations on section Arachis. G Seijo*, G Robledo, M Grabiele, D Carísimo, S Samoluk & G Lavia. Instituto de Botánica del Nordeste / FACENA Universidad Nacional del Nordeste, Corrientes, Argentina.
1	6	The races of peanuts of Peru. A Krapovickas 1*, R Vanni1, J Pietrarelli2, CE Simpson ³ . 1Instituto de Botánica del Nordeste IBONE, Corrientes, Argentina, 2Estación Experimental INTA, Manfredi, Córdoba, Argentina, ³ Texas & University, Stephenville, TX
2	1	Apply Next Generation Sequencing into Peanut Research, X Xu1*, B Yang1.BGI- Shenzhen, Shenzhen, China; PGPTechnical Steering Group
2	1	BAC clone selection using a 3D pooling strategy in <i>Arachis</i> . BS Vidigal1*, LP Muniz2, SCM Leal-Bertioli3, DJ Bertioli1 & PM Guimarães3. ¹ University of Brasília, Campus Darcy Ribeiro, Brasília, Brazil. ² Catholic University of Brasília, Campus I, Taguatinga, Brasilia, Brazil. 3Embrapa Genetic Resources and Biotechnology, Brasília, Brazil.
2	2	<u>Retrotransposons in Arachis – structure, genomic context, and phylogenetic classification</u> , S Nielen1*, B Vidigal dos Santos2, S Leal-Bertioli1, P Guimarães1 & D. Bertioli2, 1Embrapa Recursos Genéticos e Biotecnologia, Brasília-DF, Brazil. 2Universidade de Brasília, Brasília, DF, Brazil.
2	2	Dispersed and other repetitive DNA sequences in the peanut genome shown by BAC-FISH. ACG Araujo1*, J Bailey2, T Schwarzacher2, PM Guimaraes1, S Leal Bertioli1, DJ Bertioli3, C Kim4, A Paterson4 & P Heslop-Harrison2. 1Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil. 2Department of Biology, University of Leicester, UK. 3University of Brasilia, DF, Brazil.4University of Georgia, Athens, GA
2	2	Towards ultra-dense genetic maps of peanut generated by sequencing diploid and tetraploid RIL populations and a peanut diversity panel L Froenicke1, M Pandey2, H Upadhyaya2, MC Moretzsohn3, P Guimaraes3, S Leal-Bertioli3, RK Varshney2, D Bertioli3 & RW Michelmore1. 1Genome Center, University of California Davis, Davis, CA; 2International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Greater Hyderabad, Andhra Pradesh, India. 3Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.
2	2	Development of genetic linkage maps for the A and B genomes of <i>Arachis</i> , using RIL populations. EG Gouvea1,2*, SCM Leal- Bertioli1, V Penmetsa2, D Cook2, S Senthilvel3, RK Varshney3, DJ Bertioli4 & MC Moretzsohn1. 1Embrapa Recursos Genéticos e Biotecnologia, Brasília-DF, Brazil. 2University of California, Davis, CA. 3International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India. 4Universidade de Brasília, Instituto de Ciências Biológicas, Brasília-DF, Brazil.
2	3	<u>Global transcriptome analysis of peanut wild species under biotic and abiotic stress</u> . PM Guimarães1*, ACM Brasileiro1, CV Morgante1,2, PA Roberts3, GJ Pappas Jr1, OB Silva Jr1, SCM Leal-Bertioli1, A Martins4 & D Bertioli4. 1Embrapa Genetic Resources and Biotechnology, Brasília, Brazil. 2Embrapa Semi-Arid, Petrolina, Brazil, 3University of California, Riverside, CA. 4University of Brasília, Brasília, Brazil.
2	3	Denovo characterization of peanut transcriptome during gynophore development. CZ Zhao1,2, XJ Wang1,2*, AQ Li1,2, CS Li1,2. 1High-Tech Research Center, Shandong Academy of Agricultural Sciences, Key Laboratory for Genetic Improvement of Crop Animal and Poultry of Shandong Province, Ji'nan, China. 2Key Laboratory of Crop Genetic Improvement and Biotechnology, Huanghuaihai, Ministry of Agriculture, Ji'nan, China
2	3	Identification of peanut (Arachis hypogaea L.) miRNA targets through degradome sequencing. M Li1,2, XJ Wang1,2*, CZ Zhao1,2 & H Xia1,2. 1High-Tech Research Center, Shandong Academy of Agricultural Sciences, Key Laboratory for Genetic Improvement of Crop Animal and Poultry of Shandong Province, Ji'nan. 2Key Laboratory of Crop Genetic Improvement and Biotechnology, Huanghuaihai, Ministryof Agriculture, The People's Republic of China, Ji'nan.
2	6	Advance of Peanut Omics and Biotechnology in China. XJ Wang 1,2*, H Xia 1,2, CZ Zhao 1,2, AQ Li 1,2, CS Li 1,2. 1Bio-Tech Research Center, Shandong Academy of Agricultural Sciences, Key Laboratory for Genetic Improvement of Crop, Animal and Poultry of Shandong Province, Ji'nan China. 2Key Laboratory of Crop Genetic Improvement and Biotechnology, Huanghuaihai, Ministry of Agriculture, Ji'nan, China.

IPGI	PGP	Collaborative Research Projects
Goal	Goal	-
3	2	Identification of candidate genes in Arachis stenosperma involved in the interaction with root-knot nematode (Meloidogyne arenaria). LL Bride ¹ , ^{2*} , ACM Brasileiro ¹ , CV Morgante3, PA Roberts4, SCM Leal-Bertioli ¹ , DJ Bertioli 2 & PM Guimarães ¹ . ¹ Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF. ² Universidade de Brasília, Brasília, DF. ³ Embrapa Semiárido, Petrolina, PE. 4 University of California, Riverside, CA.
3	3	<u>Cloning and expression analysis of flowering regulating genes in peanut</u> . H Xia 1,2, XF Zhai 1,2, SB Wan 1,2* & XJ Wang 1,2*. 1Bio-Tech Research Center, Shandong Academy of Agricultural Sciences, Key Laboratory for Genetic Improvement of Crop, Animal and Poultry of Shandong Province, Ji'nan, China, 2Key Laboratory of Crop Genetic Improvement and Biotechnology, Huanghuaihai, Ministry of Agriculture, Ji'nan, China
3	3	Tagging of rust and late leaf spot disease resistance gene and identification of functional diversity with genic molecular markers in cultivated groundnut. S Mondal1*, AM Badigannavar1 & SF D'Souza1. 1Nuclear agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai,India.
3	3	Gene-expression profile of five runner peanut genotypes. response to short-term water defi cit utilizing controlled rainout shelters. PM Dang1*, CY Chen1, RB Sorensen1, MC Lamb1 & CC Holbrook2 . 1USDA-ARS, National Peanut Research Laboratory (NPRL), Dawson, GA. 2USDA-ARS, Crop Genetics and Breeding Research, Tifton, GA.
3	3	Large scale transcriptome analysis of wild peanut (<i>Arachis stenosperma</i>) inoculated with <i>Passalora personata</i> , the causal agent of Late Leaf Spot. ACQ Martins 1,2*, CV Morgante ³ , AK Silva ² , IC Galhardo1, GJ Pappas Jr2, OB Silva Jr2, SCM Leal-Bertioli2, DJ Bertioli1, RNG Miller1, ACM Brasileiro2 & PM Guimarães 2 ¹ University of Brasília, Campus Darcy Ribeiro, Brasília, Brazil. ² Embrapa Genetic Resources and Biotechnology, Brasília, Brazil. ³ Embrapa Semi-Arid, Petrolina, Brazil.
3	3	Differential gene expression study of wild Arachis under a water deficit induction system. TN Oliveira ^{1,2*} , PM Guimarães ¹ , M Passos ¹ , F Rodrigues ³ , A Nepomuceno ³ , DJ Bertioli ² , SCM Leal-Bertioli ¹ & ACM Brasileiro ¹ . 1Embrapa Genetic Resources and Biotechnology – Brasília-DF, Brazil, ² University of Brasília – Brasília-DF, Brazil, ³ Embrapa Soybean – Londrina-PR, Brazil.
3	5	Markers, Maps and Molecular Breeding in Cultivated Groundnut, RK Varshney, International Crops Research Institute for the Semi- Arid Tropics (ICRISAT), Patancheru, India.
3	5	Mining for induced and natural variation in peanut genes, P Ozias-Akins1*, JE Knoll1, ML Ramos1 & CC Holbrook2. 1University of Georgia and 2USDA-ARS, Tifton, GA Identification of QTLs for drought tolerance across different locations and seasons using recombinant inbred lines in peanut
3	5	(Arachis hypogaea L). I Faye1*, F Hamidou2, A Rathore3, M Pandey3, B Gautami3, V Vadez3 & R.K. Varshney3. 1Institut Sénégalais de Recherches Agricoles (ISRA)-CNRA, Bambey, Sénégal. 2International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Sahelian Center, Niamey, Niger. 3International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India.
3	5	Toward understanding molecular mechanism of peanut resistance to <i>Ralstonia solanacearum</i> . WJ Zhuang*1, DR Cook2, C Zhang1, H Chen1, GH He3, BJ Jiang1, JB Zheng1, CH Zhuang1. Fujian Province Key Lab of Plant Molecular and Cell Biology, Fujian, Agriculture and Forestry University, Fuzhou, Fujian, China. 2Department of Plant Pathology, University of California, Davis, CA. 3Department of Agricultural Science, Tuskegee University, Tuskegee, AL
3	5	Peanut gene expression induced by infection of <i>Ralstonia solanacearum</i> . B Liao1*, J Huang1, L Yan1, Y Lei1 & H Jiang1. 10il Crops Research Institute of Chinese Academy of Agricultural Sciences, Key Laboratory of Oil Crop Biology of the Ministry of Agriculture, Wuhan, China.
3	6	Genetic mapping of cultivated peanut with genomic SSR and transposon markers screened by <i>in silico</i> polymorphic analysis, K Shirasawa1*, H Hirakawa1, M Hasegawa2, H Kiyoshima2, S Kuroda2, C Kuwata2, S Suzuki2, Y Naito3, T Kuboyama4, S Tabata1 & S Isobe1, 1Kazusa DNA Research Institute, Chiba 292-0818, Japan. 2Chiba Prefectural Agriculture and Forestry Research Center, Chiba, Japan. 3Mitsubishi Chemical Medicine Corporation, Tokyo, Japan. 4Ibaraki University, Ibaraki, Japan
3	6	<u>Isolation and characterization of important genes related to embryo development</u> . H Chen 1, C Zhang 1, DR Cook 2, BJ Jiang 1, GH He 3, JB Zeng1, CH Zhuang1, TC Cai 1, WJ Zhuang1*. 1Fujian Province Key Lab of Plant Molecular and Cell Biology, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China. 2Department of Plant Pathology, University of California, Davis, CA 3Tuskegee University, Tuskegee, AL
4	1	Characterization of duplicate genes involved in oil pathways of polyploid peanut. Y Brand1, F Shilman1 & R. Hovav1. 1Department of Field Crops, Plant Science Institute, ARO, Bet-Dagan, Israel. Screening of groundnut (<i>Arachis hypogaea</i> L.) minicore collection for mutant allele of Oleoyl-PC desaturase to identify high oleic
4	2	accessions. G Mukri1, HL Nadaf 1* & HD Upadyaya 2. 1Dept. of Genetics & Plant breeding, University of Agricultural Sciences Dharwad, Karnataka, India. 2International Crops Research Institute for the Semi-Arid tropics (ICRISAT), Patancheru, Hyderabad, India.
4	3	Peanut genes involved in deic acid biosynthesis expression profiling in developing seeds at different reproduction stages. HC Liu1,2, XP Chen1, HY Liu1, EH Zhan1 & XQ Liang1*. 1Crops Research Institute, Guangdong Academy of Agriculture Sciences, Guangzhou; 2College of Life Science, South China Normal University, Guangzhou, China.
4	3	Genetic enhancement of nutritional quality traits with induced mutagenesis in groundnut (<i>Arachis hypogaea</i> L). HL Nadaf1, C Channayya1, BS Kaveri1, KG Parameshwarapp1. 1Depart of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Karnataka, India.
4	5	Identification of QTLs for Oil Content and Fatty Acid Composition in Cultivated Peanut (Arachis hypogaea L.), X Zhang*1,2, S Han1,2, F Tang1,2, W Dong1, J Xu1, M Yan2, H Liu2. IIndustrial Crops Research Institute, Henan Academy of Agricultural Sciences 2Henan Provincial Key Laboratory for Genetic Improvement of Oil Crops, Zhengzhou, China.

IPGI	PGP Goal	Collaborative Research Projects
Goal	Goal	-
4	5	Isolation and characterization of important genes toward improvement peanut resistance to Aspergillus flavus. WJ Zhuang *1, H Chen 1, PK Nancy 2, BJ Jiang1, JB Zeng1, C Zhang1, XY Chen1, Y Deng 1 & TC Cai1. 1Fujian Province Key Lab of Plant Molecular and Cell Biology, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China. 2Department of Medical Microbiology and Immunology, Department of Plant Pathology, University of Wisconsin, Madison, WI
4	5	Prevalence of aflatoxin contamination in groundnut value chains and strategies to enhance food safety in Mali. F Waliyar1*, A Traore1. V Reddy1. B Diarra2. O Kodio2 & H Sudini1. 1International Crops Research Institute for the Semi-Arid Tropics, Niamey. Niger. 2Institut d Economie Rural, Bamako Mali.
4	6	Genetic diversity of the gene Resveratrol Synthase in Arachis spp. from Embrapa active germplasm bank. ALR Santana- Pereira1*, ACM Brasileiro2, DJ Bertioli1,2, CV Morgante2,3, MA Gimenes2 & SCM Leal-Bertioli ¹ . ¹ Embrapa Genetic Resources and Biotechnology, Brasília, Brazil. ² University of Brasília, Brasília, Brazil. 3Embrapa Semi-Arid, Petrolina, Brazil
4	6	Methodology adaptation for the determination of resveratrol in peanut leaves. RM Lopes1,2, D Silveira1, MA Gimenes2, PS Vasconcelos1,3 & TS Agostini-Costa2*. 1Faculdade de Ciências da Saúde - Universidade de Brasília. 2Embrapa Recursos Genéticos e Biotecnologia. 3Universidade Estadual Paulista Julio de Mesquita Filho, Brazil
5	5	Current progress in drought tolerance work in peanut – Field and lysimetric assessments of germplasm, V Vadez1*, F Hamidou2, P Ratnakumar1, O Halilou2, O Mponda3, T. Kapewa4, E Monyo4, I Faye5, B Ntare6, SN Nigam1 & HD Upadhyaya1, 1 International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru India. 2 International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Sahelian Center, Niamey, Niger; 3 Nalendiele Research Station, Mtwara, Tanzania, 4 Chitedze Research Station, Lilongwe, Malawi. 5 CNRA Bambey, Senegal, 6 ICRISAT Bamako, Mali
5	5	Genetic engineering of groundnut for crop improvement: Current status and future prospects. KK Sharma1*, P Bhatnagar-Mathur1, V Vadez1, H Sudini1 & FW. Waliyar1. 1International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India.
5	5	Conventional breeding approaches to speed up release of new peanut varieties. GC Wright1*, D Fleischfresser2, GA Baker1 & D O'Connor1. 1P eanut Company of Australia, Kingaroy, Qld, Australia, 2AgriSciences Queensland, Department of Employment, Economic Development and Innovation, Kingaroy, Queensland, Australia.
5	5	Developing transgenic resistance in cultivated peanut (Arachis hypogaea L.) to peanut-stem- and peanut-budnecrosis viruses. T Radhakrishnan1*, M Reetu1, Y Reena1, K Abhay1, JR Dobaria1, Thirumalaisami1 & RK Jain2. 1Directorate of Groundnut Research, PO Box 5, Jungadh, Gujarat, India. 2Indian Agricultural Research Institute, New Delhi, India.
5	5	Peanut breeding program for drought resistance tolerance. S Soave1*, P Faustinelli1,2 & MI Buteler1 1Criadero El Carmen. General Cabrera, Córdoba, Argentina. 2Universidad Católica de Córdoba. Córdoba, Argentina. Molecular breeding for foliar disease resistance and quality-related traits in cultivated groundnut. MVC Gowda1*, V Sujay1, G
5	6	Mukri1, H L Nada11, RS Bhat2 & R. K. Varshney3. 1Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, India. 2Department of Biotechnology, University of Agricultural Sciences, Dharwad, India. 3International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Greater Hyderabad, India.
5	6	Development and Use of Molecular Markers to Accelerate Peanut Cultivar Development CC Holbrook1, P Ozias-Akins2* & Y Chu2. 1USDA-ARS, Tifton, GA. 2University of Georgia, Tifton, GA.
5	6	Molecular and physiological approaches to improve abiotic stress tolerance in groundnut (<i>Arachis</i> sp.). P Payton 1, 1USDA-ARS Cropping Systems Research Laboratory, Lubbock, TX
5	6	Identification and introgression of a major QTL for rust resistance in elite cultivated groundnut cultivars through marker-assisted selection. MK Pandey1, SN Nigam1, MVC Gowda2, Y Khedikar1,2, M Sriswathi1, M Govil1, V Sujay1,2, B Gautami1, HD Upadhyaya1, RK Varshney1,3*. 1International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India; 2University of Agricultural Sciences (UAS)-Dharwad, India; 3Generation Challenge Programme (GCP), c/o CIMMYT, Mexico DF, Mexico.
5	6	Selection of Arachis hypogaea breeding lines for resistance to multiple foliar diseases. JF Santos1*, IJ Godoy1, MD Michelotto2, EL Finoto2 & ALM Martins2. 1Instituto Agronômico, Campinas, SP, Brazil. 2Polo Regional APTA Centro Norte, Pindorama, SP, Brazil