

# S1.OP1.

## Results from the Peanut Genome Initiative and the Impacts on Cultivar Development

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The Peanut Genome Initiative (PGI) was a five year international effort which began in 2012. The objectives were to sequence the peanut genome and develop and apply new genomic technologies to peanut science. A large part of the funding came from the U.S. peanut industry. Their primary goal was to develop marker-assisted selection (MAS) methodologies that lead to improved cultivars. Peanut is an allotetraploid with a very large genome. One of the first accomplishment of the PGI was to sequence the genomes of the two progenitor diploid species of peanut. Recently, the sequence of the cultivated species was completed. To develop genetic markers for MAS, several structured populations were developed, genotyped, and phenotyped. Molecular markers have been developed for several economically important traits and are being implemented in breeding programs. This is having a great impact on the efficiency and effectiveness of peanut cultivar development.

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# S1.OP2.

## Modernizing the Peanut Breeding Program at ICRISAT

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Stage-gate system of product design, development, advancement and delivery are some of the key elements of modernizing peanut breeding program at ICRISAT. Peanut Network Groups of Asia and Africa represented by ICRISAT, NARS, NGO's and private sector will be a platform to for product Design, development, testing, advancement and delivery. A multi-stakeholder engagement is expected to enable designing the products to the market needs.

The strategy for product design at ICRISAT's peanut breeding program employs genetic gain estimate as a metric to measure the health of the breeding pipeline. A study conducted showed an annual genetic gain of 0.7% for pod yield equivalent to 57Kg/ha per year and indicated need to focus on shelling outturn to further enhance the genetic gain in Spanish bunch types. Process innovations such as rapid recycling of elite parents, rapid generation advancement (RGA), cost-effective genotyping, early generation testing in target sites, multi-environment testing to address G X E have contributed to enhanced rate of genetic gain in peanut Breeding and Testing Pipelines at ICRISAT in recent years. For example, the 'process innovations' resulted to drastically cut down the number of years required to develop high oleic lines in Spanish and Virginia Bunch background adapted to Africa and Asia. The hybridization stated in 2011 and in 2017, 16 high oleic lines were advanced to national release testing in India and over 200 lines were shared with partners from nine different countries.

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# S1.OP3.

## Wild *Arachis* Species, Valuable Germplasm Still to be Known

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Wild species related to agricultural crops can increase the adaptive capacity of agricultural systems around the world. They represent a large pool of genetic diversity from which to draw new allelic variation required in breeding programs. *Arachis* wild species are not the exception. New species are being discovered at a rate of almost one species a year since the Krapovickas and Gregory monograph was published in 1994. New characters were described for the genus, and the geographic distribution have been extended for most of the known species. Cytogenetic data has helped to arranged them in genome groups and genomic information was generated for a few of them including the wild relatives that originated peanut. *Arachis* wild relatives proved to be extremely valuable in providing resistant alleles to peanut commercial varieties for diverse diseases. Unfortunately, wild *Arachis* species are a threatened resource and biological knowledge on them is still very limited. Examples are the assumptions that all *Arachis* species are autogamous and that all of them have the same reproductive efficiency. Moreover, the delimitation of species is still very difficult in many cases and the intraspecific and intrapopulation variability, both morphological and genetic, is almost unknown. Here, we review and discuss (i) past and current efforts to generate knowledge on wild *Arachis* germplasm, (ii), what constraints continue to hinder increased use of wild species in breeding and (iii) what measures need to be taken to improve their protection, both in the wild and in genebanks.

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## S1.OP4.

### Could Small-seeded Wild Relatives of Cultivated Peanut be Used to Increase the Size of Peanut Seeds?

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Seed size is a major agronomic trait that has been selected in crops during the domestication process. However, many studies reported agronomically important alleles that have been left behind or lost during this process. Cultivated peanut is characterized by the paucity of DNA polymorphism. Conversely, high level of polymorphism exists with peanut wild relatives that can be harnessed together with important agronomic traits to improve the cultivated varieties. In a collaborative project with CIRAD, ISRA, UGA and EMBRAPA we developed interspecific QTL mapping populations (AB-QTL and CSSL) for identifying and tracing back the favorable wild alleles in breeding programs. We identified several genomic regions associated with seed size increase in two AB-QTL populations that shared a common recurrent parent. A CSSL population developed from the cross between Fleur11 and (*A. ipaensis* x *A.duranensis*)<sup>4x</sup> was used to validate those QTLs. The CSSL population is of particular interest as it represents the entire wild species genomes in a set of lines each carrying one or a few wild donor segments in the genetic background of the cultivated peanut. This allows mendelizing the QTLs, which ease their used in breeding programs. Several CSSLs that carry wild chromosome segments involved in seed size increase were crossed for pyramiding the QTLs. Additive effects were observed indicating that pyramiding of wild alleles has significant potential for increasing seed size in peanut. The recent release of the peanut genomes opens new avenues for understanding the genomic mechanisms that favored the loss of benefic alleles in cultivated peanut.

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# S2.OP1.

## Overview of crops to end hunger initiative

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This presentation is an introduction to the Crops to End Hunger Initiative. This new initiative is implemented by CGIAR research centers with leadership from USAID, UKAID, Australian Centre for International Agriculture Research, GIZ, and Bill and Melinda Gates Foundation. It aims to modernize and strengthen the crop improvement programs of the CGIAR in strong partnership with National Agricultural Research Programs. Efforts will include design of product profiles to guide development of improved varieties for specific geographies to support farmer productivity despite climate shocks, respond to consumer and market demands, and contribute to nutrition.

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## S2.OP2.

### Research Objectives in the Feed the Future Innovation Lab for Peanut

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The Feed the Future Innovation Lab for Peanut (Peanut Innovation Lab) is one of 22 Innovation Labs funded by USAID under the US government's Global Food Security Strategy. The University of Georgia host the management entity that provides overall leadership of the Peanut Innovation Lab. The program ultimate goal is to improve peanut production, quality and profitability for smallholder farmers in Feed the Future countries. To accomplish this goal, the innovation lab supports research partnerships between US and host country scientists working together to solve problems faced by farmers, input providers, buyers/sellers, processors and consumers along the peanut value chain. USAID has supported international peanut research since 1982, and the current program started in January of 2018. The research projects are currently being finalized with focus in varietal development, value-added gain, nutrition, gender and youth. Details on the innovation lab overall objectives and proposed research portfolio will be provided.

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## S2.OP3.

### PeanutBase: A Resource for Molecular Research and Breeding

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PeanutBase is the peanut/groundnut community resource for molecular research and breeding. It hosts the three representative reference *Arachis* genome assemblies: *Arachis duranensis* (V14167), *Arachis ipaensis* (K30076), and *Arachis hypogaea* (Tifrunner), including gene models for each of the three. The gene models have been enriched with functional information and are included in legume-specific gene families generated by the Legume Federation. Inclusion in the gene families enables comparative research across the legumes. A number of expression studies, which provide additional gene function information, are hosted at PeanutBase, including a tissue atlas, and a number of biotic and abiotic stress response expression studies. Additionally, PeanutBase holds germplasm, germplasm traits, genetic maps, markers, and QTL data. A number of browsing, visualization, and analysis tools exist at PeanutBase, including BLAST, genome browsers, a tool for exploring geographic origins of peanut accessions, and others. To improve support for breeders, PeanutBase is leading a project to genotype the US peanut core collection, and is collaborating with the Integrated Breeding Platform (IBP) and with the developers of BrAPI, a protocol that permits breeding resources to exchange data.

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## S2.OP4.

### Annotating the Peanut Transposons to Provide Resources for Peanut Improvement and Genomics

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The advancement of new sequencing technologies makes it possible and affordable to sequence the organisms with large and complex genomes. However, bioinformatics tools still do not meet the demands of many data-intensive projects. Transposable Elements (TEs) are abundant in plant genomes, especially those with large genomes such as peanut (*Arachis hypogaea*,  $2n=2x=40$ ). Given their ubiquity, the discovery of transposons and other repeats is critical to accurately annotate the peanut genome and for other genome-related research. We developed a TE annotation bioinformatics pipeline by combining *de novo* annotation and homology-based sequence searches to identify transposons and generated a peanut transposon library which includes both DNA and RNA transposons. We found that transposons contribute more than 72% of the reference Tifrunner genome. Of note, numerous potentially active transposons were identified that may be a valuable resource for the development of transposon-based markers for peanut improvement and transposon tagging for functional genomics. Furthermore, the comprehensive analysis of peanut transposons revealed the horizontal transfer of genetic materials between flowering plants and animals.

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## S2.OP5.

### Considerations for Successful Genotyping of *Arachis hypogaea* in the Modern Genomics Era

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Peanut genomics has reached a crossroads, albeit an exciting crossroads. That doesn't mean that analysis of genomes and discrete genotypes will now be easy. It will require nuance and foresight. There are reference quality genomes available now of the diploid progenitors of cultivated peanut. There are two reference quality assemblies of *Arachis hypogaea*; a runner type and a spanish type. Which is the appropriate reference to use? There are two large-scale SNP genotyping arrays. Which one is the best to use? Is it better to use sequence-based methods or to take advantage of the lower cost of the arrays. The answers to these questions will have long lasting consequences on not only the quality of genomic research, but also the transferability of results, and the efficiency of collaboration. As a global community, can we afford to let genotyping preferences divide us?

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## S2.OP6.

### The Genome Sequence of Peanut - Genetic Exchange Between Ancestral Genomes Drives Genetic Diversity

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We report the genome sequence of cultivated peanut (*Arachis hypogaea* cv. Tifrunner). As expected, it harbors essentially complete sets of chromosomes from the two ancestral species (*A. duranensis* and *A. ipaënsis*). However, we show that after its origin, the genome has evolved through mobile element activity, deletions and homeologous recombination; the flow of genetic information between corresponding chromosomes derived from the different ancestors. Uniformity of some of the patterns of recombination favors a single origin for cultivated peanut and its wild counterpart *A. monticola*. However, through much of the genome, homeologous recombination has created diversity. Using a new polyploid hybrid made from the ancestral species, we demonstrate how this can generate phenotypic change: a spontaneous change of flower color. This flow of genetic information is strongly influenced by chromosome structure and is asymmetrical: chromosomes derived from *A. duranensis* are more modified over time than the other. Homeologous recombination is ongoing and is orders of magnitude more frequent than mutation. It seems likely that this mechanism, which creates genetic diversity, helped favor the domestication of *A. hypogaea* over other diploid *Arachis* species cultivated by man.

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## S3.OP1.

### Characterization of Chinese Peanut Germplasm and Trait Mapping

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The peanut germplasm is fundamental to genetic enhancement for improved cultivars. A lot of germplasm accessions of the cultivated peanut and wild *Arachis* species have been assembled and conserved in many countries, and the Oil Crops Research Institute of Chinese Academy of Agricultural Sciences (OCRI-CAAS) is one of the major conserving agencies. A lot of peanut germplasm characterization and genetic diversity assessment work were conducted in OCRI-CAAS, and the Chinese peanut core collection, mini core collections and mini-mini collection were selected on these foundation. With the extensive and intensive germplasm characterization, elite peanut accessions with desirable traits have been identified for development of bi-parental mapping population. Using the traditional QTL mapping, association mapping and BSA-seq approaches, QTLs were identified for yield-related characters, resistance to leaf spot, rust, bacterial wilt and aflatoxin contamination, and quality-related characters such as oil content and fatty acid components. Molecular markers linked to major and stable QTLs were developed for using in marker-assisted breeding.

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## S3.OP2.

### Phenotypic and Molecular Screening of Groundnut Varieties for Cercospora Leaf Spot Diseases

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Twenty groundnut varieties were screened for leaf spots resistance using both phenotypic and molecular techniques to form the basis for resistance breeding. Phenotypically, the 20 genotypes were screened under natural field infection and artificial infection in screen house for combine resistance to early and late leaf spots. Eight SSR markers previously found to be linked to leaf spots resistance in groundnuts were also used to screen the DNAs of the 20 genotypes. Differences in disease incidence among individual plants, severity score, lesion diameter and defoliation across the 20 genotypes were highly significant ( $p < 0.001$ ) under phenotypic screening. Cluster analysis from phenotypic data grouped the genotypes into two main groups but molecular data grouped the genotypes into five groups at 70% similarity index. Significantly high and positive correlation ( $r = 0.89$ ) between artificial and natural field infection was observed. No complete resistance was found. However, 14 genotypes were moderately resistant while six were susceptible. Out of the eight SSR markers used, five (62.5%) were very promising. Two or more of these five promising markers viz pPGseq2F5<sub>280</sub>, pPGseq2B10<sub>280/290</sub>, pPGPseq17F6<sub>120/140/150</sub>, PMc588<sub>180/220</sub> and PM384<sub>100</sub> confirmed resistant genotypes at the molecular level. The resistant genotypes confirmed by the markers were ICG7878, Obolo, Oboshie, Jenkaar, Adepa, Nkosour, Azivivi, Otuhia, Nkatiesari' and 'Sumnut22'. Genotypes 55-437, Yenyawoso, and Shitaochi were susceptible. Hence, resistance to leaf spots exists among commercially grown groundnuts in Ghana. Combination of phenotypic and DNA molecular evaluation is very useful for proper identification of resistant genotypes in leaf spots resistance breeding programs.

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## S3.OP3.

### Evaluation of Advanced Breeding Lines of Groundnut (*Arachis hypogaea* L) for Foliar Disease Resistance, Drought and Productivity Traits in the Northern Dry Tract of Karnataka

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Groundnut is one of the principal oilseed crops of the world and India. The present study aimed at the evaluation of promising advanced breeding lines (ABLs) of groundnut developed at UAS Dharwad and ICRISAT through conventional and/or marker-assisted backcrossing(MABC) approaches. The ABLs comprised of Dh-243, Dh-256, Dh-257, Dh-268 and Dh-269 with drought tolerance and MABC lines DBG-1, DBG-2, DBG-A, DBG-B with foliar disease resistance (UAS Dharwad) and ICGV 03042,03043, 06420, 05155 with high oil, ICGV 16039, 16680, 16701 with high oleic; ICGV 07222, 07220, 02266, 00350, 91114 with drought tolerance and ICGV 16220 to 16254 with early maturity and drought tolerance (ICRISAT, Patancheru). These ABLs were evaluated in total of four replicated trials along with check varieties (TMV 2, JL 24, TAG 24, GPBD 4, G 2-52) for their reaction / tolerance to prevailing biotic and abiotic stresses and productivity traits during kharif 2017 at RARS, Vijayapur representing the dry tract of Northern Karnataka (Zone 3). The elite lines viz., Dh 256, Dh 257, DBG-A, ICGV 03043, ICGV 16237, ICGV 00350 were selected based on their tolerance drought and iron chlorosis, resistance to LLS and rust diseases and high yield potential. From the evaluation of 210 ABLs developed at ICRISAT for drought tolerance 32 elite lines were also selected. These elite lines are being evaluated during Kharif 2018 to confirm their superiority and identify suitable genotypes for multilocation testing and one of the drought tolerant elite line Dh 256 is under farm trial evaluation in the farmers field in the region.

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## S3.OP4.

### The hunt for leaf spot disease tolerance in groundnut: progress made at CSIR-SARI

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In Ghana, the yield of groundnut (*Arachis hypogaea*, L.) is constrained by early and late leaf spot infections. Crop varieties possessing stay-green trait are known to show resistance or tolerance to some diseases and drought conditions. This study explored the stay green trait in groundnut and its relationship with leaf spot diseases. Twenty-five (25) groundnut genotypes were phenotyped for stay-green using Leaf Area Under Greenness (LAUG), leaf spot severity using Area Under Disease Progression Curve (AUDPC) and yield. The results revealed a significant ( $p \leq 0.001$ ) positive relationship between LAUG and AUDPC for early and late leaf spot disease severity ( $r = 0.82$  and  $0.63$ , respectively). LAUG had a significant negative association ( $p \leq 0.001$ ) with chlorophyll content at pod initiation, mid pod filling and physiological maturity as well as pod yield ( $r = -0.68$ ). Based on the LAUG scores, the genotypes were grouped as stay-green or non-stay-green. Four RIL populations were developed with parents selected from the two groups and SSR markers used to confirm true hybrids. The populations are currently at the F<sub>3</sub> stage. Going forward, the RILs will be phenotyped across 5 locations in 2 years while the F<sub>4</sub> will be genotyped taking advantage of the 58k SNP 'axiom\_arachis' array. The QTLs controlling stay-greenness will be mapped through modelling of main effect QTLs and QTL-by-environment interaction. As a result, marker assisted selection will be deployed to introgress the stay-green QTL/s into our candidate varieties. Recovery of the preferred parental genome will be done through backcrossing.

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## S4.OP1.

### The Big Picture: Identifying the Links Between Drought, Development, and Aflatoxin Through Integromics in *Aspergillus flavus*

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Previous “-omics” studies into *Aspergillus flavus* responses to drought-related oxidative stress revealed the association between oxidative stress and mechanisms regulating fungal development, stress responses, host interactions, and aflatoxin production with their expression varying among isolates. Understanding the genetic causes of this variation is critical to developing novel mitigation strategies. To identify these base causes of the variation and prevention strategies, the objectives of this study were: (1) to produce pseudomolecule-level genomes of two *A. flavus* isolates with contrasting aflatoxin production, mating type, and drought-related oxidative stress tolerance, AF13 (+++, MAT1-2) and NRRL3357 (+, MAT1-1), and (2) to conduct comparative integrative analyses of the “-omics” data to answer some biological questions. PacBio sequencing generated 7.73 and 7.97Gb of data with average read lengths of 12,822 and 10,437bp for AF13 and NRRL3357, respectively. Assembly resulted in 19 and 69 contigs with N50 of 2.579 and 1.998Mb for AF13 and NRRL3357, respectively. The assembled genome sizes are 37.599Mb for AF13 and 38.645Mb for NRRL3357. Several of these contigs were found to exceed 3.0 Mb and could cover the entire length of individual chromosomes. Illumina sequencing was also used to re-sequence 11 field isolates of *A. flavus* including AF13 and NRRL3357. These will be used for assembly correction/polishing, and to identify additional structural variants and polymorphisms influencing key traits. A novel integrated “omics” (integromics) approach will also be performed correlating transcriptome, proteome, and metabolome data with genomic variations to identify factors linking aflatoxin and other traits which are useful as targets for host resistance improvement with breeding and biotechnology for possible prevention of aflatoxin contamination.

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## S4.OP2.

### Identification of QTLs for Leaf Spot and Rust Resistance in a BC<sub>3</sub>F<sub>6</sub> Interspecific Peanut Introgression Population in West Africa and Texas using SNP Markers

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Cultivated peanut is reproductively isolated from its ancestral wild species parents because of differences in ploidy and genomes, and the self-pollinating nature of the peanut. There is considerably less polymorphism among cultivated peanuts than among wild species. One way of introducing genetic diversity into cultivated peanut is through hybridization with wild species. A BC<sub>3</sub>F<sub>6</sub> population developed from a cross with the synthetic amphidiploid TxAG-6 [*A. batizocoi* x (*A. cardenasii* x *A. diogoï*)]<sup>4x</sup> as donor and Florunner as recurrent parent resulted in isolation of individual lines having high oil contents, resistance to leaf spot disease, root-knot nematodes, and rust. Genome-specific SNP-based markers were designed and used to map 63 BC<sub>1</sub> individuals for making a genetic map, and genotypes of 317 BC<sub>3</sub>F<sub>6</sub> individuals from this population were obtained on the Fluidigm Biomark system. Phenotypic evaluation was performed in Ghana, Burkina Faso, and Texas. QTLs were identified for resistance to early leaf spot, late leaf spot, and rust. Several QTLs were consistent across environments while others were environment-specific. It is expected that resistant accessions and markers will be useful for marker-assisted breeding, to introgress resistance into suitable agronomic backgrounds.

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## S4.OP3.

### Identification of Novel genes for Resistance to Tomato Spotted Wilt and Leaf spots in Peanut (*Arachis hypogaea* L.) Through GWAS Analysis

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Tomato spotted wilt (TSW), early leaf spot (ELS), and late leaf spot (LLS) are three serious peanut diseases in the United States, causing tens of millions of dollars of annual economic losses. However, the genes underlying those disease resistances in peanut were not well studied. In this study, we conducted a genome-wide association study (GWAS) for the three peanut diseases using Affymetrix version 2.0 SNP array with 120 genotypes mainly coming from the U.S. peanut mini-core collection. A total of 158 quantitative trait loci (QTLs) were identified with phenotypic variation explained (PVE) from 10.2% to 24.1%, in which 112 QTLs are for resistance to TSW, 18 QTLs for ELS, and 28 QTLs for LLS. Among the 158 QTLs, there are six, four, and two major QTLs with PVE higher than 14% for resistance to TSW, ELS, and LLS, respectively. Of the total 12 major QTLs, 10 were located on B sub-genome and only 2 were on A sub-genome, which suggested that B sub-genome has more significantly resistance genomic regions than A sub-genome. In addition, two genomic regions on linkage group B9 were found significantly resistance to both ELS and LLS. Total of 21 candidate genes were identified significantly associated with diseases, which include 15 candidate genes for TSW, 3 candidate genes for ELS, and 3 candidate genes for LLS, respectively. Most candidate genes in the associated regions are known to be involved in immunity and defense response. The QTLs and candidate genes obtained from this study will be useful to breed peanut for resistances to the diseases.

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## S4.OP4.

### Genetic Investigation and Mapping of the White Mold Tolerance Trait in Peanut

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White mold, caused by *Sclerotium rolfsii*, imposes severe losses in several peanut growing regions of Israel. Developing genetic resistance is one way to manage this problem. Yet, breeding is difficult since not much is known about genetic, biological and chemical mechanisms for tolerance. The goals of this study were to evaluate the tolerance in a RIL population derived from a tolerant X susceptible cross (both Virginia-types), to locate QTLs and to discover potential mechanisms for tolerance. In 2017, 97 RILs were analyzed in random blocks field design. Lines were artificially inoculated in the field by placing hyphal plugs near the root crown of 100-day-old plants. Other important agronomic parameters were gouged as well. Resistance parameters were found significantly correlated to brunching habit, shell strength, shell weight, pod reticulation and oil content. Trait mapping was performed by applying the new peanut Affimetrix SNP-array, containing ~2900 polymorphic SNPs among the RILs. Five QTLs were found for resistance explaining 0.14-0.26 of the total variation\*. Two lines with extreme phenotypes, B65 (tolerant) and B77 (susceptible), were further analyzed in controlled conditions. Significant differences were found in the level of mycelium and the number of healthy plants in detached inoculated shoots and small pots tests, respectively. Accordingly, RT-PCR of the fungal *Actin* gene was 13.2 and 2.6 higher in B77, respectively. Interestingly, plant developmental stage had an effect, while 20 days-old plants were significantly more resistance than 55 days-old. This study provides the first insight into resistance related mechanism in Virginia-type peanut and serves as a preview for MAS development.

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## S4.OP5.

### Using wild *Arachis* Species and Senegalese Rhizobial Strains to Improve Biological Nitrogen Fixation of Cultivated Peanut in Senegal

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In Senegal peanut is widely cultivated in rotation with cereals. Like other legumes, peanut is able to associate with soil rhizobia to form symbiotic nitrogen-fixing nodules which provide substantial amounts of nitrogen not only to the peanut plants but also to the following cereal crop. In order to optimize peanut nitrogen fixation in Senegal we are working on both symbiotic partners. On the bacterial side we characterized a collection of rhizobial strains isolated from peanut fields in Senegal. We found that in Senegal peanut is able to recruit both effective and ineffective *Bradyrhizobium* strains suggesting that peanut nitrogen fixation could be optimized by increasing the competitiveness of the effective strains. On the plant side we have explored the variability of nitrogen fixation among wild relatives of peanut and synthetic allotetraploids obtained from the wild relatives and found significant differences for some species. We have also mapped quantitative trait loci (QTLs) related to peanut biological nitrogen fixation using an interspecific CSSL mapping population developed by crossings between a synthetic tetraploid AiAd (*A. ipaensis* × *A. duranensis*)<sub>4×</sub> and the cultivated variety Fleur 11. Our results show that the introgression of wild genomes can have a strong impact on nitrogen fixation and can be used to include this trait in breeding strategies and to further understand the molecular basis of peanut nodulation.

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## S4.OP6.

### Using Wild Peanut Species for Improving Native Biological Nitrogen Fixation in Rhizobia Symbiosis

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*Arachis hypogaea* originated from South America, and was introduced and adapted in Africa and Asia centuries ago. Peanut is now one of the most widely cultivated grain legumes and the questions of its adaptation in environments that are different to its native ecosystems, particularly regarding associated-microorganisms remain unanswered. We are investigating how the adaptation of peanut to West Africa impacted the nitrogen-fixing symbiosis with rhizobia. We characterized a collection of 35 native strains isolated from peanut nodules trapped in soils coming from various regions in Senegal using the ribosomal intergenic spacer (IGS) and the *nodC* gene. These strains were also used for inoculating Fleur 11, a peanut cultivar widely grown in Senegal. Our results showed that Senegalese strains are distributed in three main clusters, affiliated to the genus *Bradyrhizobium*. Furthermore we compared Fleur11 with its wild relatives and synthetic tetraploids vis-à-vis nitrogen fixation using efficient strains isolated in Senegal or in Argentina. We failed to detect any major difference between *A. hypogaea*, its wild progenitors (*A. ipaensis* and *A. duranensis*) or the synthetic tetraploid AiAd, suggesting that the symbiotic efficiency was not affected by plant selection or by the recruitment of African symbionts. Interestingly some *Arachis* wild species showed increased or precocious nitrogen fixation compared to Fleur11 opening possibilities to improve biological nitrogen fixation in cultivated varieties using wild relatives.

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## S5.OP1.

### Genetic Diversity, Population Structure, and Botanical Variety of Global Peanut Accessions Revealed Through Genotyping-by-Sequencing

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Cultivated peanut (*Arachis hypogaea* L.) is an important oilseed crop and food source. On the basis of morphological features, crossing experiments, and seed protein electrophoretic profiles, the cultivated peanuts were classified into two subspecies (subsp. *hypogaea* and subsp. *fastigiata*) and six botanical varieties. However, these classifications were based on growth habits, morphological features, as well as seed, pod, and inflorescence characteristics. In this study, Tunable-GBS (t-GBS) and re-sequencing were applied, besides, high quality SNP markers were identified from different peanut accessions, including landraces and breeding lines worldwide. Genetic relationships of the tested peanut accessions indicated that these accessions were grouped into three clusters. Interestingly, and also supported by principal component analysis, almost all of the peanut accessions in cluster C1 were var. *fastigiata*, while clusters C2 and C3 mainly consisted of var. *vulgaris* and subsp. *hypogaea*, respectively. To our best knowledge, this study is the first to reveal the genetic relationship between var. *fastigiata* and var. *vulgaris*. Moreover, the SNPs identified in this study may be a valuable resource for peanut breeders and genetic diversity studies.

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## S5.OP2.

### The Progress of High Oleic Peanut Breeding and Application in China

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Oleic acid content is positive correlation with the quality of peanut oil and the shelf life of peanut products. High oleic acid peanut breeding has become an important direction for peanut quality improvement in China in recent years. China is one of the world's largest producers and consumers of peanuts. However, most of the peanut varieties in China are low in oleic acid content (below 55%). In recent years, great progress has been achieved in high oleic peanut breeding which is powered by marker-assisted selection combined with backcrossing approach. In addition, mutagenesis and gene editing approaches are also used for high oleic peanut breeding in China. Moreover, many other traits also improved for high oleic peanuts, such as resistance to disease and low temperature, and suitable for mechanization harvest etc. Up to now, more than 40 high oleic peanuts have been registered and the growing areas were about 100 thousand hectares for seed multiplication and for oil production. During the popularization and extension process of high oleic acid peanut, the ministry of agriculture contributed great effort in organizing and arrangement of the national wide experiments and demonstration. The high price of oil from high oleic acid peanut causes strong interest for the breeders, growers and processors. Here, the prospective and possible problems of high oleic peanut breeding and utilization in China were discussed.

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## S5.OP3.

### Development of a Next-generation, Multi-parent Advanced Generation Intercross (MAGIC), Fine-mapping Population for Advancing Genetics and Genomics Studies in Peanut

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A MAGIC population can provide an increased level of recombination and mapping resolution by integrating multiple alleles from different parents for fine-mapping of complex quantitative traits and for breeding selection of improved/diverse lines with novel genetic variation/traits. Development and phenotypic evaluation of a multi-parental MAGIC population, along with high density genotyping tools, such as SNP array and whole genome re-sequencing (WGRS), will be essential for QTL/marker/gene and trait mapping analyses. The primary goal of this project is to develop the first next-generation fine-mapping population of peanut that can be used by U.S. peanut research community for high-resolution phenotyping and trait dissection. This MAGIC population was derived from eight founders: SunOleic 97R, NC94022, Tifrunner, GT-C20, Florida 07, SPT06-06, Georgia 13M, and TifNV-High O/L. These parental selections were based on the availability of genetic and genomic information to maximize genetic diversity while meeting practical breeding objectives including high oleic content. InterCrosses (2-way, 4-way, 8-way) have been made using a simple 'funnel' breeding scheme with the founders combined in equal proportions, followed by a single seed descent (SSD) to develop the MAGIC population. Currently, this MAGIC is at the F<sub>3</sub> generation and has 3575 F<sub>2.3</sub> families in the Tifton summer nursery. Advancement and seed increase will continue using winter (Puerto Rico) and summer (Tifton) nurseries. The entire population will be genotyped. A core subset (or different core subsets) will be developed (divided) based on the genetic similarity or based on unique marker scores for different traits in preparation for phenotyping.

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## S5.OP4.

### Single Nucleotide Polymorphism (SNP) arrays and their interpretation in the context of peanut breeding

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Discovery of single nucleotide polymorphisms (SNPs) in peanut has been challenging due to the tetraploid nature of the cultivated species and the similarity between the two subgenomes. Multiple iterations of discovery pipelines were implemented to improve the discrimination of true allelic SNPs from false homeologous SNPs. Outputs from these pipelines were used to design high-density genotyping arrays for genome-wide SNP calling. The arrays have been used for genetic mapping of elite x elite populations and further exploration of homeologous recombination events as well as for identification of chromosomal regions introgressed from wild species. Array data interpretation is dependent on the set of genotypes subjected to simultaneous analysis due to single feature recognition of homeologs in most instances. Linked polymorphisms of interest can be used to design single-marker assays more applicable to large populations used in breeding programs.

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# S6.OP1.

## Characterization of *Arachis* Wild Species

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Genus *Arachis* contains about 82 species including the cultivated peanut, *A. hypogaea* L. The USDA-ARS managed Plant Genetic Resources Conservation Unit in Griffin, GA, maintains the U. S. peanut collection consisting of both cultivated as well as wild species. About 600 accessions of *Arachis* wild species are maintained mostly as seed stocks. In addition to preserving these genetic materials, a major goal of the program is to characterize the *Arachis* species for useful traits. In 2017, we selected 209 accessions of 45 wild species for laboratory analysis, primarily for seed quality traits. We measured 100-seed weight, total oil content, fatty acids and total protein content. The 100-seed weight ranged from 4 g in *A. dardani* to about 32 g in *A. glandulifera* with a mean of about 14 g for all species. The total oil content varied from 60% in *A. valida* to 44% in *A. benthamii* with a mean of 52% across all species. The fatty acid profiles displayed a large variation for individual fatty acids, however, none of the *Arachis* species were high oleic type. The oleic acid content ranged from 14% in *A. triseminata* to 56% in *A. veigae*. Similarly, the protein content ranged from 19% in *A. batizocoi* to about 37% in *A. valida*. However, we also observed significant variation for total protein content within the species accessions. In addition to the seed quality characterizations, digital images of pods, seeds, leaves from the main stem, primary branch and flowers of the accessions were also captured. The data will be uploaded into GRIN Global website for free public access.

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## S6.OP2.

### Tapping Wild *Arachis* Species for Peanut Improvement

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Narrow genetic base coupled with various biotic and abiotic stresses are the major factors affecting peanut production and productivity worldwide. Wild *Arachis* species offer enormous genetic variability, especially for biotic and abiotic stresses and can be utilized to develop cultivars having enhanced levels of resistance to key stresses and broaden the genetic base of cultivated peanut. However, frequent utilization of wild species in crop improvement is hindered due to linkage drag, need for ploidy manipulations, bridge crosses, and embryo/ovule rescue, thus making it a time-consuming and resource-demanding research endeavor. For efficient utilization of diploid wild species from section *Arachis* for the genetic improvement of tetraploid peanut, several synthetics (amphidiploids and autotetraploids) have been developed by using various A- and B- genome species. These synthetics are being utilized in crossing programs with cultigens to develop pre-breeding populations/introgressions lines (ILs) having high frequency of useful genes/alleles and good agronomic background. Sufficient genetic variability was observed in these populations for morpho-agronomic traits. Evaluation of two populations across locations resulted in the identification of ILs having late leaf spot resistance introgressed from new sources such as *A. kempf-mercadoi*, *A. hoehnei*, *A. duranensis* and *A. ipaensis* other than commonly used *A. cardenasii*. Similarly, preliminary evaluation of pre-breeding population led to the identification of stem rot resistant ILs. These pre-breeding populations provide new and diverse sources of variations and are being shared with NARS partners in India and other countries for use in peanut improvement programs to develop new cultivars with a broad genetic base.

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## S6.OP3.

### The impact of Wild Species in Peanut Breeding: Old Stories and Future Prospects

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Wild crop relatives have been used to introduce genetic diversity into elite cultivars worldwide. For peanuts, pioneering works were done in the 60's and 70's at Texas University and NCSU, where lines were created using *Arachis cardenasii* as donor parent. From the Texas program, several nematode resistant cultivars were released in the USA, benefiting enormously the US peanut industry. From the NCSU program, lines had resistances to several foliar diseases. They were shared with colleagues at ICRISAT, India in the early 80's. At the time, free germplasm exchange was possible, and exchange was done on the basis of individual agreements. As done commonly in germplasm banks, names were changed but the pedigrees were not kept. These lines were propagated at ICRISAT used for breeding and distributed to breeders in several countries, like Australia, Mali and Brazil. They were used as parents for many cultivars worldwide, and therefore, had a large impact in peanut breeding. By broadening the genetic basis of peanut, the lines also enabled the first works on marker-trait association and marker assisted selection on (then thought as) "pure" peanut. The catch is: for decades the various researchers and breeders didn't even know they were dealing with lines derived from a wild species. Here, we genotyped DNAs from different breeding lines and cultivars, looked at pedigrees, exchanged data, and a myriad of scientific articles and reports. With all this information, we pieced together the history of the amazing impact that these lines, anonymously, had on peanut breeding and genetics worldwide. This presentation will exemplify gains of a single wild accession to the peanut industry worldwide, and touch on current research that has promise to impact it in the future.

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## S6.OP4.

### Interspecific Population Development for Disease Resistance

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Peanut is plagued by many disease and insect pests for which resistance in the cultivated gene pool is lacking at sufficiently high levels to solve production losses. Globally, early and late leaf spots and rust are the most widespread and devastating diseases. Wild species of *Arachis* possess extremely high levels of resistance to these pathogens, but genomic incompatibilities and ploidy level differences impede their utilization without manipulation of chromosome numbers to restore fertility. Introgression lines ( $2n = 40$ ) derived from 'Gregory' x *Arachis diogeni* (GKP 10602) that are fully compatible in crosses with *A. hypogaea* were studied morphologically, with SNPs to detect the amount of introgression from the wild to cultivated species, and for disease resistances. Morphologically, the plants varied widely in growth habit and seed size. Significant amounts of introgression occurred into all linkage groups, with the percentage per line varying from a few percent to more than 50% of the *A. diogeni* specific SNPs. Multiple year and location disease tests for early leaf spot, late leaf spot, and Tomato Spotted Wilt Virus (TSWV) were conducted in North Carolina and Georgia. During 2017, early leaf spot was most prevalent in North Carolina (75%) and late leaf spot predominated in Georgia (90%). Defoliation was recorded multiple times using a scale of 1 = no disease to 9 = dead multiple times at each location with the final rating during mid-October after the general crop was harvested. Ten lines expressed high levels of resistance to early leaf spot at the final rating with ratings = 4 - 4.5, resistant checks = 6, cultivars = 8 - 9). SNP markers were associated with ELS defoliation on chromosomes A2, A3, A5, A6, B1, B4, B5, B8 and B9. One line had a rating of 3.3 for late leaf spot in Georgia (checks = 6 - 9). SNP marker associations with late leaf spot defoliation were found on chromosomes A2, A3, A4, A6, B1, B2, and B9 and for the number of lesions on B10. Up to 63% of field plots had TSWV in North Carolina. Four lines did not express symptoms in North Carolina, three lines in Georgia, and one line (IL 51) was disease free at both locations. SNP associations with TSWV were observed on nine chromosomes, with the strongest associations on A9 and B9. Similar results were observed in 2018. Additional studies are in progress to better associate SNPs with all five diseases.

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## S7.OP1.

### Groundnut Improvement for Sub-Saharan Africa and Asia: What Strategy, What Targets?

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With the recent sequencing of the groundnut genome, and the wealth of knowledge gathered on groundnut genetics in the past 15 years or so, the challenge remains to bring these advances in form of crop improvement to the farm gate. This presentation is an attempt to cast, or re-cast, strategies and priorities for groundnut improvement. In the past couple of years, the Excellence in Breeding Platform (EiB) has initiated an effort to modernize breeding in the CGIAR, starting by a “breeder non-centric” definition of breeding product profiles, which sets the main characteristics of demand-led varieties that need to be developed. Under EiB principles, breeding becomes a concerted project co-owned by a group of people encompassing a varied skill set, in which crop genetics is only one of the multi-disciplinary components. The presentation will explore how breeding targets can be better defined by using crop simulation to properly characterize the target population of environments (TPE). Results in that domain will show how water stress adaptation, considered to be a must-have trait in groundnut breeding for Africa, should only be considered critical in well-defined portions of these TPE’s. This new strategy is also a way to better integrate best agronomic practices. For instance, the recommended sowing density has remained unchanged for the past 4-5 decades although groundnut plant type has evolved from spreading types with profuse foliage requiring lower density, to now mostly erect types of early duration with much smaller foliage and needing a higher seed rate. Other simulations will give insights on potential genetic traits for future peanut improvements within the context of optimal agronomic practices. The presentation will also give insight on potential future targets for breeding. For instance, considering that groundnut haulm to feed cattle has an increasing importance in the groundnut value chain and that there is genotypic variation for both the haulm productivity and haulm nutritional quality. In relation to this, results will be presented on the symbiotic nitrogen fixation potential of groundnut, as an advocacy to add this trait as a potential breeding target, thinking of groundnut as a critical component of cereal-legume based systems in the semi-arid tropics.

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## S7.OP2.

### Strengthening Groundnut Regional Variety Trials Networks in West and Central Africa

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The groundnut breeding program for ICRISAT WCA works to identify improved groundnut varieties which are high yielding, tolerant/resistant to drought, foliar diseases, with high oil and preferred by farmers and consumers targeting the Sahel, Sudan and Guinea savannah agro-ecologies. Regional variety trial is among the important components of activities to evaluate the performance of advanced breeding lines for target environments. Two sets of regional variety trials containing 48 varieties were conducted using 4 x 4 and 6 x 6 partial lattice designs in Burkina Faso, Ghana, Mali and Nigeria between 2016 and 2018 in partnership with national programs. The number of locations per country per season varied from 2 to 6. Preliminary ANOVA results for the 4 x 4 trial indicated mean pod yield range of 0.8t/ha at Kita, Mali to 4.5t/ha at Damongo, Ghana during 2016. In both trials, varieties with significant high yield compared with checks were identified. The regional variety trials enabled evaluation of advanced breeding lines in a larger number of locations. Hence, the networks of regional variety trials will be strengthened with new varieties incorporated in the testing program every year. ICRISAT will support NARS program to facilitate the release of best bet varieties in target environments so that farmers adopt new varieties.

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## S7.OP3.

### Drought Tolerance Physiology for Improving Groundnut Adaptation and Productivity in Semi-arid Zones

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Groundnut productivity is still adversely challenged by drought stress in 70% of its grown area. Although great progress has been achieved in improving groundnut tolerance to drought through genomic and biotechnology, further understanding of drought tolerance mechanisms still needed for groundnut improvement success notably in Sahelian zones. For dissecting groundnut response to explore traits and understand the underlying mechanisms of drought tolerance we investigated large number and subset of groundnut genotypes using forward and reverse phenomics approaches. Several years and Multilocation field phenotyping was conducted to identify the best genotypes having desirable traits contributing to yield while lysimetre phenotyping led to explore what make these genotypes the best. Genotype and genotype by environment (GGE) biplot analysis revealed significant genotype and year (GxY), and genotype by environment (GxE) interactions and suggested that under drought conditions marker assisted recurrent assisted selection and genome wide selection need to take care of high GxY interaction. Our findings revealed also that, water saving traits were relevant for drought tolerance in groundnut grown in Sahelian sandy soil. Water extraction, transpiration efficiency, canopy temperature depression, root volume, length density and dry matter investigated showed different pattern of water use among drought tolerant genotypes. Under drought conditions, water extracted was not related to root traits but highly dependent on stomatal adjustment which was driven by water transport pathways. Aquaporins play key roles in water transport pathway modifications, QTLs identification and genes expression study could contribute in advancing groundnut improvement to drought tolerance and high yield in Sahelian environment.

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## S7.OP4.

### ICRISAT's Efforts Toward Understanding and Translating Genomic Information from Genome to Field in Groundnut

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Groundnut has now entered in post-genome sequencing era and several resequencing efforts are in progress across the world. These resources have also made available millions of genetic markers and high density genotyping arrays for conducting high resolution genetic diversity, trait mapping and candidate gene discovery. In parallel during the last five years, ICRISAT has successfully developed multi-parent advanced generation intercross (MAGIC), training population and nested-association mapping (NAM) populations for high resolution trait mapping and breeding. Significant progress has been made by ICRISAT with its partners on trait mapping for agronomically important traits such as resistance to rust, late leaf spot, stem rot and aflatoxin contamination, and several other yield and oil content/quality related traits. Much emphasis has now been paid in using high throughput genotyping and sequencing-based trait mapping approaches for faster discovery of genomic regions and candidate genes for above mentioned traits in addition to other new traits namely allergens, fresh seed dormancy and seed weight. For improving qualitative traits, marker-assisted backcrossing (MABC) successfully developed molecular breeding lines for foliar fungal diseases and high oleic acid. The promising MABC lines are currently in final year of testing in the All India Coordinated Research Project on Groundnut for evaluation and release. For improving complex and quantitative traits, genomic selection (GS) has been initiated and the different GS models have been tested in the training population consisting of 340 elite lines. Our efforts are focused further to accelerate trait discovery and deployment pipeline in addition to optimizing GS breeding for achieving higher genetic gains in groundnut.

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## S8.OP1.

### Promising Virginia Bunch Groundnut Varieties for Target Ecologies of India

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India is one of the major groundnut growing countries with the largest area under cultivation and second largest production in the world after China. Maharashtra is one of the major groundnut growing states of India. Many groundnut growing regions of Maharashtra falling in the catchment area of South-West and North-East monsoon that are suitable for cultivation of Virginia bunch varieties. In many of these regions, the crop suffers from severe biotic stresses such as rust and late leaf spot diseases resulting in low yields. To overcome these production constraints, a breeding program was undertaken at Agricultural Research Station (MPKV), Kasbe Digraj, District Sangli in collaboration with ICRISAT Patancheru. Two advanced breeding lines, ICGV 04168 designated as Phule Morna and KGD 128 designated as Phule Warna were identified as most promising after testing in on-station, state multi-location and All India Coordinated Research Project on Groundnut (AICRP-G) trials. On the basis of their superior performance in AICRP-G trials over controls, Phule Morna (KDG 123) and Phule Warna (KDG 128) were released in 2016 for cultivation in Zone II (Gujarat and Southern Rajasthan), Zone IV (West Bengal, Jharkhand, Orissa and Manipur) and Zone V (Southern Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu). On farmers' fields in Maharashtra, these varieties performed exceedingly well under high incidence of diseases giving an additional 15 to 25% pod yield over the traditional popular varieties, which are now being rapidly replaced by these new varieties.

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## S8.OP2.

### Present Status and Future Possibilities of Groundnut Cultivation in Different Zones of Vietnam

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Groundnut is one of the major food legume crop in Vietnam's agriculture. Domestic production satisfies about 75% of industry demand and rest is imported from neighboring countries. The total groundnut area was declined from 219.3 to 195.8 thousand hectares whereas the yield was increased from 2140 to 2360 kg/ha during 2012 to 2017. The production is mainly concentrated in five regions of Vietnam *viz.*, Northern East, Northern Midland Mountain, Middle North, Red River Delta, South East and Cuulong River Delta. Among these, middle region has the largest area under cultivation (94.1 thousand hectares) followed by Northern Midland Mountain (47.7 thousand hectares). The South East regions has recorded highest yield in the country (3.87 tons/ha) followed by the Red River Delta (2.5 tons/ha). The majority of groundnut area in the country is under drought and/or salinity stress along with saviour infestation of bacterial wilt responsible for low yield potential in these regions. The large gap between the seed demand and supply of improved variety seeds is also one of the major production constraint in some areas. The countries ongoing groundnut improvement program with targeted traits such as high yield, high oil content, early maturity duration, resistant to bacterial wilt, tolerant to drought and salinity offers a hope for better future of groundnut in the county. Apart from this, the increased demand of food industries for high oleic groundnuts will also play a key role in expansion of area in the country upon release of high oleic varieties in Vietnam.

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**S8.OP3.**

## Mapping GRD using DART sequencing

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# POSTERS

# Poster 1.

## Screening of U.S. Germplasm for Resistance to Peanut Smut

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Peanut smut, caused by *Thecaphora frezzii*, was first reported in Brazil, but has since spread to other countries including Argentina where it has become established and is now found in 100% of the country's peanut growing regions. Disease severity varies with location, but yield reductions as high as 51% have been reported. Although peanut smut is not currently found in the U.S., immediate proactive measures will ensure that the industry will not be threatened should this disease reach the U.S. The first step in breeding efforts for peanut smut is to identify sources of resistance. Therefore, the objective of this study was to identify sources of resistance to *T. frezzii* that can be used to incorporate smut resistance into cultivars optimized for key areas of U.S. peanut production. In 2017, 106 genotypes, including mini-core accessions from the USDA Peanut Germplasm collection and a selection of U.S. elite breeding lines and cultivars, were planted in a test plot with high levels of *T. frezzii* inoculum near the town of General Deheza (Córdoba Province). Plots were arranged in an augmented grid design with three replicates and were maintained for weeds and other diseases throughout the growing season. Upon harvest, pods were air dried and opened by hand to rate for the presence or absence of *T. frezzii*. For screening purposes, entries were retained for further testing if they scored 10% or less disease incidence. Of the 106 test entries, 35 potential sources of peanut smut resistance were identified. Thirteen entries had 0% disease incidence, 9 entries had between 0 and 5% disease incidence, and 13 entries had between 5% and 10% disease incidence. Seventy-one (71) of the entries tested had greater than 10% disease incidence and have been eliminated from future testing. Entries demonstrating strong resistance over multiple years can be used to incorporate peanut smut resistance into cultivars suitable for U.S. production areas.

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## Poster 2.

### **Integrated small RNA and mRNA Expression Profiles Reveals miRNAs and Their Target Genes in Response to *Aspergillus flavus* Infection in Peanut Seeds**

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Peanut is vulnerable to the threat of aflatoxin contamination. The molecular basis between peanut and *A. flavus* compatible interaction is elusive. MicroRNAs have been found to be an important regulator of plant immune system. Here, we employed small RNA, transcriptome and degradome sequencing approaches to systematically investigate the regulatory roles of miRNAs in resistant and susceptible genotypes of peanut under fungus infection. A total of 30 miRNAs, 447 genes and 21 miRNA/mRNA pairs were differentially expressed significantly after infected with *A. flavus*. Moreover, a total of 62 miRNAs, 451 genes and 44 miRNA/mRNA pairs exhibited differential expression profiles between resistant and susceptible genotypes. GO analysis showed that metabolic-process related GO terms were enriched, such as “metabolic process” and “catalytic activity”. KEGG pathway analysis further supported the GO results, in which the majority of enriched pathways were related to biosynthesis and metabolism, such as “biosynthesis of secondary metabolites”, and “metabolic pathways”. Correlation analysis of small RNA, transcriptome and degradome results indicated that the differential expressed miR156/SPL pairs regulated the accumulation of flavonoids in resistant and susceptible genotypes. The miR482/2118 family regulated NBS-LRR defense genes, such as Aradu.168L7, which has the higher expression level in resistant genotype when compared with that in susceptible genotype. These results suggested that both miR156/157/SPL and miR482/2118/NBS-LRR pairs play crucial roles in peanut-*A. flavus* interaction and lead the difference resistance between two varieties. In summary, our study provides a comprehensive information for our understanding of peanut-*A. flavus* interaction.

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## Poster 3.

### Assessing INTA's *Arachis hypogaea* Core Collection for Reaction to Peanut Smut

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There is a high intensity of peanut smut in Argentina's main peanut area. As chemical control and crop rotation have shown modest results controlling the disease, genetic resistance appears a more promising approach. Thus, increased efforts to detect sources of genetic resistance to *Thecaphora frezzii* are needed. INTA's Manfredi Exp. Stn. developed a Core Collection of *A. hypogaea* (CNM) that includes entries from the six botanical varieties and its entries account for a 4% of the total entries in the entire Manfredi Peanut Collection. Molecular analysis showed that the genetic diversity in CNM is high (0.61). Population structure was inferred through Bayesian analysis. Highest data probability was attained at K=2 suggesting entries can be assigned to two groups coinciding with the subspecies taxonomic level. Lack of structure within each group was also observed implying CNM has great potential for association mapping for peanut smut. Additionally, two years of seed increase of an association mapping population (made out of a single plant from each entry in the CNM) has been done. Seeds from each plant in this population along with resistance and susceptible checks were assessed in a heavily smut infested field in General Deheza, Province of Córdoba, Argentina. Augmented grid design (akin to Early Generation Variety Trials Designs) was used by placing experimental units in a 2D layout (row-column). Experimental units were individual plants of each entry in the mapping population. R & S checks were deployed diagonally across the test. Smut resistance was assessed by estimating "smut incidence" as a ratio "infected pods/total pods". First season results (summer 2017-18) show some entries display resistant reaction similar to the resistant standard. GLMM models are being adjusted for better analysis of the data obtained during the first season. Additionally, DNA samples for each entry were extracted for genotyping with Axiom *Arachis*2 (58 K SNP array) thus increasing the likelihood of finding alleles for smut resistance.

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## Poster 4.

### Tracking of Wild Allele Introgressions in a Peanut Chromosome Segment Substitution Line Population

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Cultivated peanut arose from the hybridization of the diploids *Arachis duranensis* (A genome progenitor) and *Arachis ipaensis* (B genome progenitor), followed by spontaneous chromosome doubling to yield the current allotetraploid state (AABB;  $2n=4x=40$ ). This genetic heritage, short period since polyploidization, self-pollinating breeding system, and domestication bottleneck have resulted in a crop with reduced diversity. In order to harness polymorphism from its wild relatives, a chromosome segment substitution line (CSSL) population was created via the tetraploid route to interspecific hybridization. The CSSL population was derived by crossing the A and B genome progenitors, *doubling the chromosomes of the cross, and introgressing chromosome segments from the resultant synthetic allotetraploid into the background of a cultivated variety (Fleur 11)*. Through SNP genotyping, we have developed high-resolution sets of markers that have enabled us to precisely delineate the regions of wild genetic introgression. In addition, we have observed evidence of tetrasomic recombination events in the population. By comprehensively phenotyping the population, we have uncovered significant variation in canopy, below ground, as well as seed composition traits. Analysis of the genotype and phenotype data has enabled us to propose how chromosome segments from the wild may alter the expression of traits in the cultivated genetic background. This study improves our understanding of how the wild relatives of peanut can be used to confer beneficial traits to cultivated peanut varieties.

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## Poster 5.

### Global methylome and Gene Expression Analysis During Early Peanut Pod Development

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Early development of peanut pod is an important process of peanut yield development. DNA methylation modes during early peanut pod development are still unclear now. To investigate the functions of the dynamic DNA methylation during peanut early pod development, global methylome and gene expression analysis were carried out by MeDIP-seq and Illumina high throughput sequencing. Differentially methylated genes were identified during three stages, S1, S2 and S3 of early peanut pod development, for examples, nodulin, cell number regulator-like protein, and senescence-associated genes. The expression levels of many gibberellins-related genes were changed during this period of pod development. From S1 to S2 gynophore, expression levels of two key methyltransferase genes, DRM2 and MET1, were up-regulated, which may lead to global DNA methylation changes between these two stages. The differentially methylated and expressed genes identified in S1, S2 and S3 gynophores involved in different biological processes, such as stem cell fate determination, response to red, blue and UV light, post-embryonic morphogenesis, and auxin biosynthesis. The expression levels of many genes were correlated to their DNA methylation levels. In addition, our results showed that the abundance of some 24-nt siRNAs and miRNAs were positively associated with DNA methylation levels of their target loci in peanut pods. The identified methylation changes during peanut early pod development provide useful information for understanding the roles of epigenetic regulation in peanut pod development.

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## Poster 6.

### **Assessing the Genetic Diversity of 15 Groundnut (*Arachis hypogaea* L.) Genotypes Among Which the Most Widely Cultivated Varieties in Senegal**

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Groundnut (*Arachis hypogaea* L.) is the most important grain legume in Senegal. However, its production is constrained by a myriad of biotic and abiotic stresses which necessitate the development and use of superior varieties for increased yield. Germplasm characterization both at the phenotypic and molecular level is important in all plant breeding programs. The aim of this study was to characterize 15 selected advanced breeding groundnut lines with different phenotypic attributes using simple sequence repeats (SSR) markers. The selected lines are contrasting for different traits including drought tolerance, pre-harvest aflatoxin contamination, seed quality traits, earliness, diseases resistance and yield. A total of 300 SSR markers were screened and hundred and sixty SSR markers were found polymorphic. The averaged mean of alleles per a locus was 3 alleles while the highest number of alleles per locus was 7. The markers TC11H06, Seq19D06, IPAHM103, Seq9A07, Seq14H06, Seq3A08, TC25G11, Seq15C10, TC27H12, TC23H09, Seq9A08 and PM050 were the most polymorphic markers revealing a least 5 alleles among the panel of genotyped lines. These highly polymorphic markers are being used for background and foreground selection to advance new populations.

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## Poster 7.

### A Comparative Analysis of the Complete Chloroplast Genome Sequences of Four Peanut Botanical Types

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*Arachis hypogaea* L. is an economically important oilseed crop worldwide and there are in total six botanical varieties within this species that have considerable morphological and molecular differences. The available chloroplast genome data within species is still limited. The complete chloroplast (cp) genome sequences of four representative botanical varieties (var. *hypogaea*, var. *hirsuta*, var. *fastigiata* and var. *vulgari*) were obtained by next-generation sequencing (NGS). The high throughput sequencing data were assembled, annotated and comparative analyzed. The total cp genome lengths of the studied *A. hypogaea* were 156,354 bp (for var. *hypogaea*), 156,878bp (for var. *hirsuta*), 156,718bp (for var. *fastigiata*) and 156,399bp (for var. *vulgaris*), respectively. Comparative cp genome sequence analysis of these four types revealed that their gene content, gene order and GC content were highly conserved, with only a total of 46 SNPs and 26 InDels identified among them. Most of these variation is restricted to non-coding sequences, especially, the highly variable region (*trnI*-GAU intron) was detected and will be useful for future evolutionary studies. These four cp genome sequences acquired here will provide valuable genetic resources for distinguishing *A. hypogaea* botanical types and determining the genetic relationship.

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## Poster 8.

### Peanut Mutant Induction, Screening and Utilization

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Germplasms innovation through mutagenesis is an important way for crop genetic improvement and gene functional analysis. Peanut mutant population was generated by treating mature seeds of 12 cultivars from China and the United States, including 10 erect type and 2 spreading type peanuts, with EMS, <sup>60</sup>Co  $\gamma$  ray and fast neutron. Totally, more than 60,000 M<sub>1</sub> lines were obtained. M<sub>2</sub> lines with different phenotypes from the parental cultivars were grown to generate M<sub>3</sub> plants for further confirmation of the mutant phenotypes. Through screening of more than 30,000 lines, a large number of mutants with stable phenotypes were identified, for example, mutants with high protein contents, high oil contents, high oleic acid contents, more branches, dwarf, big and small fruits, shrinking seed, crack seed coat, purple seed coat, shortened dormancy period, and late maturity. Currently, a small seeded mutant and a semi-dwarf mutant were used for further study. These mutants were used to cross with the parental cultivars to generate F<sub>1</sub> and then constructed F<sub>2</sub> populations. F<sub>2</sub> populations were used to identify genes that caused the phenotypes by BSA sequencing and map-based cloning. Digital gene expression profiles of mutants and wild type plants were carried out to understand the global influence of the mutated genes. A large number of genes were found to be differentially expressed in the mutants compare with the wild type controls. The contents of endogenous hormones including GA, IAA and ABA in the mutants and the wild type plants were also analyzed.

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## Poster 9.

### Using an Interspecific Population to Improve Biological Nitrogen Fixation of Cultivated Peanut (*Arachis hypogaea* L.)

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Biological nitrogen fixation (BNF) is an important economic and environmental process, which remains integrated in legumes breeding programs. Groundnut (*Arachis hypogaea* L.) is an allotetraploid grain legume cultivated for oil and food uses. Groundnut is mainly cultivated by poor farmers in Africa without fertilizers and in soil with low fertility, showing particularly N and P deficiency. Improving biological nitrogen fixation in groundnut could be of great interest to increase yield and lift-up soil fertility. In this study we used an interspecific mapping population to identify quantitative trait loci (QTLs) involved in BNF traits in groundnut. A subset of 83 chromosome segment substitution lines (CSSLs) developed at CERAAS by crossings between a synthetic tetraploid AiAd (*A. ipaensis* × *A. duranensis*)<sup>4×</sup> and cultivated variety Fleur 11 was evaluated for BNF under glasshouse conditions. Three conditions were tested: - N, + N and - N + inoculation with an efficient *Bradyrhizobium* strain (ISRA 400). BNF traits such as chlorophyll content, shoot and root dry weight were recorded. A significant variation of response to inoculation was observed for all traits and a significant positive relationship was found between chlorophyll content and biomass traits. A total of 25 QTLs were mapped only in inoculated condition for BNF traits whose positive or negative effects were associated with alleles of the wild parents. These results suggest new possibilities to improve BNF using wild species and could be exploited to understand the genetic mechanism of BNF in groundnut.

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## Poster 10.

### Toward Fine-mapping of a Wild Genomic Region Involved in Seed Size Reduction on Chromosome A07 in Peanut (*Arachis hypogaea* L.)

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Cultivated peanut (*Arachis hypogaea* L.) is a tropical grain legume with allotetraploid genome. In a previous work, we found that the introgression of a wild segment of 5.9 cM length located at the top of chromosome A07 of the cultivated species has major effect in reducing seed size. Attempts to refine this segment with new SSR developed, using *A. duranensis* sequences and a large F<sub>2</sub> population, were not successful due to the lack of polymorphism and SSR transferability in the region. Moreover, in silico analysis of this region showed a deletion of 0.8 Mb in the cultivated species genome. We therefore developed 50 SNPs in the target region based on GBS data and the "Axiom-Arachis" SNP array, and used them to genotype a F<sub>3:4</sub> segregating population. The SNPs were first validated on a set of samples with known genotype. A total of 23 SNPs were polymorphic. Then, 490 F<sub>3:4</sub> individuals were genotyped, using two polymorphic SSR and 4 SNPs evenly distributed on the segment to identify recombinant individuals, and 4 additional SNPs were used on the recombinant individuals to precise the location of the recombination events. As results, 40 recombinant individuals were identified. Among them, a subset of 11 NILs, homozygous for the wild and the cultivated genotypes, were selected. These NILs are very good genetic material to precise the position of the seed size QTL. They are being phenotyped under field conditions and genotype/phenotype associations will be conducted to narrow down the size of the QTL region.

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## Poster 11.

### DNA Methylation Pattern Among the Diverse Genotypes of Peanut (*Arachis hypogaea* L.)

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Low DNA sequence polymorphism despite enormous phenotypic variations in peanut indicate the possible role of epigenetic variations. In this study, an attempt was made to analyze DNA methylation pattern and its influence on gene expression towards identifying the epialleles for foliar disease resistance and productivity traits in peanut. Bisulfite sequencing of eleven peanut genotypes, differing for the foliar disease resistance and productivity traits, after 21 days of sowing showed that CHH regions recorded the highest DNA methylation sites (10,52,94,579) followed by CHG (6,98,64,108) and CpG (5,87,23,667) across the 11 genotypes. JL 24 recorded the highest DNA methylation sites (8,21,37,767) while TMV 2 had the lowest sites (6,90,44,110). In general, B sub-genome exhibited higher DNA methylation sites (13,92,82,071) than the A sub-genome (9,39,22,286). Overall, the DNA methylation was more frequent in inter-genic regions than in the genic regions. Parent (TMV 2) versus its EMS mutant (TMV 2-NLM) and parent (ICGV 86855) versus its progeny (GPBD 4) differed for 650 and 649 DNA methylation sites, respectively. When compared to the foliar disease [late leaf spot (LLS) and rust] resistant genotypes (GPBD 4, VG 9514, ICGV 86855, ICGV 86699 and ICGV 99005), the susceptible genotypes (TAG 24, TMV 2 and JL 24) showed significant differential DNA methylation at 766 sites corresponding to 25 genes. Of these genes, two showed significant differential expression (FPKM) between the resistant and susceptible genotypes. Interestingly, four differentially DNA methylated sites mapped to the QTL region (for LLS) on A02 and one mapped to the QTL region (for rust) on A03. However, the functional relevance of these sites need to be investigated for an efficient use of marker-assisted breeding for foliar disease resistance.

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## Poster 12.

### Investigating the Potential of the Wild Species *A. valida* to Enlarge the Cultivated Peanut Genetic Basis

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The allotetraploid (AABB) cultivated peanut (*A. hypogaea* L.) is a legume with low genetic variation at the DNA level attributed mostly to its recent monophylogenetic origin. For broadening the genetic basis of the cultivated species, several interspecific mapping populations are being developed using various combinations wild diploid species. Here we reported the development and characterization of an AB-QTL population involving the crossing between Fleur11 and ISATR52B. ISATR52B is a synthetic allotetraploid provided by ICRISAT that combines the A and B genomes of the wild species *A. duranensis* and *A. valida* respectively. A genetic map was developed using 150 BC<sub>1</sub>F<sub>1</sub> and 50 BC<sub>1</sub>F<sub>1</sub> were used to produce up to 200 BC<sub>2</sub>F<sub>4</sub> for QTL mapping purpose. The 200 BC<sub>2</sub>F<sub>4</sub> lines along with the two parents were genotyped with 128 polymorphic SSR markers and with the 30 K SNP Affymetrix-Axiom version 2. Our results showed a distorted population structure with a limited level of recombination between the B genomes resulting in low introgression rate of the alleles of *A. valida*. QTL analysis was performed for pod and seed characteristics as well as flowering time. Several QTL were mapped and lines that carry one or several favorable QTL alleles were identified. Additionally, for about half of the 38 QTL identified for agronomic traits, increase of the phenotypic value was associated with the wild parent. The present work contributed to identify good new source of alleles among peanut wild species.

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## Poster 13.

### Can Pod and Seed Size be Improved by Pyramiding Wild QTLs Alleles in Cultivated Peanut?

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Groundnut (*Arachis hypogaea* L.) is an important legume (AABB,  $2n = 4x = 40$ ), native to South America. A low level of DNA polymorphism is observed in cultivated germplasm. Peanut wild species are interesting sources of beneficial alleles that can be used to enlarge the genetic basis of the cultivated species particularly for traits of agronomic interest. In a previous study, we developed AB-QTL and Chromosome Segment Substitution Lines (CSSL) populations using a marker-assisted backcrossing strategy. A synthetic tetraploid species (*Arachis ipaensis* x *Arachis duranensis*)<sub>x4</sub> used as donor has been crossed with a cultivated cultivar (Fleur 11), and several wild alleles contributing positive variation to pods and seeds size were identified. These QTLs were subsequently validated in CSSL population. To test whether the pyramiding of these QTLs would increased pods and seeds size (length and width), we developed 42 lines by crossing two lines, one harboring a pod length QTL on chromosome A09 and the other a pod width QTL on chromosome B06. These 42 lines were assessed in three locations (Darou, Nioro, Sinthiou Malème) in Senegal during the rainfall season (2016). Dunnett's test revealed significant differences between the lines and their parents for several traits measured including 100-pod and 100-seed weights and pod and seed lengths and widths (PL; PWI; SL and SWI). Our results show that wild species can be used to improve agronomic traits in peanut. They also highlight the usefulness of QTL pyramiding strategy and pave the way for the creation of new peanut varieties by this strategy.

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## Poster 14.

### Evaluation of 36 Peanut Varieties for Adaptability, Pod Yield and Resistances to Foliar Disease in Burkina Faso

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In Western Burkina Faso where rainfall normally exceeds 1000 mm per year, peanut production is recurrently affected by foliar diseases. These diseases such as early (ELS) and late (LLS) leaf diseases, rust and rosette are major problems limiting peanut productivity. In this context, multiyear, multilocation field trials are essential in the process of varietal selection.

During the rainy season (July-October 2018), we used 34 lines from an advanced breeding population developed at ICRISAT and two check varieties (Fleur 11 and Sereba1), for on-farm trials in two locations (Bobo and Dédougou) in Western Burkina Faso. The experiments were set up in a 6 x 6 incomplete lattice design with three replicates. The genotypes were evaluated for adaptability, pod yield and resistances to ELS/LLS, rust and rosette diseases. Here we report the main findings of these trials and explore the implication of the results in the selection of varieties that will be tested in a larger number of locations in 2019.

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## Poster 15.

### Insights on the Composition and Evolution of the Satellitome in the A and B *Arachis* Genomes

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Satellite DNA (satDNA) is a major component of the heterochromatic regions of eukaryote genomes and usually shows a high evolutionary dynamic, even among closely related species. Cytogenetic and molecular evidences suggest that *A. duranensis* (A-genome) and *A. ipaensis* (B-genome) are the wild diploid species ( $2n=2x=20$ ) most likely involved in the origin of the allotetraploid *A. hypogaea*. One of the most striking karyotypic differences between these progenitors is the relative content of heterochromatin; i.e. while *A. duranensis* have conspicuous centromeric bands in most chromosomes, *A. ipaensis* chromosomes are deprived of them. The aim of this study was to provide information on the major changes underwent by satDNAs (the satellitome) during their genome differentiation. SatDNAs were identified using a high-throughput analysis using the satMiner pipeline. In addition, the chromosome distribution of the most abundant satDNA families was analyzed by Fluorescent in situ hybridization (FISH). Our results suggest that both, A and B genomes, share most of the satDNAs but they differ in the representation of some families, in accordance with the satDNA 'library hypothesis'. Only few of the isolated satDNAs were amplified and constitute the major components of the heterochromatic bands. This research provides complementary information to the genome analyses that are being carried out in *Arachis*, and constitutes the basis for understanding the satDNA evolutionary dynamics in diploid and allotetraploid *Arachis* genomes and their impacts on karyotype evolution.

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## Poster 16.

### Multi-resistant RILs Population Assessed for Peanut Blight Caused by *Sclerotinia minor*

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Peanut blight caused by *Sclerotinia minor* became a concern of farmers in Argentina for its high impact on yield losses. The current strategies to control soilborne diseases by decreasing the amount of inoculum adopting long term rotations and no-till practices have been insufficient. Thus, the development of resistant peanut varieties arose as a need for peanut producers in Argentina. Several accessions of wild *Arachis* species showed no symptoms of peanut blight during three seasons (2003-2005) of field trials at "Criadero El Carmen" in General Cabrera, Córdoba, Argentina, and were considered resistant to peanut blight. A fertile amphidiploid derived from a multiple cross ((*A. cardenasii* x *A. correntina*) x *A. batizocoi*)<sup>4x</sup> was also considered resistant after a multiple year field assay. On these bases, a RIL population developed from the cross of this amphidiploid with an elite high-oleic line (initially developed to test resistance to the peanut smut) was evaluated for resistance to peanut blight. A total of 93 recombinant inbred lines (RILs) and their parents were field inoculated with an inoculum suspension generated in the laboratory. This experimental approach was used to provide an intense inoculum pressure, which ensured high infectivity and pathogenicity. After the first season (2017) of peanut blight field trials, statistically significant differences (P=0.05) of disease incidence and severity were detected among RILs and their progenitors. The results suggest that these RIL population has promissory materials to be used in following pre-breeding programs focused on obtaining multi-disease peanut resistant lines.

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## Poster 17.

### Graph-based Clustering and Characterization of rDNA Genes of Allotetraploid Species of *Arachis* Section (Peanut and *A. monticola*) and Their Diploid Parentals by Next-generation Sequencing

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The ribosomal DNA repeats are an important fraction of the repetitive DNA of the genomes, which have a direct role in gene expression. 45S rDNA repeat unit contains the sequence for the 26S, 5.8S and 18S rRNA genes, as well as two transcribed spacers (ITS1 and ITS2) and a large intergenic spacer (IGS). Likewise, the 5S rDNA is organized in a transcribable region and a non-transcribable (NTS) region. Both rDNA repeats are organized into arrays and contains hundreds to thousands of repeats. Here, we analysed the structure of the ribosomal genes present in *A. hypogaea* (AABB), *A. monticola* (AABB) and their diploid parental species, *A. duranensis* (AA) and *A. ipaënsis* (BB) for future studies on amphiplasty and other effects of polyploidization. The characterization of the structures of the rDNA units of the 5S loci was highly conserved. However, the structure of the 45S sequence showed some species-specific differences between diploids. A small fraction of 79 bp towards the 5' and another 300 bp fraction towards the 3' of the promoter were exclusive of *A. ipaënsis*. Differences in the composition and structure of another repetitive region were also observed in the ETS. Both 45S rDNA versions were recovered from the allopolyploids. Ongoing research is centered in quantifying the copy number of both 45S versions in the allotetraploids to provide additional information for the analysis of genome specific NOR activation.

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## Poster 18.

### **Nourishing Groundnut Productivity: Case of the Community Seed Bank and Farmer Research Network Technology Transfer Models in Malawi**

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Agricultural productivity is constrained by lack of access to quality technologies such as seed of improved varieties. Despite the availability of a wide range of groundnut technologies in the era of genomics, adoption rates by small scale farmers remain low in Malawi chiefly because of less effective technology-transfer methods that support learning and decision making. The development and strengthening of farmer managed institutions was therefore envisaged as key to an effective and efficient technology dissemination approach that would contribute to adoption. Two approaches embedded within multidisciplinary partnership to accelerate diffusion of technologies were explored. These included establishment of “community seed banks” aimed at enhancing access of improved varieties seed; and second the “Farmer Research Network”, a farmer to farmer extension system to improve diffusion of productivity enhancing technologies. By 2015, 5 years from the inception, the number of seed banks grew to 174 with a total of 15,000 farmers reached. A follow up study in 2016/2017 showed an increase in land under improved varieties, and seed banks were indicated to be the main source of seed. Through the Farmer to Farmer approach, a total of 45000 had been reached. The study clearly indicated that community seed banks were an important model to ensure access to quality seed by rural farmers and play a vital role in promoting adoption of new technologies. The Farmer to Farmer extension system when supported with training has also shown to be an effective platform that can increase the reach and local anchorage of agro-innovations.

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## Poster 19.

### **Molecular Diversity and Marker-trait Association for Resistance to Foliar Fungal Diseases and Nutritional Quality Traits in Genomic Selection Training Population of Groundnut**

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Molecular diversity and marker-trait association in a set of 336 genotypes of genomic selection training population (GSTP) comprising 223 individuals from *ssp. fastigiata* and 113 from *ssp. hypogaea* were performed with 14 SSR markers linked to rust, late leaf spot (LLS) and nutritional quality traits. The population was also phenotyped for resistance to rust and LLS, yield and nutritional quality traits across four environments during 2015-16. The results revealed that 8% of the total molecular variance was due to among the sub-species variation whereas 92% was due to variation within the sub-species. Fourteen markers detected a total 462 alleles in 336 genotypes. The neighbor-joining tree grouped 336 genotypes into five major clusters. The cluster means along with pedigree and botanical information indicated that the molecular markers clearly differentiated the genotypes based on botanical classification, place of breeding and expression of traits. Marker-trait associations (MTA) analysis reported a total of 311 significant MTAs for 18 traits with 79 MTAs explaining >30% phenotypic variance. The SSR markers GM 1009, GM 2301 and TC6H03 were associated with LLS whereas IPAHM 103 and GM 2301 were associated with rust resistance across the environments. Markers IPAHM 103 was reported to be associated with oil and protein content across the environment. The MTAs explaining high phenotypic variance will be useful to develop markers for rust, LLS and nutritional quality traits for use in breeding programs.

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## Poster 20.

### Fatty Acid Accumulation in *ahFAD* Mutant High Oleic Groundnut Lines Across Developmental Stages and its Effect on Seed Quality Parameters

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Fatty acids (palmitic, oleic, and linoleic acid) accumulated during seed development govern the physical and chemical properties of groundnut seeds and its derived food products. Nine *ahFAD* (fatty acid desaturase) mutants with high oleic content (>75%) and four *ahFAD* wild types with normal oleic acid content (35-50%) were evaluated in the field condition during *rainy* and *post-rainy* 2017-18. Fresh seeds harvested at three different developmental stages *viz.*, 30, 60 and 90 days after fertilization (DAF) were analysed for gene expression using qRT-PCR and fatty acid profiling using near-infrared reflectance spectroscopy. The results of qRT-PCR revealed that the gradual down-regulation of *ahFAD2A* and *ahFAD2B* genes in FAD mutants resulted in elevated accumulation of oleic acid (80.1%) at 90 DAF. All the FAD mutants showed gradual increase in oleic acid content across the developmental stages. The declined accumulation of palmitic acid and linoleic acid was observed in *ahFAD* mutants at 90 DAF. Palmitic acid concentration was low in the *ahFAD* mutants whereas high in *ahFAD* wild types. The accumulation of high oleic acid had non-significant effect on oil and protein content in *ahFAD* mutants. The results of the current study suggest that the crop should be harvested at proper physiological maturity to get desirable composition of fatty acids along high oil yield in *ahFAD* mutants.

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## Poster 21.

### **Genetic Variability for Resistance to Peanut Bud Necrosis Disease in a Recombinant Inbred Line Population of Groundnut (*Arachis hypogaea* L.)**

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Peanut bud necrosis disease (PBND) is one of the economically important viral diseases of groundnut in India, China, Nepal, Sri Lanka and Thailand. The present investigation involved phenotyping for peanut bud necrosis disease (PBND) reaction, yield and associated traits in recombinant inbred line (RIL) populations. The population comprising of 318 RILs and parents from the cross TAG 24 × ICGV 86031 were evaluated at UAS, Raichur, Karnataka, India during rainy seasons 2014 and 2015 under natural disease epiphytotic. Individual season analysis revealed significant variation for all the studied traits. In the pooled season analysis significant variation was observed between the two seasons and genotype × season (g × s) interaction for all the traits except for hundred seed weight and PBND incidence at 30 days after sowing (DAS). High PCV and GCV were found for pod yield per plot, haulm yield per plot, percent of disease incidence at 30, 60 and 90DAS in individual rainy season 2014, 2015 and in pooled season. Variation was highly heritable for pod yield per plot, shelling percent, hundred seed weight in individual rainy seasons 2014 and 2015 and in pooled season. High heritability was observed for PBND disease parameters, days to 50% flowering, days to maturity, haulm yield per plot, during rainy season 2014 whereas, in rainy season 2015, moderate heritability was recorded for these traits. Per cent disease incidence at 30, 60 and 90DAS showed strong positive genotypic and phenotypic correlation with each other and strong negative correlation with haulm yield per plot. The variability observed among the RILs for disease and yield parameters enables selection of lines to recycle as parents in the peanut breeding program to enhance genetic gain for PBND resistance.

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## Poster 22.

### Relative Ranking and Risk to Sustainability of Pest Management Tools in the Virginia-Carolina Region of the United States

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Successful peanut production systems require genetic resources delivered with sound production and pest management practices. Limitations in both areas can adversely affect yield and sustainability of peanut-based farming systems. Traits that have been introgressed into peanut for improved insect management, nutrient use efficiency, and other limitations to production are not a focus of most breeding programs. In the U.S. Virginia-Carolina region there are many pests that must be managed with cultural practices, cultivars, and chemicals. Many factors influence effectiveness of controls. Of the 18 pests or groups of pests considered, chemicals were the primary tool for management (4 groups of weeds, 4 groups of arthropods, and 4 pathogens or viruses for disease). Cultivar resistance was listed as the primary tool for *Cylindrocladium* black rot (CBR) while a close second in terms of management for tomato spotted wilt, leaf spot, stem rot, and Sclerotinia blight. Cultural practices including crop rotation, plant population, planting date, and irrigation were primary tools for tomato spotted wilt suppression while crop rotation was essential for nematode management. Risk to sustainability of management through chemicals for leaf spot, southern corn rootworm, and thrips was listed as moderate to high while spider mites, Palmer amaranth, and common ragweed were listed as being at high risk for chemical management. Evolved resistance by pests was a major source of risk for maintaining chemical control for caterpillars and worms, common ragweed, Palmer amaranth, seedling disease, and thrips. Other resources that could partially impact availability of tools for pest control include EPA regulations, international acceptance of residues in peanut products, and manufacturer investment. This study suggests that many of the pests that limit yield require significant integration of cultural practices and chemicals for yield maintenance. Farmers use a wide range of practices to manage a broad spectrum of biotic and abiotic practices to protect yield. Moving forward, research programs will need to explore new approaches to managing pests, especially where key tools are vulnerable, in particular pesticides and fumigants, and where cultivars have limited impact.

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## Poster 23.

### Association of Pod Shell Thickness and Kernel Weight with Shelling Outturn in a MAGIC Population of Groundnut

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The present study was undertaken to determine the use of shell thickness and hundred kernel mass as surrogates to select for shelling outturn. The genetic gain assessment conducted in Spanish type groundnut varieties at ICRISAT indicated need to improve shelling outturn to further enhance the genetic gain for pod yield. Association of hundred kernel weight and shell thickness with shelling percent was studied in 983 lines of three multi-parent advanced generation inter cross (MAGIC) populations of groundnut developed at ICRISAT, Patancheru. The data were recorded on hundred kernel weight, average shell thickness and shelling percent from two replications during rainy 2017. The results of analysis of variance revealed highly significant differences among genotypes for all the traits under studied. The phenotypic coefficients of variation (PCV) were higher than the corresponding genotypic coefficients of variation (GCV) indicating the role of environment in the expression of characters. All the traits recorded high GCV and PCV along with high heritability ( $h^2$ ) and genetic advance as percentage of mean. The genotypic correlation coefficients were generally higher than the corresponding phenotypic correlation coefficients, showing the inherent association between the characters. Shelling percent exhibited significant negative genotypic and phenotypic association with shell thickness (-0.35 to -0.69) across the populations. Association between shelling percent and hundred kernel weight was not significant indicating the possibility of improving the shelling outturn across various sizes of kernels. Early generation selection for shell thickness, and selection for greater proportion of sound mature kernels in advance generations can contribute to enhanced genetic gain for shelling outturn.

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## Poster 24.

### A Survey of Physiological and Genetic Responses of *Arachis* Species to Water Deficit

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Wild *Arachis* species show a wide variability of agronomic traits and are potential sources of alleles for breeding programs aiming plants with better performance under environmental stress conditions. This work aimed at characterizing physiological and genetic mechanisms of *Arachis* species in response to water deficit (WD). The physiological parameters herein evaluated for four wild species of *Arachis* and cultivated peanut were leaf relative water content; gas exchange; electrolytic leakage and content of malondialdehyde, photosynthetic pigments and proline in plants kept in pots with 20, 45 and 75% of water capacity, for five days, in a controlled environment greenhouse. Results of gas exchange, chlorophyll fluorescence and pigment contents pointed to a non-stomatic photosynthesis limitation, negatively related to the increase of cell membrane damage in all species analyzed. Transcription factors (TF) were identified and those putatively related to WD responses were selected through *in silico* analyses of predicted proteomes of *A. duranensis* and *A. ipaensis* genes and predicted amino acid sequences generated from preliminary RNA sequencing data for *A. hypogaea* and *A. stenosperma* submitted to drought stress. The conservation of the TF family types and frequencies indicates that the regulatory pathways might be shared by these species. Furthermore, analyses of predicted orthologues of drought-responsive genes revealed the conservation of TF regulatory pathways among *Arachis* spp. and *Arabidopsis*. The expression modulation of eight of these TF genes were different among the *Arachis* spp., whenever water was limited in soil, suggesting variances in transcriptional regulation of TF genes, which might lead to diverse acclimatization mechanisms to drought stress in *Arachis* spp.

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## Poster 25.

### Brazilian Indian Landraces of *Arachis hypogaea*: Cytogenetic Evidences of its Origin

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*Arachis hypogaea* has been cultivated for a long time by the Kayabi indigenous tribe, inhabiting the Xingu Indigenous Park, Brazil. Local germplasm displays many characteristics morphologically different from the taxonomic variation described for cultivated peanut, raising questions about the origin and evolution of Xingu landraces. Further cytogenetic characterization aiming to take any doubts about their origin and better understand of domestication processes were carried out for three Xingu landraces morphologically very different. Analysis of CMA and DAPI bands, distribution of rDNA loci by FISH and genomic affinities with diploid *Arachis* species evidenced by GISH were determined and patterns were compared to those in cultivated peanut and wild *A. monticola*. The analysis of the Xingu landraces confirmed the similarity in the number and general morphology of their chromosomes, and with those of cultivated and wild allotetraploids; number and position of DAPI+ bands; 5S and 45S rDNA loci; predicted dominance of A subgenome chromosomes for NORs. The Indian landraces differed from the other tetraploids only in the number of CMA+ bands. Using probes of the genomes of *Arachis* diploid species (A and B genome types), GISH showed evident signals, with *A. duranensis* and *A. ipaensis* probes, for all three Xingu genotypes, stronger than those produced by *A. stenosperma* and *A. magna* probes. Although it is evident the contribution of *A. stenosperma* for the A subgenome of these Xingu types, here it is corroborated that *A. duranensis* and *A. ipaensis* are the progenitor species

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## Poster 26.

### Proteomic Analysis of *Arachis stenosperma* and *Meloidogyne arenaria* Interaction

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*Arachis stenosperma* displays high levels of resistance against the root-knot nematode *Meloidogyne arenaria* and exhibits the hypersensitive response (HR) as a response to nematode attempt to establish the feeding site. Proteomic survey of *A. stenosperma* inoculated roots, using 2-DE and 2D-NanoUPLC-MSE, identified a plethora of proteins (1,400) potentially involved in this HR response. Among them, 222 proteins were differentially abundant (DAPs) between inoculated and control roots. Analysis of protein-protein interactions using STRING highlighted functional categories involved in plant defense such as stress, glycolysis, redox and tricarboxylic acid cycle, whilst KEGG analysis revealed metabolic pathways responsible for biosynthesis of secondary metabolites and microbial metabolism. Overall, more than half of the DAPs showed a compatible profile of protein abundance and transcripts expression (RNA-Seq). The expression profiles of genes codifying for 18 DAPs identified by both proteomic approaches, were also validated by RT-qPCR showing that 50% were up- and 17%, down regulated during the nematode infection. Among those DAPS with increased abundance, some were related to plant defense such as, MLP-like protein 34 (MLP34), an uncharacterized protein containing the PLAT/LH2 domain (PLAT/LH2); copper transport protein ATX1 (ATX1); enolase (ENO); mitochondrial-processing peptidase subunit beta (MPP $\beta$ ); protein disulfide-isomerase (PDI); alcohol dehydrogenase (ADH) and eukaryotic translation initiation factor (eIF). The further functional validation of these candidates *in planta* might contribute for the development of new RKN control methods and provide new insights into the molecular basis of the interactions between plants and nematodes.

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## Poster 27.

### Selection of Lines Related to the Wild Parents with Useful Variability for the Peanut Breeding Program in Brazil

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Peanut (*Arachis hypogaea* L.) production and commercialization in Brazil experienced much progress in the last two decades. The technology leading this successful business are runner market-type cultivars, with high yield and market quality. However, susceptibility to leaf spots is common among these cultivars. The main sources of variability to leaf spots rely on wild species. In 2011, BC1 runner market-type lines related to the wild parents with variability for yield, seed size, and leafspots partial resistance have been selected and used as parents in the peanut breeding program at Embrapa. The main result is the cultivar BRS 425, adapted to the main growing regions of Brazil. Considering that *A. duranensis* has good resistance to leaf spots, we decided to evaluate the 88 F9 RIL population Runner IAC 886 x [*A. ipaënsis* x *A. duranensis*]<sub>4x</sub> for leaf spots resistance, in the field, in 2015. Morphological traits were also evaluated: height of the main stem, growth habit, flower color, stem and peg pigmentation, stem and leaflet surface, pod constriction. Among the ten RILs selected, resistance equivalent to *A. duranensis*, pod shape similar to the cultivated peanut, high yield potential and erect growth habit were observed. New breeding populations were developed since then, allowing the selection of new progenies and lines for the runner market-type in Brazil.

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## Poster 28.

### Development of peanut lines that incorporate resistances to leafspots and root-knot nematode from *Arachis* wild species

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Fungal foliar diseases are the main constraints to peanut production in Brazil. Root-knot nematode, *Meloidogyne arenaria* race 1, is an important pathogen in peanut-producing areas worldwide. Race 1 has not been detected in Brazil, but it was identified in Argentina, and this increases the risk of its appearance in Brazil. Although moderate sources of resistance to these pathogens have been found in cultivated peanut (*A. hypogaea*), its wild parents have levels of resistance considerably higher. During last years, we have developed tools for efficient introgression of wild genes into peanut. These tools include synthetic amphidiploids; molecular markers, genetic and physical maps; and the identification of markers associated to QTLs for traits of interest. By using these tools and *A. cardenasii* as donor, we developed high oleic peanut lines, now in F<sub>7</sub>, adapted to Brazilian growing conditions with resistance to root-knot nematode. Also using *Arachis cardenasii* as donor parent, lines with strong late leaf spot and rust resistance were obtained. One line, IAC-322, will be released as a cultivar. IAC-322 do not conform to market type and its use will be limited to niche markets. However, using marker-assisted breeding, these resistances are being incorporated into elite cultivars. By using marker-assisted breeding and *A. magna* and *A. stenosperma* as donors, BC<sub>3</sub>F<sub>4</sub> lines with very promising resistances to late leaf spot, rust, and probably new genes for resistance to nematode have been produced. Their growth habits and pod and seed morphologies are essentially indistinguishable from peanuts of pure pedigree.

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## Poster 29.

### Cytogenetic characterization of new induced allotetraploids of *Arachis*

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Peanut (*Arachis hypogaea*) is a recent allotetraploid originated from the hybridization between the diploid wild species, *A. duranensis* and *A. ipaensis* (AABB genome type). Since most wild species of *Arachis* are diploid, and display higher resistance to biotic and abiotic stress, induced allotetraploids are obtained aiming improved tetraploids to be used for peanut breeding. Among the analyses needed to characterize them, here it is presented some cytogenetic data on the induced allotetraploid ValSten (*A. valida* x *A. stenosperma*)<sup>4x</sup> and MagDur (*A. magna* x *A. duranensis*)<sup>4x</sup>, both with BBAA genome type. The number of chromosomes ( $2n=4x=40$ ); 5S rDNA loci; DAPI+ bands on centromeres of A subgenome are additive characters, shared by both allotetraploids, as well as the lack of DAPI bands on centromeres of B subgenome chromosomes. The number of 45S rDNA loci in ValSten was also an additive character, but not in MagDur that had one less loci than the sum of those present in the progenitor species. NORs were detected only on cyt-A10, for both hybrids, supporting the hypothesis of the nucleolar dominance of A subgenome after allopolyploidization. MagDur and other former studied induced allotetraploids, IpaDur1 and IpaDur2 (*A. duranensis* x *A. ipaensis*)<sup>4x</sup> have undergone alterations in their B subgenome organization, as demonstrated by the loss of the 45S rDNA loci, which is not observed in the spontaneous allotetraploids, *A. hypogaea* and *A. monticola* that have the expected loci number corresponding to the sum of the progenitor species.

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## Poster 30.

### Evaluation of Root-knot nematode resistance from the peanut wild relative *Arachis stenosperma* incorporated in allotetraploid backcrossed lines

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*Arachis hypogaea* (peanut or groundnut) is a very important oilseed, food and fodder crop cultivated worldwide. It is an allotetraploid species with very low genetic diversity and high susceptibility to root-knot nematode (RKN) *Meloidogyne arenaria*. This pathogen is an important constraint for peanut production that causes yield losses, reduces pod and grain quality and increases production cost. Some released cultivars harbor single resistance derived from the wild species *A. cardenasii*, but this is a single source of resistance and may be overcome leading to devastating consequences for peanut production. Therefore, additional sources of resistance are urgently needed. The peanut wild relative *A. stenosperma* has been described as very resistant to RKN. Previously, three chromosome locations conferring resistance were mapped in the *A. stenosperma* genome (A02, A04 and A09). In order to incorporate RKN resistance from *A. stenosperma* into peanut cultivars, lines derived from the cross of a induced allotetraploid ((*A. batizocoi* K9484 x *A. stenosperma* V10309)<sup>4x</sup>) and *A. hypogaea* Runner1AC886 were firstly selected base on SNP markers linked to the resistance, and good agronomic traits; and secondly they have been crossed and backcrossed with peanut elite breeding lines from Tifton, GA. Advanced backcrossed lines have been developed and phenotypic evaluation for resistance was completed recently, which allowed us to confirm their resistance to RKN. Additionally, genotyping with the Affymetrix SNP array is underway to closely define the chromosomal segments conferring resistance and select the most resistant individuals for further crosses and advancement.

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## Poster 31.

### **Development of a High-density Genetic Map Based on Specific Length Amplified Fragment Sequencing and its Application in Quantitative Trait Loci Analysis for Yield-related Traits in Cultivated Peanut**

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High-density genetic maps (HDGMs) are very useful for genomic studies and quantitative trait loci (QTL) mapping. However, the low frequency of DNA polymorphisms in peanut has limited the quantity of available markers and hindered the construction of a HDGM. This study generated a peanut genetic map with the highest number of high-quality SNPs based on SLAF-seq technology and a newly constructed RIL population ('ZH16' × 'sd-H1'). The constructed HDGM included 3,630 SNP markers belonging to 2,636 bins on 20 linkage groups (LGs), and it covers 2,098.14 cM in length, with an average marker distance of 0.58 cM. This HDGM was applied for the following collinear comparison, scaffold anchoring and analysis of genomic characterization including recombination rates and segregation distortion in peanut. For QTL mapping of investigated 14 yield-related traits, a total of 62 QTLs were detected on 12 chromosomes across 3 environments, and the co-localization of QTLs was observed for these traits which were significantly correlated on phenotype. Two stable co-located QTLs for seed- and pod-related traits were significantly identified in the chromosomal end of B06 and B07, respectively. The construction of HDGM and QTL analysis for yield-related traits in this study provide useful information for fine mapping and functional analysis of genes as well as molecular marker-assisted breeding.

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## Poster 32.

### Discovery of Genomic Regions and Candidate Genes for Stable QTLs Controlling Shelling Percentage Using QTL-seq Approach in Cultivated Peanut (*Arachis hypogaea* L.)

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Cultivated peanut (*Arachis hypogaea* L.) is an important grain legume providing high-quality cooking oil, rich proteins and other nutrients. High shelling percentage is an important quantitative trait reducing waste and increasing economic value of peanut. Deployment of diagnostic markers through genomics-assisted breeding (GAB) can accelerate the process of developing improved varieties with enhanced shelling percentage. In this context, we deployed the QTL-seq approach to identify genomic regions and candidate genes controlling shelling percentage in a recombinant inbred line population (Yuanza 9102 × Xuzhou 68-4). Four libraries (two parents and two extreme bulks) were constructed and sequenced, generating 456.89–790.32 million reads and achieving 91.85%–93.18% genome coverage and 14.04–21.37 X average read depth. Comprehensive analysis of two sets of data (Yuanza 9102/two bulks and Xuzhou 68-4/two bulks) using the QTL-seq pipeline resulted in discovery of two overlapped genomic regions (2.75Mb on A09 and 1.1Mb on B02). Nine candidate genes affected by ten SNPs with nonsynonymous effect or in UTRs were identified for shelling percentage by detailed analysis. Cost-effective KASP markers were successfully developed for one SNP on A09 and three SNPs on B02. Genotyping of the mapping population with these four allele-specific markers revealed that the QTLs located in the two genomic regions had major and stable expression across five environments. The identified candidate genomic regions and genes for shelling percentage further provides opportunity for gene cloning and deployment of diagnostic markers in molecular breeding for peanut improvement.

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## Poster 33.

### Identification and Functional Analysis of the *KCS* Gene Family in Peanut (*Arachis hypogaea* L.)

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$\beta$ -ketoacyl-CoA synthase (*KCS*) is the key enzyme for biosynthesis of very long chain fatty acids (VLCFA) in plant. In this study, a total of 33 *KCS* genes were identified and characterized. Twenty of them were expressed in 22 tissues, and most of them exhibited expressing tissue preference. *AhKCS13*, *AhKCS15*, *AhKCS20*, *AhKCS21*, *AhKCS27* and *AhKCS31* were highly expressed in leaves, while *AhKCS1*, *AhKCS13*, *AhKCS18*, *AhKCS20*, *AhKCS27* and *AhKCS30* were highly expressed in roots. Almost every *KCS* gene was expressed in flowers. In developing seeds, *AhKCS1*, *AhKCS6*, *AhKCS10*, *AhKCS15*, *AhKCS18*, *AhKCS25*, *AhKCS30* and *AhKCS31* were most abundant members. Notably, only *AhKCS15* exhibited seed-specificity. These eight *KCS* genes were cloned and heterologously expressed in yeast, respectively. All the eight *KCS* genes could produce VLCFAs in yeast, but different substrate preferences of them were observed. *AhKCS10*, *AhKCS15* and *AhKCS31* only added two carbons to oleic acid and formed arachidonic acid, but other three copies added two more carbons to oleic acid and generated nervonic acid. All the results indicating that each *KCS* gene in peanut played different role in different tissues at different developing stages. The identified *KCS* genes provide valuable information for improvement of oil quality and enhancement of defense in peanut.

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## Poster 34.

### Identification of Genomic Regions and Diagnostic Markers for Resistance to Aflatoxin Contamination in Peanut (*Arachis hypogaea* L.)

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Aflatoxin contamination caused by *Aspergillus flavus* is a major constraint to peanut industry worldwide due to its toxicological effects to human and animals. Developing peanut varieties with resistance to seed infection and/or aflatoxin accumulation is the most effective and economic strategy for reducing aflatoxin risk in food chain; however, it is challenging because the genetic basis is still poorly understood. To identify QTLs for resistance to aflatoxin contamination, a recombinant inbred line (RIL) population was developed from crossing Zhonghua 10 (susceptible) with ICG12625 (resistant). The percent of seed infection index (PSII), the contents of aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) of RILs were evaluated by a laboratory kernel inoculation assay. Two QTLs were identified for PSII including one with 11.32-13.00% phenotypic variance explained (PVE). A total of 12 QTLs for aflatoxin accumulation were detected by unconditional analysis, and four of them (*qAFB1A07* and *qAFB1B06.1* for AFB1, *qAFB2A07* and *qAFB2B06* for AFB2) exhibited stable effects across multiple environments with 9.32%-21.02% PVE. Furthermore, *qAFB1A07* and *qAFB2A07* were co-localized in the same genetic interval on LG A07, and *qAFB1B06.1* was co-localized with *qAFB2B06* on LG B06. Conditional QTL mapping also indicated a strong interaction between resistance to AFB1 and AFB2 accumulation, suggesting that *qAFB1A07* and *qAFB1B06.1* interacted additively to reduce the accumulation of aflatoxin B1 and B2. Additionally, two markers were validated to be associated with resistance to aflatoxin accumulation in diversified germplasm collection. The identified QTLs and associated markers exhibit potential to be applied in improvement of resistance to aflatoxin contamination.

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