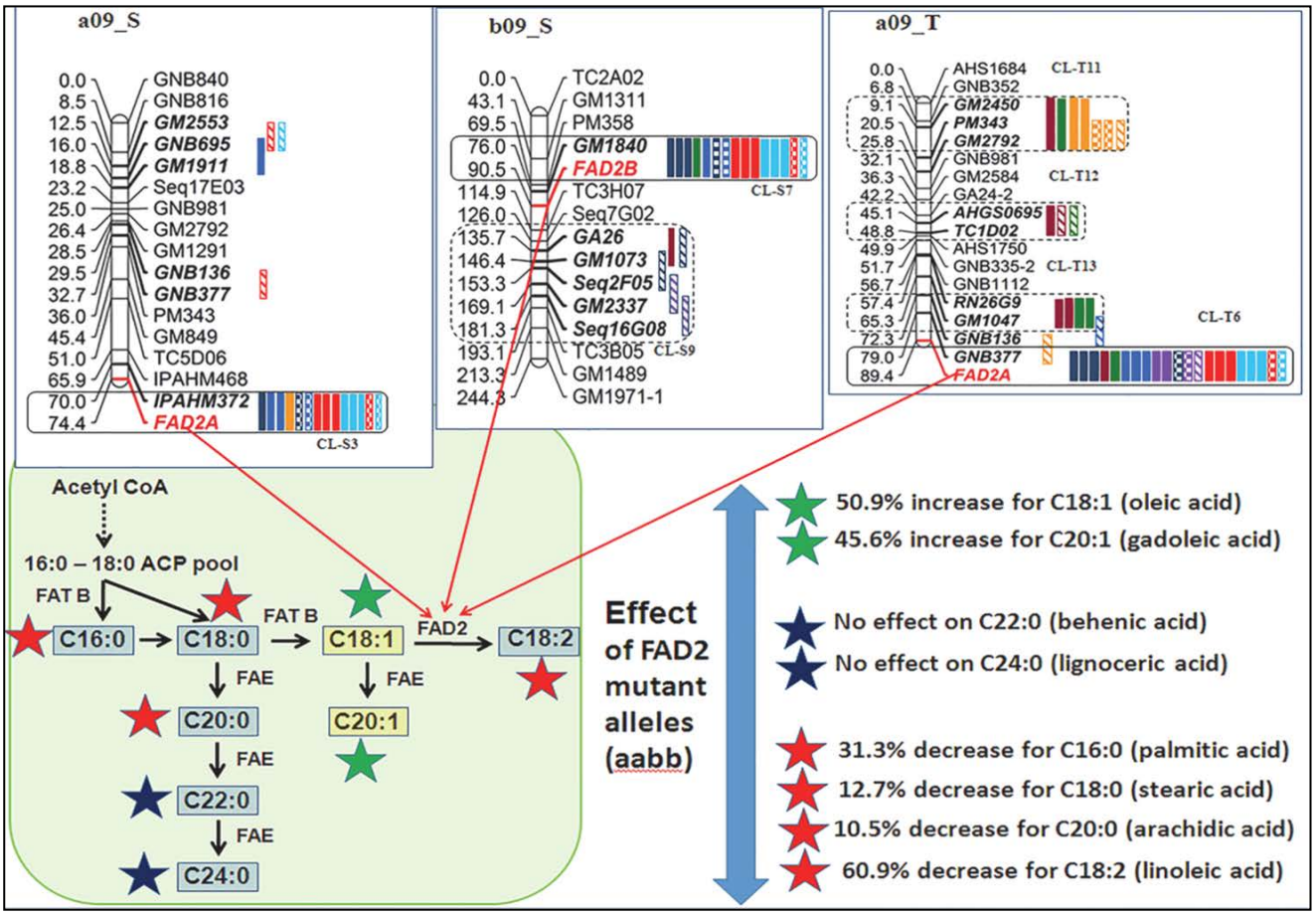
The Peanut Foundation, USA
EMBRAPA, Brazil
ICRISAT, India
ICRISAT, West Central Africa
USDA- ARS, USA
Gates Foundation, USA
ICAR, India
HAAS, Zhengzhou
China
CAAS, Wuhan China
SAAS, Shangdong, China
Peanut Company Australia
ARO, Volcani Center, Israel
Kazusa Research Institute, Japan
Intstit Botanica Nordeste, Argentina

International Peanut Genomic Research Initiative

Strategic Plan for 2017 to 2021

Integration of Germplasm & Genomic Resources

March 2016 v 3.0



Wang, et al., PLOS ONE 2015 doi:10.1371/journal.pone.0119454

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Cover page Image:

The figure on the cover page was chosen to present an integrated vision of how markers are used to create maps and locate genes that impact phenotypic trait expression in breeding populations.

Citation: Wang ML, Khera P, Pandey MK, Wang H, Qiao L, Feng S, et al. (2015) Genetic Mapping of QTLs Controlling Fatty Acids Provided Insights into the Genetic Control of Fatty Acid Synthesis Pathway in Peanut (Arachis hypogaea L.). PLoS ONE 10(4): e0119454. doi:10.1371/journal.pone.0119454

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, and develop germplasm resources that facilitate genotypic/phenotypic characterization of genetic traits. Integrated application of these resources will establish a knowledge platform for achieving enhanced quality, disease management, and yielding ability in varieties that enhance economic competitiveness and help improve global food security.

The Program in a Nut Shell:
One of the biggest challenges for the U.S. peanut industry is the ability to compete with other crops for production. Most growers today are focused naturally on dollar value per acre and peanuts have often been uncompetitive in regards to yield and production costs. As an industry, the best way to compete is to enhance our peanut varieties for disease resistance, quality and yield potential. This can best be done with genomic tools that help maximize yield while minimizing inputs. The International Peanut Genomic Research Initiative (IPGI) provides those tools which help define the future direction of peanut breeding.

The IPGI generates research findings that enable three avenues of investigation: 1) Generating Detailed Maps of the peanut genome, 2) development of Tools for Marker Assisted Selection, and 3) Application of Markers and Maps in Breeding programs.

Generating Detailed Genome Maps. Because cultivated peanut is a very young crop, the two wild parent genomes present in cultivated peanut are very similar to each other (cultivated peanut is believed to be only 10,000 years old which is not enough evolutionary time to accumulate a large number of genetic differences in key genes). Maps of the cultivated peanut genome are needed to show the location of all genes in both sub-genomes and to discover useful DNA markers for those genes. These markers are the tools breeders need to select superior varieties.

Tools for Marker Assisted Selection. As more DNA markers are discovered in peanut, a better way was needed to screen hundreds of lines in a breeding population simultaneously with thousands of markers. A device called a 'chip' was created that holds 60,000 DNA fragments that give dense coverage of gene rich regions in both genomes. Now breeders can track a wide range of genes through each generation of a hybrid population. This helps remove a lot of the mystery in deciding which lines to keep and which to advance as candidate varieties.

Application of Markers & Maps in Breeding. Marker Assisted Selection has been shown to reduce the time needed to add a new trait to a current cultivated variety. Good markers have been found for resistance to late leaf spot (LLS), early leaf spot (ELS), tomato spotted wilt virus (TSWV), root knot nematode (RKN), and high oleic acid. More useful markers are being developed through genome mapping for traits such as cylindrocladium black rot (CBR), white mold (WM), peanut rust and drought tolerance. 'Chip' technology facilitates new breeding strategies for stacking all these traits in improved varieties for each market type and geographic production area. Many breeders are beginning to use these markers in their breeding programs to create new varieties that help reduce the cost of production, enhance peanut quality and ensure an adequate/safe supply of peanut products.

Continued work on markers and maps as outlined in the *IPGI Strategic Plan for 2017 to 2021* will help breeders address the most critical needs of producers, shellers, manufacturers and consumers. The outcome will be a proud and enduring legacy to all segments of the peanut value-chain.

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. An integrated international multi-disciplinary cooperative research workforce of world class scientists and institutions in the U.S., China, Brazil, India, Japan, W. Africa, Australia, and Israel has been organized to accrue the resources needed to meet this challenge. By virtue of the latest advances in genomic technology, IPGI has accomplished a majority of the priorities outlined in the *IPGI Strategic Plan for 2012-2016*, and is well positioned to

deliver a high quality reference genome sequence of cultivated peanut, detailed genomic maps, tools for marker assisted selection, and to deploy these technologies to overcome existing situations and emerging threats to peanut production such the outbreak of as peanut smut in Argentina.

The *International Peanut Genomic Research Initiative: Strategic Plan for 2017-2021* has evolved through a lengthy process that captured and integrated input based upon the progress demonstrated in the annual research accomplishment reports; research presentations at AAGB-2013 (Zhengzhou, China), AAGB-2014 (Savannah, GA, USA), AAGB-2015 (Brisbane, Australia); annual meetings of The Peanut Foundation; annual meetings of the American Peanut Research & Education Society; and numerous ad hoc evaluations by scientists and stakeholders among all segments of the peanut industry. The resulting product, presented herein, serves as the framework for IPGI research. 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 and assessment of program performance toward accomplishment of IPGI goals.

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

http://www.peanutbioscience.com

Background

Cultivated peanut (*Arachis hypogaea* L.) is grown on about 24 million hectares world-wide, and is a principle source of human nutrition in Asia and Africa. Peanut is distinguished by 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 and folic acid, also may improve cardiovascular health.

Although peanut is an economically important source of nutrition, genetic challenges have impeded research progress toward the development of technologies that help decrease crop production costs and improve dietary health benefits in peanut food products. For example, cultivated peanut has a large 2,800 Mb genome, comparable in size to the human genome; and cultivated peanut is a polyploid species, where duplicate sets of chromosomes from two different Arachis species make genetic analysis difficult. However, breakthroughs in DNA sequencing technology encouraged the peanut research community to set a lofty goal of developing genetic maps and assembling a genetically-anchored physical map of gene-rich regions. Both research and funding strategies were required to implement such plans for discovery of alleles that influence peanut quality, nutritional value and productivity.

The genomics research communities of related cool and warm season legumes in the U.S. and abroad had mutual needs and goals. Therefore an alliance was forged among the soybean, dry bean, pea, lentil, chickpea, alfalfa and peanut genomic research communities. This coalition 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.

<u>Development of the International Peanut Genomic Research Initiative (IPGI)</u>

USDA-Foreign Agricultural Service databases show that world supply of peanut ranks third on par with cottonseed and sunflower. Although soybean and canola dominate global oilseed supply, the apparent long-term status quo on peanut supply is just not acceptable, especially in the 22 countries in Sub-Saharan Africa (SSA) where food security is a serious problem. USDA forecasts suggest a two-fold increase in global dietary consumption of peanuts and peanut products by 2050. Meeting that target will require substantial increases in peanut production in China, India, South America, the USA and SSA. However, continuation of current trends in harvested area and yield cast doubt on the ability of traditional technologies to meet that target. Harvested land area hasn't changed appreciably in the past decade, thus a two-fold expansion does not appear feasible. Traditional crop improvement technology also has limitations. There has been little positive variation in average yields if geographic regions during the past decade; and two-thirds of global land resources (mostly in food insecure regions) produce only 40% of world peanut supply due to low yields. The situation is exacerbated by United Nations predictions that the world population will reach 9.3 billion by 2050. Half of that total will reside in peanut producing countries. Population in SSA alone is expected to double,

Global Outlook for Oilseed Peanut

Major Peanut Producing Countries	2014	2050	10 yr mean (2005-2014)	
	Production (MMT)		Harvested Area (Mha)	Yield (MT/Ha)
	Current	Forecast		
China	17.0	32.9	4.4 ± 0.3	3.4 ± 0.2
India	5.5	6.8	5.8 ± 0.6	1.0 ± 0.1
SubSaharan Africa	9.9	18.4	10.0 ± 1.1	1.0 ± 0.0
South America	1.5	2.1	0.4 ± 0.1	3.1 ± 0.2
USA	2.3	4.0	0.5 ± .01	3.9 ± 0.5
% World Total	90.0	97.8	90.6	

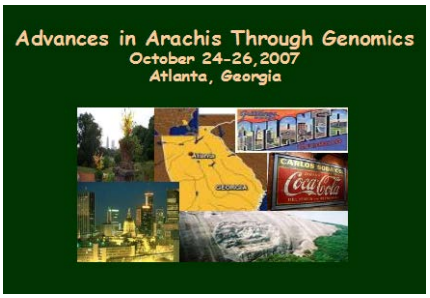
to 1.1 billion. If SSA has to rely on traditional technology, per capita consumption of peanuts will erode substantially and cause a greater nutritional deficient because peanut virtually is the only source of vegetable protein and oil in SSA.

Efforts to overcome this eminent challenge are impeded by losses in productivity and quality attributed to diseases, pests, environmental stresses and 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 was needed to facilitate more rapid discovery of genes that confer a remedy to these constraints and the incorporation of those genes into elite germplasm/ varieties 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 launch this program, it was necessary to establish an infrastructure for genomic research with a coordinated 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 U.S. contingent launched the Peanut Genomic Research Initiative (PGI) at the Atlanta workshop. An advisory committee, representing the broad interests of industry and the peanut research community, drafted the *Strategic Plan for the Peanut Genome Initiative 2004-2008*, a document that outlined research goals objectives, performance measures and significant near-term milestones to guide the development of this emerging organization.

In June 2004, the PGI advisory committee tasked a writing team to develop an Action Plan to implement initial high priority research program needs. The *National Action Plan for the Peanut Genomic Research 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 Action Plan was amended to include ancillary performance measures specific to the immunology of peanut proteins in model systems (PGI Action Plan v-2.4, March 2006).

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, wa*s another committed step toward bringing elite members of the international peanut community together in a manner that fostered research collaboration on high priority issues.



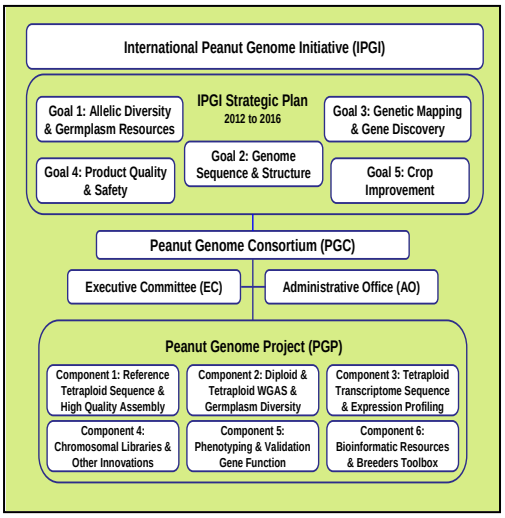
The first *IPGI Strategic Plan for 2008-2012* was developed during AAGB-2007 in Atlanta, GA. The conference was organized by the leaders of the Peanut Genomics Initiative in association with the Peanut Foundation, the International Crops Research Institute for the Semi-arid Tropics (ICRISAT), and representatives of three institutes in China (Shandong Academy of Agricultural Sciences, Henan Academy of Agricultural Sciences, and Guangdong Academy of Agricultural Sciences). Seventy-three participants 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 CSREES.

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).

An internal review of progress toward research priorities in the *IPGI Strategic Plan for 2008-2012* was documented in 2010 and published on-line as the *IPGI: 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, Richard Wilson, 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.

An IPGI workshop on emerging research needs was convened during the 5th *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 the 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 *IPGI Strategic Plan for 2012 to 2016: Characterization of the Peanut Genome.* Plans also were initiated to expand IPGI strategic goals and research priorities to accommodate the genome sequencing under *The Peanut Genome Project*.



The Peanut Genome Project (PGP): The central thrust of the *IPGI Strategic Plan for 2012 to 2016* was 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.* Founding members were: 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 (**Co-*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 Company 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. **Founding Ex Officio** members were: 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, ICRISAT.

Original PGP goals were: 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. PGP Research Accomplishment Reports are submitted to The Peanut Foundation annually and posted on-line at *PeanutBioscience.com/*. Highlights of Most Important PGP Accomplishments to date include:

- Public release of the first chromosomal-scale assemblies for two peanut species (*A. duranensis and A. ipaensis*) that are the ancestral progenitors of cultivated peanut (*A. hypogaea*)
- Evidence that the diploid progenitor species (A & B) genome sequences are highly identical to tetraploid sub-genomes and serve as a useful guide for the reference tetraploid assembly
- Major rearrangements found only in the tetraploid A-subgenome are due to recombination with alleles from the B-subgenome, an unexpected event
- Enhanced assemblies of the tetraploid transcriptome that have led to the identification of candidate genes for expressed traits
- The *A. ipaensis* (B-genome) is a near perfect match for the B-subgenome of cultivated peanuts, and likely is the actual progenitor parent that contributed the tetraploid B-subgenome
- Cultivated peanut originated in northern Argentina about 10,000 years ago

IPGI Strategic Plan for 2017-2021.
The present strategic plan has evolved through a lengthy process that has captured and integrated input based upon the progress demonstrated in the annual research accomplishment reports; research presentations at AAGB-2013 (Zhengzhou, China), AAGB-2014 (Savannah, GA, USA), AAGB-2015 (Brisbane, Australia); annual meetings of The Peanut Foundation; annual meetings of the American Peanut Research & Education Society; and numerous ad hoc evaluations by stakeholders among all segments of the peanut industry. The resulting product, presented herein, serves as the framework that will guide IPGI action beyond the PGP. This plan ensures that research conducted by the IPGI is relevant to the future needs of the global peanut industry, provides a basis for project implementation, and establishes benchmarks for assessment of program performance toward accomplishment of IPGI goals.

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Strategic Research Goals for Peanut Genomics Research

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 and special populations facilitates discovery of useful DNA markers for genome assembly and comparative analysis. Ability to transfer useful genes from wild to cultivated species enables effective use of the genetic diversity resident in germplasm.

Goal 1: Characterize genetic diversity and facilitate transfer of useful genes among Arachis species

Performance Measures

1.1 Conservation of *A. hypogaea* and wild peanut species collections. International collections of wild and cultivated peanut contain 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. Multiple locations preserve these valuable genetic resources.

Milestones and Deliverables:

- Collect and organize existing phenotypic information in collections worldwide
- Phenotype all lines in major collections focusing on core collections initially
- Back-up working collections of A. hypogaea and wild accessions held by the U.S., ICRISAT, China, EMBRAPA and other institutions
- International adoption of on-line comprehensive uniform standards for hebarium and photo-documented peanut phenotypic descriptors.
- A digital record of descriptive data for each germplasm line in GRIN, PeanutBase and other web accessible International databases
- Catalog genotype x phenotype of each germplasm line in a collection.

1.2 Development of *A. hypogaea* populations for QTL discovery. Core and mini-core collections and special populations have been developed in the U.S., ICRISAT, and China to evaluate genetic diversity and genetic variation of crop protection and crop quality traits. These resources are the direct foundation of future variety development.

Milestones and Deliverables:

- Maintenance and genotypic characterization of diversity panels in India, U.S. and China
- Genotype all accessions in major collections and special populations with SNP-chip or comparable platforms
- Maintenance & use of specialized germplasm resources for demonstration of gene function, such as TILLING populations

- Maintenance & characterization of recombinant inbred lines (RIL) harboring genes for key traits (Bacterial Wilt, Peanut Smut, Early leafspot, Late leafspot, Rust, Tomato spotted wilt virus, Sclerotinia blight, White mold, CBR, PAC, Drought, Yield, Oil quality, Oil content, Seed dormancy, Maturity)

1.3 Use of interspecific resources to transfer genes from wild to cultivated germplasm.

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 in response to a wide range of abiotic and biotic stresses, and provide a rich source of variation in agronomic traits; but sterility barriers often hamper the use of wild species in breeding. However, synthetic amphidiploids of wild Arachis species that incorporate different genomes may produce fertile hybrids with cultivated 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 specific genomes in addition to resources such as (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.
- Development & use of technologies to track the introgression of genes from wild to cultivated species

Genomic Resources

The *A. hypogaea* nuclear genome contains approximately 3 billion base pairs, about 30,000 unique genes and is similar to the size of the human genome. Chromosomal scale sequences of the two diploid progenitor species of cultivated peanut are useful guides in the development of a draft reference sequence of a tetraploid peanut genome. These resources enable characterization of gene-rich genomic regions and the generation of genomic maps, gene markers, and other technologies that will help capitalize on the genetic potential for variety improvement.

Goal 2: Develop & implement genetic and genomic tools that facilitate peanut improvement

Performance Measures

2.1 Genome sequencing and assembly

Non-coding repeating-elements account for more than 75% of the cultivated peanut genome (*Arachis hypogaea L. Fabaceae*). These features complicate accurate and complete genome assembly. Continual evaluation and testing of new or improved genome assembly and annotation technologies are needed to facilitate assembly of the reference sequence for tetraploid peanut.

Milestones & Deliverables

- Improved genome sequences of the diploid progenitors of cultivated peanut
- Completion and refinement of the tetraploid peanut genome reference sequence
- Evaluation and application of emerging sequencing, assembly and annotation technologies
- Improved annotations (predictions of genes and other genetic elements) for the diploid progenitor genomes and the tetraploid reference genome sequence

2.2 High-density genetic maps & gene markers

Because cultivated peanut is a very young crop, the two wild parent genomes present in cultivated peanut are very similar to each other since there has not been enough evolutionary time to accumulate genetic differences in key genes. Maps of the cultivated peanut genome are needed to show the location of all genes in both sub-genomes and to discover useful DNA markers for those genes. Markers are tools breeders need to select superior varieties.

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.
- Improved arrays of validated markers for tetraploid peanut accessions of core collections and special populations
- Genetic and consensus maps for diploid, amphidiploid and tetraploid peanut
- 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
- Customized chip platforms with up to 60,000 validated high-quality SNP markers for specific breeding objectives
- SNP maps correlated with the variation captured in the diversity panels and germplasm collections.

2.3 Discovery of candidate genes & function

Validated DNA markers enable identification of QTL and sequence analysis reveals gene sequences within QTL that may mediate a specific trait. A variety of techniques ranging from chemical mutagenesis to genome editing may be used to identify the functional association of a candidate gene with trait expression. This information leads to generation of a higher class of DNA markers that pinpoint specific alleles within given A- or B-genomes. These allele specific markers enhance breeding efficiency and improve understanding of probable biological mechanisms that mediate the trait.

Milestones & Deliverables

Integrated mutagenesis, knockout, and expression data for functional annotation of gene sequences

Identification of candidate genes for induced and constitutive traits and their placement on genetic maps

Correlation of expression profiles with QTL to measure both copy number and allelic variation.

Confirmation of predicted expressed genes in A, B and tetraploid transcriptomes

Identify genes that mediate resistance to diseases and pests, such as: bacterial wilt, peanut smut, 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)

Identify 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 genes, alternative splice products, the identification of co-regulated genes and gene networks.

Methods for genome editing to determine or validate gene function

2.4 A central on-line database and portal to integrate genetic and genomic information

Bioinformatic resources provide access to interactive programs for sequence analysis. Genome user-guidelines for genomic data submission and application also establish a unified means for implementing a breeder-friendly database.

Milestones & Deliverables

- Unified or centralized databases that connect DNA sequences to linkage groups, chromosomes, QTL, candidate genes, polymorphisms & phenotypic traits
- A HapMap browser that connects the sequence to polymorphisms for traits of interest
- Ability to overlay transcriptome, genetic and physical maps from A, B, amphidiploid and whole tetraploid genome sequences
- Unified or centralized databases that link genomic information to metabolic pathways governing biological processes
- Tools for the identification of candidate genes underlying QTLs.
- Develop and provide user-friendly, affordable genomic services and tools to all peanut breeders.

Integration of IPGI Resources for Crop Improvement

Marker Assisted Selection has been shown to reduce the time needed to add a new trait to a current cultivated variety. Good markers have been found for resistance to late leaf spot (LLS), early leaf spot (ELS), tomato spotted wilt virus (TSWV), root knot nematode (RKN), and high oleic acid. More useful markers are being developed through genome mapping for traits such as cylindrocladium black rot (CBR), white mold (WM), peanut rust, bacterial wilt, peanut smut and drought tolerance. 'Chip' technology facilitates new breeding strategies for stacking all these traits in improved varieties for each market type and geographic production area. Many breeders are beginning to use these markers in their breeding programs to create new varieties that help reduce the cost of production, enhance peanut quality and ensure an adequate/safe supply of peanut products.

Goal 3: Enhancing crop improvement using genetic and genomic tools

Performance Measures:

3.1. Improved methods for phenotyping germplasm resources

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 & Deliverables:

- Guidelines and protocol for standardizing phenotypic description of germplasm resources
- High-throughput systems for screening genetic variation for physical and chemical traits that impact product quality
- Improved methods for phenotyping flavor and sensory trait profiles in germplasm resources

- Effective systems for characterization of genetic variation in bacterial wilt, peanut smut, early leafspot, late leafspot, rust, tomato spotted wilt virus, Sclerotinia blight, white mold, aflatoxin production, drought tolerance; yielding ability, maturity in breeding populations

3.2 Improved understanding of biological mechanisms that mediate important traits

As genomics become more efficient, improved understanding of how genes interact and how gene expression is regulated within biological pathways will guide next generation strategies for crop improvement.

Milestones & Deliverables

- Determine the biological basis for genetic correlations among protein & oil or other seed constituents
- Characterize regulatory mechanisms that mediate resistance to diseases and pests, such as: bacterial wilt, peanut smut, tomato spotted wilt virus (TSWV), leaf spot (early - Cercospora arachidicola; late - Cercosporidium personatum), rust (Puccinia arachidis), white mold (Sclerotium rolfsii), and nematode (Meloidogyne arenaria)
- Characterize regulatory mechanisms that mediate tolerance to abiotic stresses, such as: drought, temperature (cold, heat), and nutrient deficiency
- Characterize genes associated with substantial reduction of pre-harvest aflatoxin contamination
- Characterize genes that mediate genetic variation in endogenous levels of antioxidants & essential nutrients
- Establish genetic associations that help predict the expression of flavor components in roasted peanuts
- Characterize the genetic basis for variation in kernel size/grade & market types

3.3 Improved methods for pyramiding desired gene combinations in breeding lines

As genomics becomes more efficient, innovative approaches are needed to associate molecular markers with phenotypic traits in a wide range of genotypes, and to enhance the efficiency of introgressing multiple traits into an elite cultivar.

Milestones & Deliverables:

- Implement IBP databases in peanut breeding
- Deploy innovative breeding methods, such as MAGIC and MARS
- Deploy innovative breeding methods for tracking the introgression of genome segments from interspecific parents

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